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# Organic Fertilizers

## From Basic Concepts to Applied Outcomes

*Edited by Marcelo L. Larramendy*
*and Sonia Soloneski*

# ORGANIC FERTILIZERS - FROM BASIC CONCEPTS TO APPLIED OUTCOMES

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Edited by Marcelo L. Larramendy and Sonia Soloneski

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**Notice**

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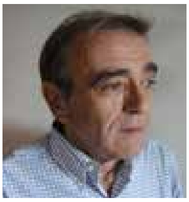
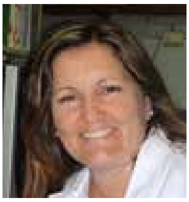
# Meet the editors



Marcelo L. Larramendy, Ph.D., serves as Professor of Molecular Cell Biology at the School of Natural Sciences and Museum (National University of La Plata, Argentina). He has been appointed senior researcher of the National Scientific and Technological Research Council of Argentina. He is also former member of the Executive Committee of the Latin American Association of Environmental Mutagenesis, Teratogenesis and Carcinogenesis. He is author of more than 470 contributions, including scientific publications, research communications and conferences worldwide. He is recipient of several national and international awards. Prof. Larramendy is a regular lecturer at the international A. Hollaender Courses organized by the IAEMS and is former guest scientist at the NIH, USA, and University of Helsinki, Finland. He is expert in genetic toxicology and is, or has been, referee for more than 30 international scientific journals. He is also member of the International Panel of Experts at the International Agency for Research on Cancer (IARC, WHO, Lyon, France) in 2015 for the evaluation of DDT, 2,4-D, and Lindane. Presently, Prof. Dr. Larramendy is Head of the Laboratory of Molecular Cytogenetics and Genotoxicology at the UNLP.



Sonia Soloneski is Ph.D. in Natural Sciences and Professor Assistant of Molecular Cell Biology at the Faculty of Natural Sciences and Museum of La Plata, National University of La Plata, Argentina. She became a member of the National Scientific and Technological Research Council (CONICET) of Argentina in Genetic Toxicology field. Presently, she is a member of the Latin American Association of Environmental Mutagenesis, Teratogenesis and Carcinogenesis (ALAMCTA), the Argentinean Society of Toxicology (ATA) and the Argentinean Society of Genetics (SAG). She has authored more than 290 scientific publications in the field, including scientific publications in research papers, reviewed journals and conferences worldwide. She is a referent for issues related to Genetic Toxicology, Mutagenesis and Ecotoxicology field.

# Contents

# Preface

According to the United Nations, the world population of 6.7 billion is likely to reach 9.2 billion by 2050. The UN Millennium Project report indicated that to keep up with population and economic growth, food production will have to increase by 70% by 2050 to help solve the current food crisis. This increased food production will have to occur in less available arable crops, and this can only be accomplished by intensifying the production. Agriculture in the twenty-first century faces several challenges, such as meat production without growing animals, better irrigation management for agricultural processes, the development of genetic engineering for drought-tolerant and higher yielding crops, the improvement of agricultural precision and aquaculture, the sustainable development of biofuels and the promotion of the organic agriculture around the world, among others. However, intensifying food production must be done in an environmentally safe manner through ecological intensification to increase the yield per unit of land, approaching the reachable yield of farming systems, with minimal or no negative environmental impact.

The world will not be able to meet its food production goals without the use of fertilizers. Actually, fertilization is responsible for 40–60% of the world's food production. In this way, the government's responsibility is in developing best management practices that use fertilizers in an effective, efficient and safe manner, ensuring that good production and environmental goals are met not only in industrialized nations, but also in developing countries. According to the Food and Agriculture Organization (FAO), global fertilizers have played an important role in increased crop production, especially cereal yields, and will continue to be the key in the future. In the FAO report "World Fertilizer Trends and Outlook to 2018," they report that global fertilizer use is likely to rise above 200.5 million tons, 25% higher than that recorded in 2008. At the same time, the global capacity of synthetic fertilizer products, intermediates and raw materials will increase with the production of the main three soil fertilizers, namely, nitrogen, phosphorus and potash. The doubling of global production during the past 35 years was related to a 6.9-fold increase in nitrogen fertilization and a 3.5-fold increase in phosphorus fertilization. Agriculture practices contribute to over 20% of global anthropogenic greenhouse gas emissions. Furthermore, agricultural intensification has had major detrimental impacts on worldwide ecosystems.

Agricultural practices are constantly changing in nature, and fertilization procedures vary with time due to the emergence of remarkable innovations in crop production practices under sustainable crop production systems. Since climate change has a direct impact on agriculture systems, environmentally sound farming practices need to be quickly developed. Organic agriculture offers a major potential to diminish the emissions of agricultural greenhouse gases. This is regarded as a sustainable agricultural system, and taking into consideration soil fertility conservation for the establishment of an adequate crop system that is economically acceptable, environmentally sustainable and technically practicable is the goal of agricultural agronomists, farmers and producers. Dependence on organic nutrient sources is a central characteristic of organic agriculture, which uses organic nutrient sources such as livestock and green manure and several types of compost even to meet the crop demand in intensive cereal production. One of the advantages to the use of organic fertilizers is that they provide their nutrients to crops over a long period of time in a slow release process. Accordingly, more research on improving efficiency and minimizing losses from organic natural resources is needed to determine benefits, costs and adequate agricultural practices to avoid the necessity of using synthetic inorganic fertilizers.

This book, *Organic Fertilizers – From Basic Concepts to Applied Outcomes*, is intended to provide an overview of emerging researchable issues related to the use of organic fertilizers that highlight recent research activities in applied organic fertilizers toward a sustainable agriculture and environment. We aimed to compile information from a diversity of sources into a single volume to give some real examples extending the concepts in organic fertilizers that may stimulate new research ideas and trends in the relevant fields.

This book comprises nine general chapters describing issues related to the use of several manures and other farming derivative products. The first chapter describes the current status of the composting process, the development of novel spectroscopy techniques for assessing compost maturity and the improvement of soil fertility by organic fertilizer amendments. The second chapter aims to provide information on organic fertilizer sources, including poultry farms and fish farms, as well as a discussion of the composition, transformation and crop response in selected soils. The third chapter is an update about the difficulties and limitations involved in the use of green manure and the use of crop residues in managing soil fertility, and the main factors influencing the decomposition and mineralization processes in tropical crops. The fourth chapter provides information about experiences using different organic amendments, including tankage, chicken manure and seaweed as potential organic fertilizers, in different tropical soils. The fifth chapter focuses on the importance of economical and sustainable sources of phosphorus and the comparative efficacy in the use of organic fertilizer containing rock phosphate for legumes crops. The sixth chapter aims to provide information about the role of integrated biofertilizers, animal manures and phosphorous management for improving crop productivity under semiarid conditions. As a case study, the seventh chapter discusses the use of soil amendments for agricultural production using different waste applications, such as municipal sewage sludge, chicken manure, horse manure and cow manure. The eighth chapter depicts the impacts of organic and inorganic fertilizers on physicochemical properties of red soils. Finally, the ninth chapter is an interesting overview attempting to unveil a conflict between the traditional knowledge of biochar fertilizer and the new knowledge of soil science. Then, three chapters discuss the use of different derivatives of the meat industry sector as potential organic fertilizers. The influence of different rates of slaughterhouse organic residues applied alone or together with synthetic mineral fertilizers in diverse crops as an efficient strategy to reduce costs and increase the carbon levels, providing agronomic and environmental benefits, are evaluated. Similarly, the use of organic waste such as tannery sludge, which has high organic matter, nitrogen, and phosphorus content, as an organic fertilizer for improving soil fertility in semiarid soils and for the remediation of abandoned mine sites is described. Also, the effects of two pasteurized nitrogen-enriched sludge loadings on corn crops for grain production and soil modification evaluation are analyzed. The next three chapters include a discussion on an important contribution to leguminous intercropping that includes soil organic matter enhancement and fertility building, biological nitrogen and other plant nutrition availability; a study of the effect of organic and inorganic fertilizers on the total phenolics content and fatty acid levels of five common chicory varieties and research about the production of forage grass fertilized with poultry manure applied to the soil with and without soil chiseling. Finally, this book includes a last chapter discussing the possibilities of recycling foodborne pathogens and residual antibiotics through agricultural crop practices as potential constituents of organic fertilizers, stressing the potential risk for human populations.

The contributions made by the specialists in this field of research are gratefully acknowledged. The publication of this book is of high importance for those researchers, scientists, engineers, teachers, graduate students, agricultural agronomists, farmers, and crop producers who make use of these different investigations to understand the advantages of the use of organic fertilizers. Future agricultural practices will irreversibly shape the Earth’s land surface, including its species, geochemistry and disponibility of surface to the people living on it. We hope that the information presented in this book will be of value to those directly engaged in the handling and use of organic fertilizers, and that this book will continue to meet the expectations and needs of all those interested in the different aspects of the use of organic fertilizers to achieve a sustainable agriculture without compromising environmental integrity.

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Chapter 1

# Compost Process and Organic Fertilizers Application in China

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Additional information is available at the end of the chapter

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## Abstract

Composting is an inexpensive and sustainable treatment for solid wastes. The composting industry has been growing rapidly because of a boom in the animal industry in China over the past decades. In this chapter, we introduce composting process and status in China, especially in Jiangsu Province. Meanwhile, the developed novel spectroscopy techniques are also introduced, which are more suitable for assessment of compost maturity than the conventional techniques in view of ease of sample preparation, rapid spectrum acquisition, and nondestructive nature of the analysis. These novel spectroscopy techniques include near-infrared reflectance spectroscopy (NIRS)—partial least squares (PLS) analysis and fluorescence excitation–emission matrix (EEM) spectroscopy—parallel factor (PARAFAC) analysis. In addition, organic fertilizer amendments can not only improve soil fertility but also offset chemical fertilizers' nanoscale changes. Emerging cutting-edge technologies of synchrotron-based X-ray absorption fine structure (XAFS) spectroscopy and nanoscale secondary ion mass spectrometry (NanoSIMS) were used to identify the composition of organic carbon and minerals and their correlations, respectively. Recently, investigators have shown that organic fertilizer amendments could enhance the production of highly reactive minerals, for example, allophane, imogolite, and ferrihydrite, which further benefit for soil carbon storage and soil fertility improvement.

**Keywords:** compost process, compost maturity, China, solid waste treatment, soil fertility, spectroscopy techniques, nanominerals, short-range ordered minerals, soil carbon storage

## 1. Introduction

Composting is an inexpensive, efficient, and sustainable treatment for solid wastes. In China, the composting industry has been growing rapidly, owing to a boom in the animal industry over the past decades. Because an immature compost applied to soil results in seed germination inhibition, root destruction, and a decrease in the $O_2$ concentration and redox potential [1, 2], assessing organic fertilizer maturity is critical. By the way, a main difference between common composts and commercial organic fertilizers is the complexity and unpredictability of the raw materials of the latter.

In recent decades, livestock numbers have increased dramatically in China. The quantity of manure generated by China's livestock has increased significantly as a result of the rapid increases in livestock numbers. The quantity increased by at least fourfold between 1980 and 2005, to an annual estimated total of 3060 million tons (Mt, fresh weight of manure) in 2005. [3] It was estimated that manure generation in 2010 was ca. 2800 Mt (fresh weight). [3] In addition, organic fertilizer amendment has been shown to be an effective way of increasing soil organic matter (SOM) content and reducing environmental pollution. However, the mechanism of storage of SOM remains largely unknown. Recently, some investigators have shown that organic fertilizer amendments could enhance the production of highly reactive short-range ordered (SRO) minerals, which further benefit for SOM storage and soil fertility improvement. [4–6]

In this chapter, compost process and status, novel spectroscopy techniques in assessing compost maturity, and improvement of soil fertility by organic fertilizer amendments in China are introduced.

## 2. Compost process and status in China

Traditionally, farmers in China were mainly depending on organic fertilizers, for example, animal manures and agricultural residuals. In 1950s, farmers also began to apply some of chemical fertilizers (**Figure 1**). In 1980s, the application of chemical fertilizers and organic fertilizers had a very similar percentage. However, the application of chemical fertilizers in 2010 was over 90%. As a result, soil acidification is a major problem in soils of intensive Chinese agricultural systems. Two nationwide surveys showed that soil pH declined significantly ($P < 0.001$) from the 1980s to the 2000s in the major Chinese crop-production areas. [7] Therefore, the replacement of chemical fertilizers by organic fertilizers in a certain percentage is urgent.

During the last decade's development of composting in China, numerous large-scale animal farms with more than 10,000 pigs or 5000 cattle have been established. As a result, a large amount of animal manure is produced, which is a major pollutant if untreated [8]. On the other hand, this is also a perfect resource of organic fertilizers. For example, more than 100 factories produce over 5000 tons of commercial organic fertilizers each year in Jiangsu Province, China (**Figure 2**). Correspondingly, the Jiangsu government now subsidizes the composting factories

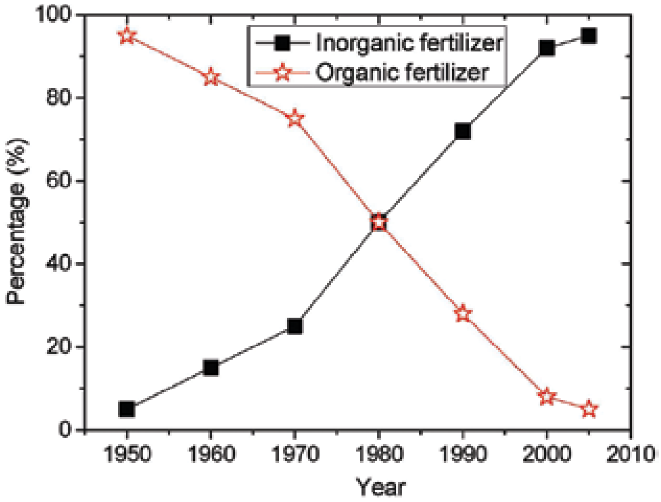

**Figure 1.** Percentage of fertilizers used in China from 1950 to 2005.

with 200 RMB per ton. As a result, the price of commercial organic fertilizers has decreased from 550 to 350 RMB. Therefore, the farmers are pleased to replace chemical fertilizers with commercial organic fertilizers. Presently, the total amount of commercial organic fertilizers produced by subsidized composting facilities is more than 2 million tons per year in Jiangsu Province, China (**Figure 2**). Thereof, the government of Jiangsu Province plays a critical role in promoting the production and application of organic fertilizers by the farmers.

**Figure 2.** Summary of factory locations to produce commercial organic fertilizers in Jiangsu Province, China.

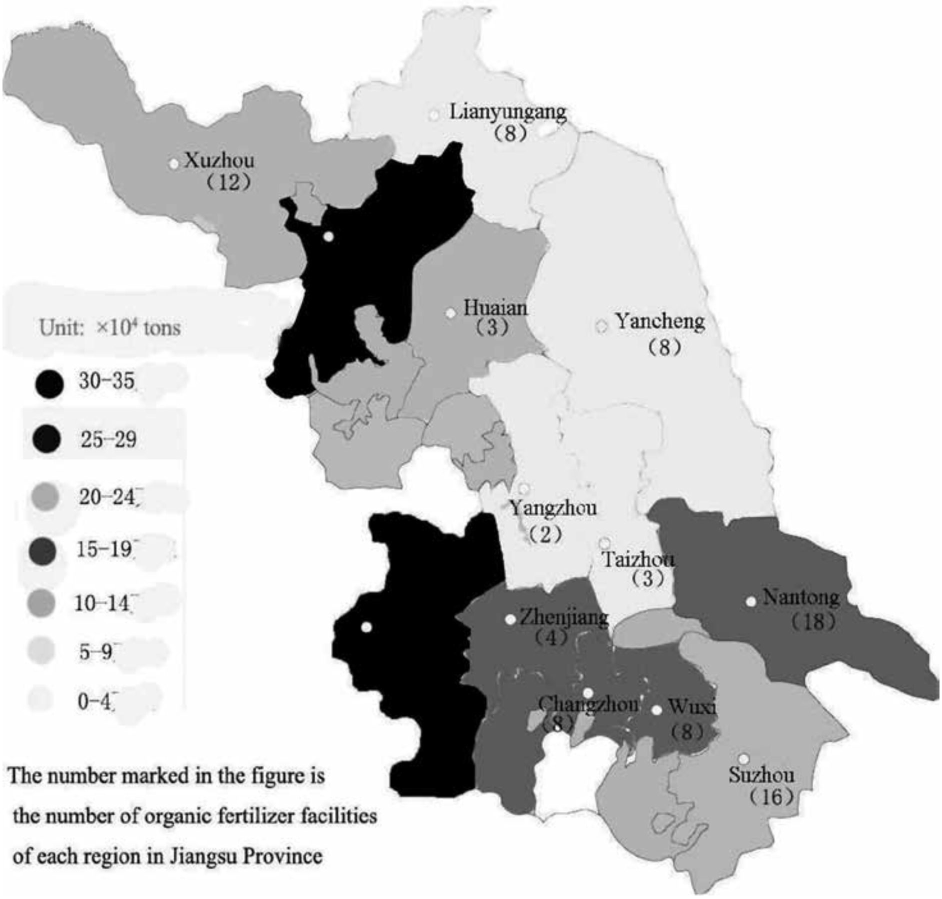

**Figure 3.** Trough composting system (a) and windrow composting system (b) in China.

In China, trough composting system and windrow composting system are the main composting processes, with windrow composting system being more popular in Jiangsu Province (**Figure 3**). Windrow composting consists of placing the mixture of raw materials in long narrow piles that are agitated or turned on a regular basis. The turning mixes the materials during composting and enhances passive aeration. Generally, the heights of windrows are in a range of 90 to 180 cm. Correspondingly, the width of them varies in a range of 100 to 300 cm. In general, the size, shape, and spacing of the windrows are determined by the turning equipment. During aeration, the rate of air exchange depends on the porosity of the windrow. Therefore, the size of a windrow is determined by its porosity.

## 3. Assessment of compost maturity by spectroscopy techniques

Various parameters are commonly used to evaluate compost quality. [9] In general, these parameters include germination index (GI), water-soluble organic carbon (WSOC), water-soluble organic nitrogen (WSON), pH, electrical conductivity (EC), moisture, and total organic matter (TOM) content. It is accepted that any sole parameter cannot determine compost maturity, which must be assessed by a combination of different physical, chemical, and biological properties (**Table 1**). However, all these approaches are expensive or time-consuming when a large number of samples are involved. [1,10] It is reported that spectroscopy techniques have many advantages over traditional chemical analyses, such as its ease of sample preparation, rapid spectrum acquisition, nondestructive analysis, and portability. [11]

| **Physical** | **Odor, color, temperature, particle size and inert materials** | |
|---|---|---|
| Chemical | Carbon and nitrogen analyses | C/N ratio in solid and water extract |
| | Cation exchange capacity | CEC, CEC/Total organic-C ratio, etc |
| | Water-soluble extract | pH, EC, organic-C, ions, etc |
| | Mineral nitrogen | $NH_4$-N content, $NH_4$-N/$NO_3$-N ratio |
| | Pollutants | Heavy metals and organics |
| | Organic matter quality | Organic composition: lignin, complex carbohydrates, lipids, sugars, etc. |
| | Humification | Humification indices and humic-like substances characterization: elemental and functional group analyses, molecular weight distribution, E4/E6 ratio, pyrolysis GC-MS, spectroscopic analyses (NMR, FTIR, Fluorescence, Raman, etc.) |
| Biological | Microbial activity indicators | Respiration ($O_2$ uptake/consumption, self-heating test, biodegradable constituents) |
| | | Enzyme activity (cellulase, phosphatases, dehydrogenases, proteases, etc) |
| | | ATP content |
| | | Nitrogen mineralization-immobilization potential, nitrification, etc. |
| | | Microbial biomass |
| | Phytotoxicity | Germination and plant growth tests |
| | Others | Viable weed seed, pathogen, and ecotoxicity tests |

**Table 1.** Current criteria evaluated in the literature to characterize compost quality [9]

Near-infrared reflectance spectroscopy (NIRS) has been shown to rapidly (within 1 min) assess compost quality. [12–14] However, it is unclear whether NIRS can also be applied to rapidly determine the quality of commercial organic fertilizers, owing to the complexity and unpredictability of the raw materials of the commercial products. **Figure 4** shows a distinct appearance of commercial organic fertilizers with composting samples. A total of 104 commercial organic fertilizers were collected from full-scale compost factories in Jiangsu Province, China. These factories treat organic matter from animal manure and other agricultural organic residues. These factories produce approximately 5000–150,000 tons of commercial organic fertilizers per year.

**Figure 4.** Typical commercial organic fertilizers, including powered (A, C) and granular (B, D) fertilizers. These photos suggest that the commercial organic fertilizers are more even than samples from the composting process.

We can see that all the NIR spectra collected from commercial organic fertilizers in Jiangsu Province, China (**Figure 5**) were divided into two groups of signals with different slopes under 1400 nm: one group has an increased curvature with a significant absorbance peak at a wavelength of approximately 1420 nm, while another is more flat and has only a small absorption at this position. This is because the second significant spectral peak is mainly at approximately 1950 nm (**Figure 5**). The band at 1420 nm is associated with the O–H and aliphatic C–H, while that at 1950 nm is assigned to the amide N–H and O–H. Because the NIR spectrum contains all strength information of the chemical bond, chemical composition, electronegativity, etc, the absorption peaks are heavily overlapped. In addition, other interference information, such as scattering, diffusion, special reflection, refractive index, and reflected light polarization, also has an important influence on the NIR spectrum. Thus, the quantitative predictions are difficult directly through NIR spectra alone.

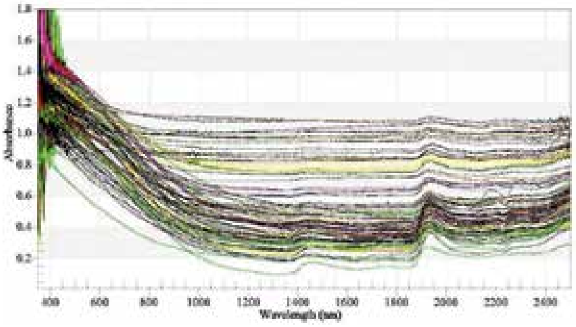

**Figure 5.** Spectra of NIR of a total of 104 commercial organic fertilizers. [10]

Multivariate analyses are required to discern the spectral characteristics of commercial organic fertilizers with the support of chemometric methods, for example, partial least squares (PLS) analysis in this study. The results of the NIRS calibration and validation for the quality indices of commercial organic fertilizers are listed in **Table 2** and Figures 6 and 7. The NIR calibrations allowed accurate predictions of WSON, TOM, pH, and GI ($R^2 = 0.73–0.93$ and RPD = 1.47–2.96). However, the results were less accurate for moisture ($R^2 = 0.91$, $r^2 = 0.79$, RPD = 2.22), TN ($R^2 = 0.98$, $r^2 = 0.80$, RPD = 2.25), and EC ($R^2 = 0.99$, $r^2 = 0.74$, RPD = 2.27). In addition, the WSOC had the worst prediction ($R^2 = 0.88$, $r^2 = 0.76$, RPD = 2.10). Therefore, predictions were moderately successful for TOM, TN, WSON, pH, EC, GI, and moisture, but failed for WSOC.

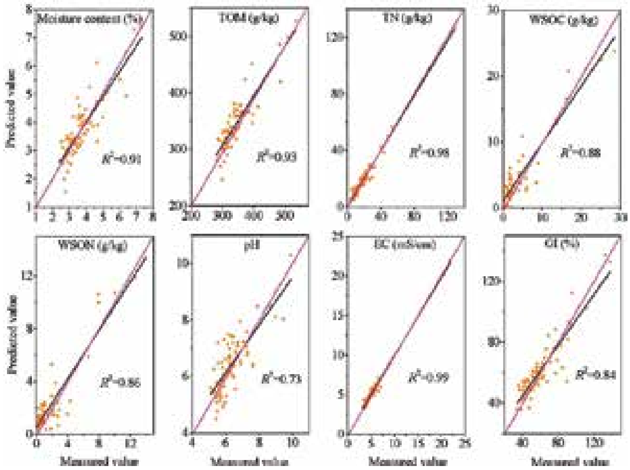

**Figure 6.** The measured and predicted values of the quality indices of commercial organic fertilizers in the calibration data set. [10] The best fit is shown by red line.

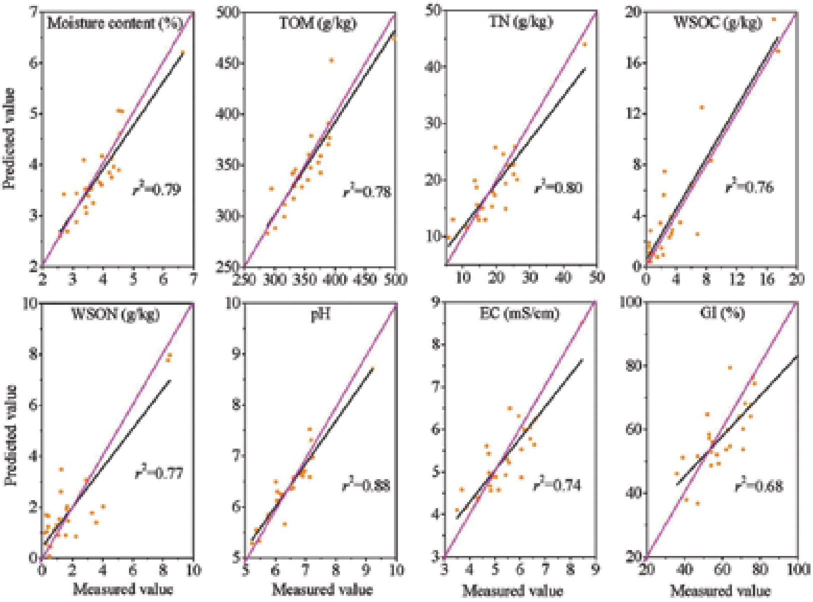

**Figure 7.** The measured and predicted values of the quality indices of commercial organic fertilizers in the prediction data set. [10] The best fit is shown by red line.

| Parameters | Calibration set | | | Validation set | | | | |
|---|---|---|---|---|---|---|---|---|
| | PC | $R^2$ | RMSECV | $r^2$ | RMSEP | RPD | Bias | Slope |
| Moisture content (%) | 19 | 0.94 | 0.23 | 0.67 | 0.36 | 2.27 | −0.03 | 0.76 |
| TOM (g/kg) | 12 | 0.85 | 17.69 | 0.69 | 24.76 | 1.71 | −9.03 | 0.72 |
| TN (g/kg) | 17 | 0.98 | 3.08 | 0.80 | 3.63 | 2.25 | −0.29 | 0.77 |
| WSOC (g/kg) | 7 | 0.85 | 3.82 | 0.55 | 3.21 | 1.43 | 0.06 | 0.69 |
| WSON (g/kg) | 8 | 0.86 | 1.81 | 0.77 | 1.60 | 1.47 | 0.05 | 0.74 |
| pH | 9 | 0.86 | 0.36 | 0.75 | 0.41 | 1.96 | −0.10 | 0.73 |
| EC (mS/cm) | 17 | 0.99 | 0.46 | 0.74 | 0.54 | 2.27 | −0.04 | 0.78 |
| GI (%) | 18 | 0.84 | 4.81 | 0.68 | 9.52 | 1.73 | −2.68 | 0.63 |

TOM, total organic matter; TN, total nitrogen; WSOC, water-soluble organic carbon; WSON, water-soluble organic nitrogen; EC, electrical conductivity; GI, germination index; PC, number of principal components; $R^2$, the coefficient of determination for the calibration set; RMSECV, the root mean squared error in cross-validation; $r^2$, the coefficient of determination for the validation set; RMSEP, room mean squared error of prediction; RPD, the ratio of the standard deviation in the validation set over the room mean squared error of prediction.

**Table 2.** NIRS calibration and validation results for quality indices of commercial organic fertilizers [10]

Similar to NIRS, fluorescence excitation–emission matrix (EEM) spectroscopy is extensively utilized to detect protein-like, fulvic-acid-like, and humic-acid-like substances. These materials are directly proportional to fluorescence intensity at low concentrations and thus are applied to assess compost maturity [15]. Fluorescence spectroscopy has been widely used as a tool to assess compost maturity, owing to high instrumental sensitivity. [16] However, analysis of fluorescence EEM has generally been limited to visual identification of peaks or development of ratios of fluorescence intensities in different regions of the spectrum. These techniques lack the ability to capture the heterogeneity of samples. It has been reported that as opposed to individual main peak positions analysis, analyzing the full fluorescence EEMs can provide much information. [17] Additionally, the composition complexity of WEOM in compost samples often results in the overlapped fluorophores in the EEM spectra. As a result, the EEM spectra are difficult to interpret.

Recent work has demonstrated that parallel factor (PARAFAC) analysis can be used to decompose full fluorescence EEMs into different independent groups of fluorescent components. [18] Therefore, EEM-PARAFAC analysis is able to assess compost maturity and also to be a potential monitoring tool for rapidly characterizing compost maturity. For this purpose, 62 full-scale compost facilities in nine Provinces of China, yielding compost from animal manures and other industrial organic residues of different maturities, were selected. Then, these compost samples were used to extract water-soluble OM (WEOM) and characterized by fluorescence EEM spectra. EEM-PARAFAC analysis was then conducted for assessment of compost maturity.

**Figure 8** shows the typical fluorescence EEM contours of WEOM. The EEM contours of WEOM between mature and immature composts were obviously distinct. Specifically, the former had only two peaks (A and B), while the latter exhibited four peaks (A, B, C, and D) (**Figure 8**).

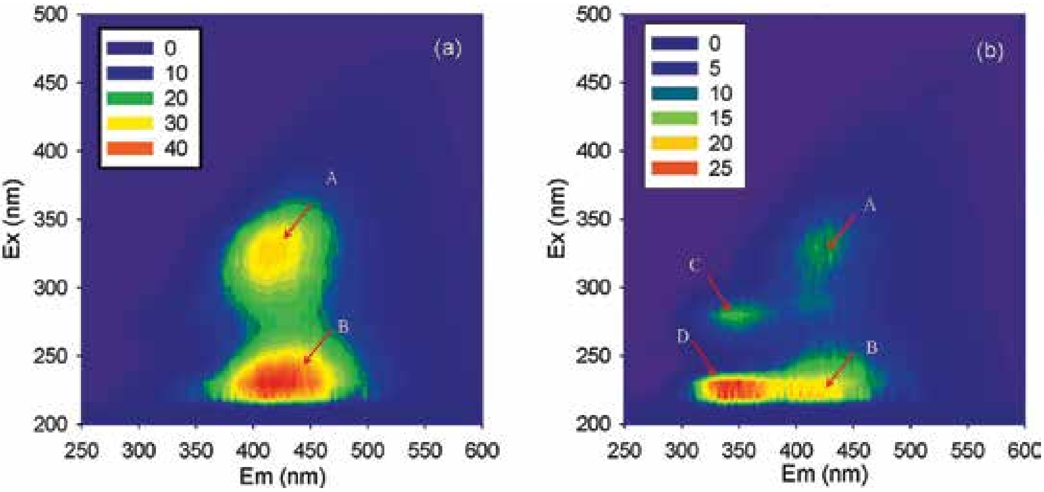

**Figure 8.** Typical EEM contours of mature (a) and immature (b) composts, [1] normalized to 1 mg DOC/L. Note: The arrows refer to the location of peaks. Specifically, two peaks at Ex/Em of 230/420 (B) and 330/420 (A) were presented in mature composts, whereas four peaks at Ex/Em of 220/340 (D), 280/340 (C), 220/410 (B), and 330/410 (A) were detected in the immature composts.

The fluorescence EEM contours were interpreted using the protocol of the previous literature. [17] The typical mature compost contained humic-acid-like substances (i.e., Peak A: Ex/Em = 330/420) and fulvic-acid-like substances (i.e., Peak B: Ex/Em = 230/420) in the WEOM of compost. The typical immature WEOM of compost, however, in addition to containing humic acid–like substances (Peak A) and fulvic acid–like substances (Peak B), also contained tryptophan-like substances (i.e., Peak D: Ex/Em = 220/340) and a few soluble microbial by-product–like (SMP) substances (i.e., Peak C: Ex/Em = 280/340). The above results were consistent with those of Marhuenda-Egea et al. in that the composting process was a degradation of the original tyrosine-like and tryptophan-like materials and an increase in the humic-like and fulvic-like materials. [19] As composting time increased, molecular heterogeneity decreased, while aromatic polycondensation, level of conjugated chromophores, and humification degree increased, as shown by fluorescence EEM spectra. [20] Therefore, fluorescence EEMs of the WEOM from composts had the potential to be used as a monitoring tool for rapidly assessing the maturity of compost.

Preprocessing of the fluorescence EEMs is essential to acquire a correct component number before PARAFAC analysis. After removing the Rayleigh and Raman scatters, the sum of squared residuals in the excitation (Ex) and emission (Em) directions for three different models are plotted in **Figure 9a**. By comparing the two-, three-, and four-component models, it is clear that the step from the two- to three-component model showed great improvement of fit, whereas the step from the three- to four-component model offered only some enhancement of fit. This result suggests that three components are adequate for this data. Split-half analysis further validated the close-to-perfect correspondence between the Ex and Em loadings for the three components, using the four independent random halves (**Figure 9b**). Thus, three components were suitable for all of the examined samples. The EEM contours are shown in

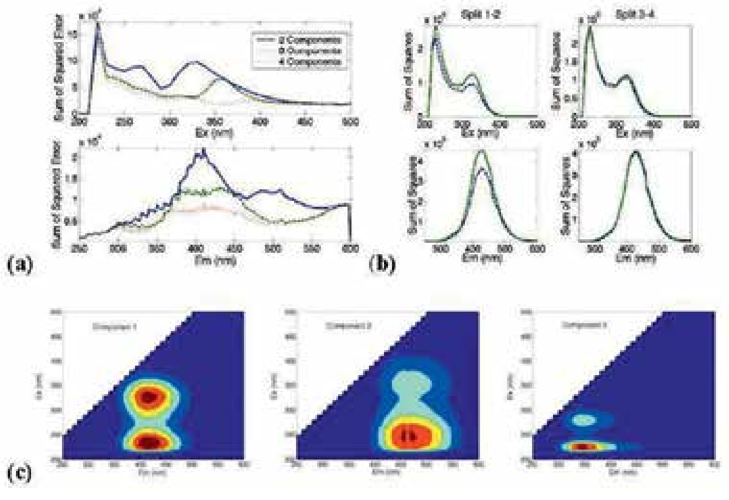

**Figure 9.** PARAFAC analysis of 60 compost samples. (a) Sum of squared error for determining the component numbers. (b) Split-half analysis for model validation. (c) EEM contours of identified components. [1]

**Figure 9c**. All the EEMs in this study could be decomposed into a three-component model by PARAFAC analysis, with component 1 [Ex/Em = (230, 330)/410], component 2 [Ex/Em = (250, 350)/450], and component 3 [Ex/Em = (220, 280)/340]. The three components belonged to humic-like, fulvic-like, and protein-like substances, respectively.

Clearly, component 1 was humic fluorophores derived from both terrestrial-like and marine-like, and component 2 was terrestrial-like humic fluorophores. In contrast, component 3 was a tryptophan-like substance. In addition, all the components displayed the same Em wavelength at different Ex wavelengths, possibly attributable to the rapid internal conversion of excited electrons to the lowest vibration level of the first excited state. **Table 3** shows that component 1 was strongly correlated with components 2 and 3, whereas components 2 and 3 had no significant correlation, indicating that component 1 has a common source with components 2 and 3, but components 2 and 3 do not have the same source.

| | Component 1 | Component 2 | Component 3 | TOC | C/N | TOC/TN | OM | MI | GI | OUR | CER |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Component 1 | 1 | 0.40[b] | 0.89[b] | 0.35[b] | –0.05 | –0.15 | 0.40[b] | –0.22 | –0.39[b] | 0.40[b] | 0.43[c] |
| Component 2 | | 1 | 0.18 | 0.57[c] | –.16 | –0.11 | 0.20 | –0.11 | –0.22 | 0.30[a] | 0.37[b] |
| Component 3 | | | 1 | 0.51[c] | 0.04 | –0.04 | 0.54[c] | –0.29 | –0.37[b] | 0.40[b] | 0.39[b] |
| TOC | | | | 1 | –0.11 | 0.07 | 0.53[c] | –0.32[a] | –0.38[b] | 0.35[b] | 0.33[a] |
| C/N | | | | | 1 | 0.13 | 0.25 | –0.15 | 0.38[b] | –0.08 | –0.04 |
| TOC/TN | | | | | | 1 | –0.06 | 0.19 | 0.31[a] | 0.13 | 0.04 |
| OM | | | | | | | 1 | –0.76[b] | –0.06 | 0.11 | 0.06 |
| MI | | | | | | | | 1 | 0.18 | –0.06 | –0.03 |
| GI | | | | | | | | | 1 | –0.27[a] | –0.29[a] |
| OUR | | | | | | | | | | 1 | 0.84[c] |
| CER | | | | | | | | | | | 1 |

[a] Correlation is significant at the 0.05 level (two-tailed).
[b] Correlation is significant at the 0.01 level (two-tailed).
[c] Correlation is significant at the 0.001 level (two-tailed). OM, organic matter; MI, mineralization index; GI, germination index; OUR, oxygen uptake rate; CER, $CO_2$ evolution rate.

**Table 3.** Pearson correlation between log (scores) of PARAFAC components and selected stability indices ($n$ = 60) [1]

In addition, components 1 and 3 had a strong correlation ($R > 0.35$, $p < 0.01$) with TOC, OM, CER, GI, and OUR. But component 2 was only significantly ($R > 0.37$, $p < 0.01$) correlated with TOC and CER, and weakly ($R = 0.30$, $p < 0.05$) correlated with OUR (**Table 4**). Therefore, components 1 and 3 are more suitable to assess compost maturity than component 2. Furthermore, the regression equations in **Table 4** indicated that the composts could be identified as mature when the log scores of components 1 and 3 were higher than 3.69 ± 0.06 and 3.49 ± 0.09, respectively.

| | **Indices ($x$)** | **Regression equation** | **Maturity value in the literature [9]** | **Calculated log (scores) of components** |
|---|---|---|---|---|
| Component 1 ($y$) | TOC | $y = 3.7 + 7.5 \times 10^{-3}x$ | 10 mg/g-DM | 3.78 |
| | OM | $y = 3.3 + 1.1 \times 10^{-2}x$ | 30% | 3.63 |
| | GI | $y = 4.0 - 6.8 \times 10^{-3}x$ | 50% | 3.66 |
| | OUR | $y = 3.7 + 3.6 \times 10^{-3}x$ | 10 mg $O_2$/g-OM/d | 3.74 |
| | CER | $y = 3.6 + 8.9 \times 10^{-3}x$ | 4 mg C-$CO_2$/g-OM/d | 3.64 |
| Component 3 ($y$) | TOC | $y = 3.5 + 1.2 \times 10^{-2}x$ | 10 mg/g-DM | 3.62 |
| | OM | $y = 2.9 + 1.6 \times 10^{-2}x$ | 30% | 3.38 |
| | GI | $y = 3.8 - 6.8 \times 10^{-3}x$ | 50% | 3.46 |
| | OUR | $y = 3.5 + 3.9 \times 10^{-3}x$ | 10 mg $O_2$/g-OM/d | 3.54 |
| | CER | $y = 3.4 + 8.8 \times 10^{-3}x$ | 4 mg C-$CO_2$/g-OM/d | 3.44 |

TOC, total organic matter; OM, organic matter; GI, germination index; OUR, oxygen uptake rate; CER, $CO_2$ evolution rate.

**Table 4.** Calculated log (scores) of components from regression equations ($n = 60$) [1]

Some investigators have shown great interest in the assessment of compost maturity using fluorescence intensity. Our results demonstrate that log scores of components 1 and 3, identified by the DOMFluor–PARAFAC approach, can be applied to assess the maturity of composts which cover a large range of waste sources. This is attributable to the fact that the full fluorescence spectroscopy analysis provides a basis for capturing subtle changes in the fluorescence spectra. In summary, the assessment of compost maturity by the DOMFluor–PARAFAC approach is not wastesource–specific.

## 4. Impact of application of organic fertilizers on the soil properties

It is well known that organic fertilizer amendments can enhance soil fertility, increase soil aggregation, and increase soil pH (e.g., acidic soils). [21–23] For example, long-term (over 20 years) organic fertilization or organic plus inorganic fertilization could markedly improve soil organic carbon (SOC) content when compared to no fertilization (Control) and chemical fertilization (NPK) (**Figure 10**). Using Nano-CT, the structure of long-term fertilized soils at the Jinxian Experiment station was examined (**Table 5**). It was found that long-term organic amendments improved soil aggregation by decreasing the number of pores, pore throats, and paths between adjacent nodal pores in soil aggregates (**Table 5**). [24]

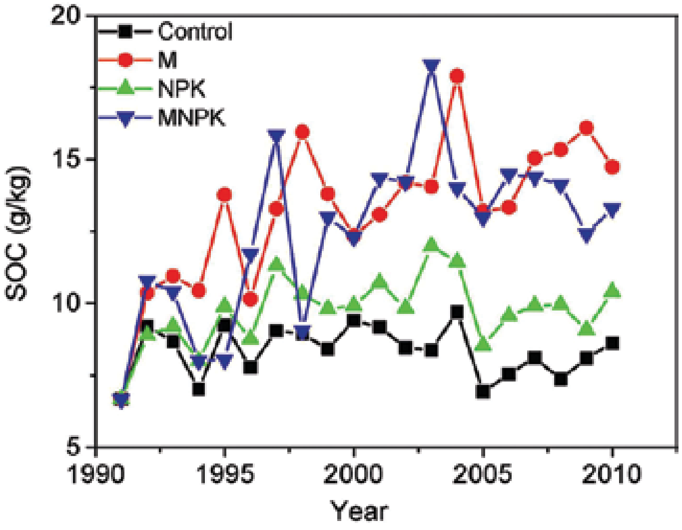

**Figure 10.** Dynamics of soil organic carbon (SOC) with fertilization time at the Qiyang long-term fertilization experiment station.

| Pore properties | No fertilizer input | Organic plus chemical amendments | Chemical fertilizer input |
|---|---|---|---|
| Porosity (%) | 14.2 (1.7)a | 13.2 (1.3)a | 14.9 (2.3)a |
| Macroporosity (>500 μm) (%) | 3.4 (0.6)b | 6.7 (0.4)a | 3.6 (0.7)b |
| Mesoporosity (≤500 μm) (%) | 10.8 (1.1)a | 6.5 (0.4)b | 11.3 (1.6)a |
| Total no. of pores | 3308 (313)a | 2288 (502)b | 2695 (267)ab |
| No. of interior pores | 2676 (407)a | 1881 (352)b | 2189 (268)ab |
| No. of boundary pores | 632 (250)a | 404 (68)b | 505 (87)ab |
| Fraction interior pores (%) | 55.2 (5.2)a | 33.0 (7.2)b | 29.3 (2.9)b |
| Fraction boundary pores (%) | 44.8 (5.2)b | 67.0 (7.2)a | 70.7 (2.9)a |
| Total no. of throats | 3709 (704)a | 1526 (257)b | 2698 (432)ab |
| Mean area of throats ($\mu m^2$) | 2913 (337)a | 3148 (342)a | 3803 (469)a |
| Specific surface area ($\mu m^{-1}$) | 8.50 (1.50)a | 5.80 (2.62)a | 7.80 (1.81)a |
| Total no. of paths | 6218 (1028)a | 3277 (988)b | 4927 (1346)a |
| Average length of paths (μm) | 158 (14)a | 175 (19)a | 169 (27)a |

Values in parentheses represent the standard deviation of the mean. Different letters following values between different fertilization treatments indicate significant differences at $P < 0.05$ (LSD). The soil at the Jinxian Experiment station is red soil.

**Table 5.** Summary of soil microstructure properties from three contrasting fertilization treatments at the Jinxian Experiment station, Jiangxi Province, China [24]

However, very few studies are conducted on how organic fertilizer amendments affect the morphology and coordinate state of nanominerals or organomineral associations in soil. High-resolution transmission electron microscopy (HRTEM) coupled to selected area electron diffraction (SAED) technique could be a promising tool to observe the morphology/appearance of nanominerals in soil dissolved organic matter (DOM). Under three fertilization treatments at the Qiyang Experiment station, HRTEM observation showed two regions (i.e., black and gray regions) of soil DOM with distinctive percentage presented under Control (no fertilization), NPK (chemical nitrogen, phosphorus, and potassium fertilization), and NPKM (NPK plus manure fertilization) at the 22 years' long-term fertilization site (**Figures 11–13**). The electron diffraction patterns indicated that the obtained nanominerals in the black and gray regions had a determinate crystalline and amorphous pattern, respectively. Elemental maps further confirmed that crystalline nanominerals were dominated by Fe and O, while amorphous nanominerals were mainly composed of Al, Si, and O (**Figures 11–13**). These results showed that after 22 years' fertilization, crystalline nanominerals were predominant under NPK, while amorphous nanominerals under both the Control and NPKM.

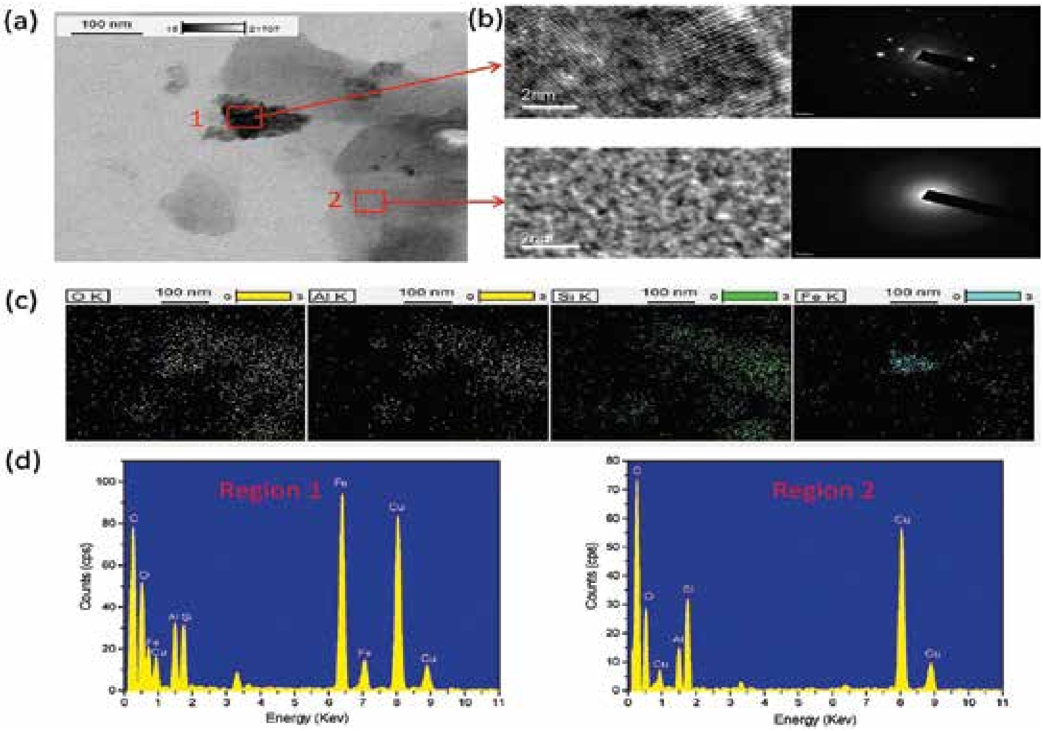

**Figure 11.** High-resolution transmission electron microscopy (HRTEM) images of soil dissolved organic matter from Control fertilization test in the long-term (22 years) location experiment. [4] (a) TEM image; (b) HRTEM images and selected area electron diffraction (SAED) pattern of the two regions indicated by blue squares, indicating that the black region is completely crystalline, while the gray region remains amorphous; (c) elemental maps; (d) EDS image. Control, no fertilization.

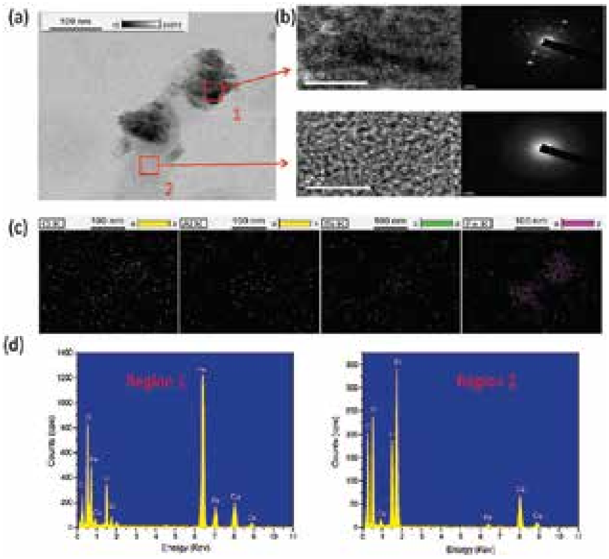

Figure 12. High-resolution transmission electron microscopy (HRTEM) images of soil-dissolved organic matter from NPK fertilization test in the long-term (22 years) location experiment. [4] (a) TEM image; (b) HRTEM images and selected area electron diffraction (SAED) pattern of the two regions indicated by blue squares, indicating that the black region is completely crystalline, while the gray region remains amorphous; (c) elemental maps; (d) EDS image. NPK, chemical nitrogen, phosphorus, and potassium fertilization.

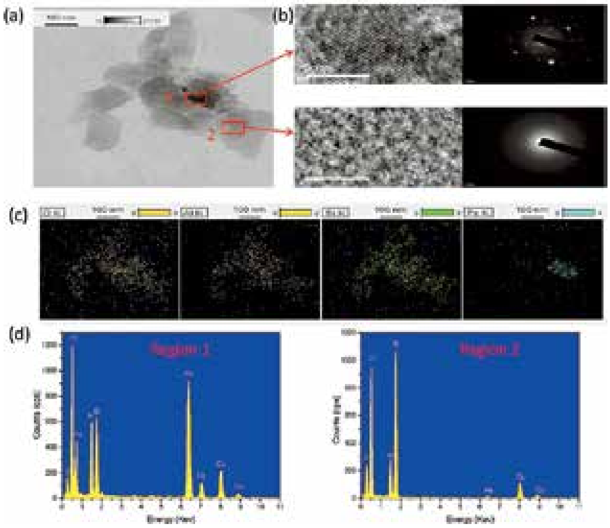

Figure 13. High-resolution transmission electron microscopy (HRTEM) images of soil-dissolved organic matter from NPKM fertilization test in the long-term (22 years) location experiment. [4] (a) TEM image; (b) HRTEM images and selected area electron diffraction (SAED) pattern of the two regions indicated by blue squares, indicating that the black region is completely crystalline, while the gray region remains amorphous; (c) elemental maps; (d) EDS image. NPKM, NPK plus manure fertilization.

To understand whether fertilization practices can affect the local coordination state and the environment of Al and Si, the $^{27}$Al and $^{29}$Si nuclear magnetic resonance spectroscopy (NMR) spectra of soil water-dispersible colloids were used (**Figure 14**). The results showed that octahedrally coordinated aluminum ($^{VI}$Al), with a peak at approximately 0 ppm, was the only type of aluminum in the soil water-dispersible colloids under the 22-year long-term Control, M, and NPKM treatments (**Figure 14**). Although the octahedrally coordinated Al was also dominant in soil colloids under the NPK, NPKCa (NPK + lime), N, and NCa (N + lime), small amounts of distorted tetrahedrally coordinated aluminum ($^{IV}$Al, 46 ppm) and pentahedrally coordinated aluminum ($^{V}$Al, 25 ppm) were observed. Distorted $^{IV}$Al and $^{V}$Al are usually present in well-characterized crystalline minerals. [25–27] This finding strongly implies that chemical fertilization modified the local coordination state and environment of Al, with a small part of $^{IV}$Al replaced by distorted $^{IV}$Al and $^{V}$Al, whereas organic fertilization or organic plus chemical fertilization did not influence the local coordination state and Al environment. The results from the high-resolution $^{27}$Al NMR spectra support the finding that amorphous Al is more present in organic fertilizations (i.e., M and NPKM) than in chemical fertilizations (i.e., NPK, NPKCa, N, and NCa) (**Figure 15**). $^{29}$Si NMR spectra also confirmed the presence of amorphous Al as allophane and imogolite in the soils under control, M, and NPKM, but not under the four chemical fertilizations (N, NCa, NPK, and NPKCa). These results from $^{27}$Al and $^{29}$Si NMR spectra are consistent with our previous publication, in which nanominerals were directly observed by HRTEM images of soil DOM. [4]

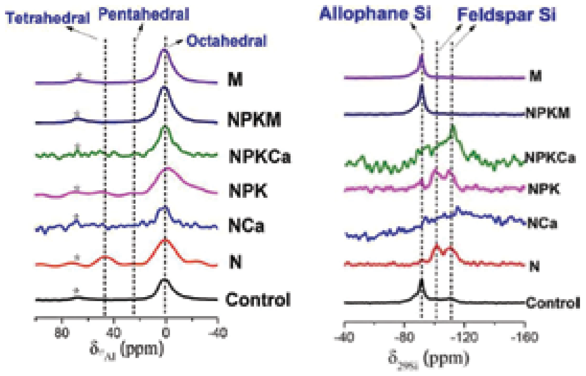

**Figure 14.** High-resolution $^{27}$Al and $^{29}$Si NMR spectra of water-dispersible colloids from the long-term fertilized soils. [28] Control, no fertilization; N, chemical nitrogen; NCa, chemical nitrogen plus lime; NPK, chemical nitrogen, phosphorus, and potassium; NPKCa, chemical nitrogen, phosphorus, and potassium plus lime; NPKM, NPK plus swine manure; and M, swine manure. The results of $^{29}$Si NMR spectra support the presence of nanominerals in organic (i.e., NPKM and M) rather than chemical (i.e., N, NCa, NPK, and NPKCa) fertilization treatments.

Selective extraction also showed that the Al fractions were significantly ($P < 0.05$) altered by the long-term fertilization treatment (**Figure 10**). Significantly higher amorphous Al concentrations among the fertilizers were ranked as M ≈ NPKM > NPK ≈ NPKCa > N > NCa > Control (**Figure 10**). Significantly higher strongly organically bound Al concentrations ranked as NPKM > Control > NCa > NPK > NPKCa > M > N; significantly higher weakly organically bound Al concentrations ranked as NCa ≈ NPKCa > NPKM ≈ NPK ≈ Control > M > N. In addition, significantly higher exchangeable Al concentrations ranked as N > NPK > NPKCa > NCa > Control > M ≈ NPKM. These four fractions followed the pattern: organically bound Al > amorphous Al fraction > exchangeable Al. The results demonstrated that organic fertilization treatments increased amorphous Al and reduced exchangeable Al compared with chemical fertilization treatments. The addition of lime significantly ($P < 0.05$) increased the weakly organically bound Al and reduced exchangeable Al, suggesting that lime amendment transferred Al fractions from exchangeable Al to the weakly organically bound Al. These trends definitely affected soil C sequestration and soil pH.

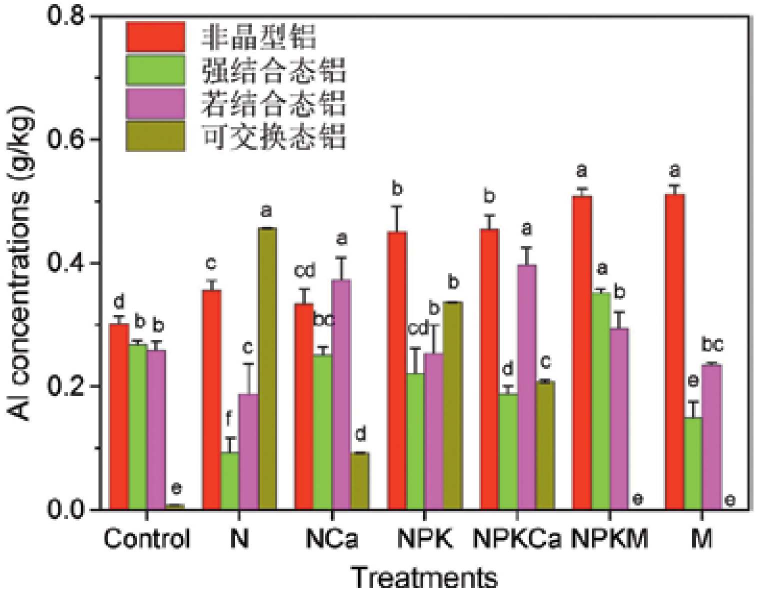

**Figure 15.** Aluminum fractions in the different fertilization treatments from the site of the long-term fertilization experiment, obtained by selective dissolution techniques. [28] Significant differences between fertilization treatments were determined using one-way ANOVA followed by Duncan's multiple range test at $P < 0.05$, in which conditions of normality and homogeneity of variance were met. The data are shown as mean ± SD ($n = 3$). Control, no fertilization; N, chemical nitrogen; NCa, chemical nitrogen plus lime; NPK, chemical nitrogen, phosphorus, and potassium; NPKCa, chemical nitrogen, phosphorus, and potassium plus lime; NPKM, NPK plus swine manure; M, swine manure.

Meanwhile, Fe K-edge X-ray absorption fine structure spectroscopy (XAFS) is used for both identification and quantification of different mineral phases present in soil colloids.[5,29] Linear combination fitting (LCF) of soil colloids (**Figure 16** and **Table 6**) showed that goethite (56.8–67.0%) and hematite (14.9–25.0%) were prominent under all three fertilizations. The remaining Fe phases were composed of the less crystalline ferrihydrite species. The percentage of ferrihydrite was the highest under NPKM (18.0 ± 0.02%), followed by Control (16.0 ± 0.03%) and NPK (6.30 ± 0.02%). In view of the better C binding and potential preservation capability of ferrihydrite when compared to goethite and hematite,[5,30-32]. Fe minerals under organic fertilization should have a greater C loading than chemical fertilization.

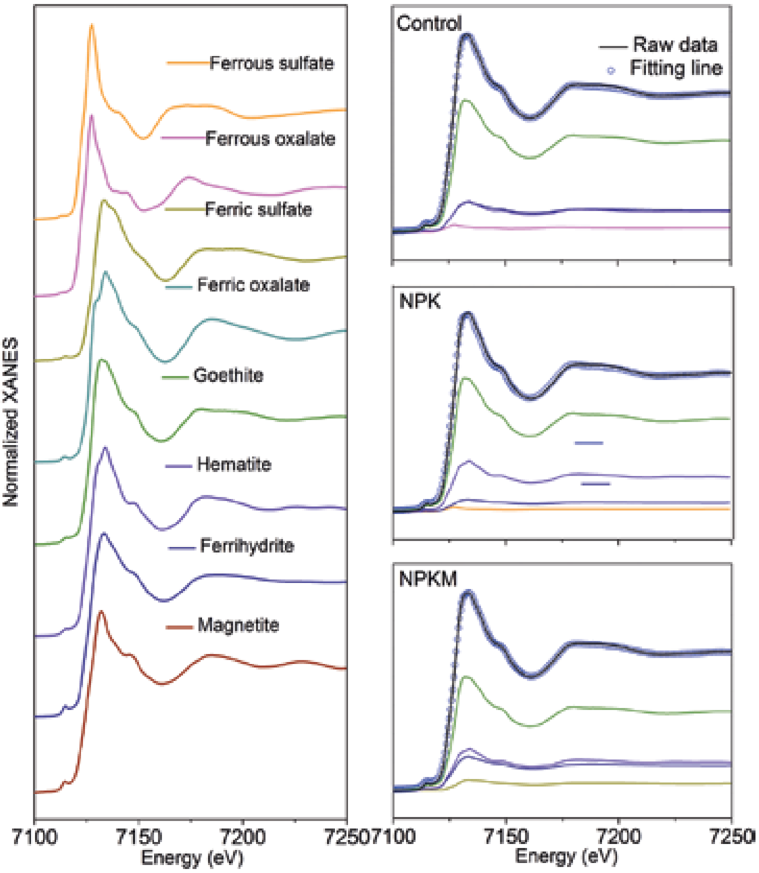

**Figure 16.** Fe K-edge XANES spectra of reference materials and soil colloids from three contrasting long-term (1990–2014) fertilization treatments. [29] The scattered circles represent the linear combination fitting (LCF) results of the sample spectra. Control, no fertilization; NPK, chemical nitrogen, phosphorus, and potassium fertilization; NPKM, chemical NPK plus swine manure fertilization.

| **Treatment** | **LCF results (%)** | | | | | | **LCF parameters** | |
|---|---|---|---|---|---|---|---|---|
| | **Goethite** | **Hematite** | **Ferrihydrite** | **Ferric sulfates** | **Ferrous citrates** | **Ferrous sulfates** | ***R*-factor** | **Chi-square** |
| **Control** | 66.0 ± 0.025 | 14.9 ± 0.000 | 16.0 ± 0.025 | ND | 3.10 ± 0.012 | ND | 0.000052 | 0.00437 |
| **NPK** | 67.0 ± 0.025 | 25.0 ± 0.000 | 6.30 ± 0.020 | ND | ND | 1.70 ± 0.008 | 0.000051 | 0.00426 |
| **NPKM** | 56.8 ± 0.025 | 20.4 ± 0.000 | 18.0 ± 0.017 | 4.8 ± 0.018 | ND | ND | 0.000051 | 0.00436 |

Note: Control, no fertilization; NPK, chemical nitrogen, phosphorus, and potassium fertilization; NPKM, chemical NPK plus swine manure fertilization; ND, not detected. Determination of parameters of fit (i.e., *R*-factor and Chi-square) indicated that the LCF results are convincing.

**Table 6.** Linear combination fit (LCF) results of Fe K-edge XANES spectra of the soil colloids from three separate long-term (1990–2014) fertilization treatments [29]

Nanoscale secondary ion mass spectrometry (NanoSIMS) has the potential to examine the spatial integrity of soil microenvironments and has been designed for high lateral resolution (down to 50 nm) imaging, while still maintaining high mass resolution and high sensitivity (mg $kg^{-1}$ range).[5,33]. NanoSIMS images, combined with the region of interests (ROIs) analysis, were used to explore the C-binding capability of Al and Fe minerals. Based on the pixel value of secondary $^{12}C^-$ ion mass in all spots from each sample, the selected ROIs were identified. The selected ROIs were further divided into $^{12}C^-$-rich and $^{12}C^-$ less-rich ROIs. The area percentage of the $^{12}C^-$-rich or $^{12}C^-$ less-rich ROIs accounted for 7.47 or 40.18%, 10.80 or 27.64%, and 8.23 or 37.99% under Control, NPK, and NPKM, respectively. Interestingly, the box plots (**Figure 17**) of $^{12}C^-/^{27}Al^{16}O^-$ (a, b) and $^{12}C^-/^{56}Fe^{16}O^-$ (c, d) ratios showed that both the median and the mean values were higher under NPKM than under NPK. These results suggest that Al and Fe minerals under NPKM can bind more organic C than those of NPK.

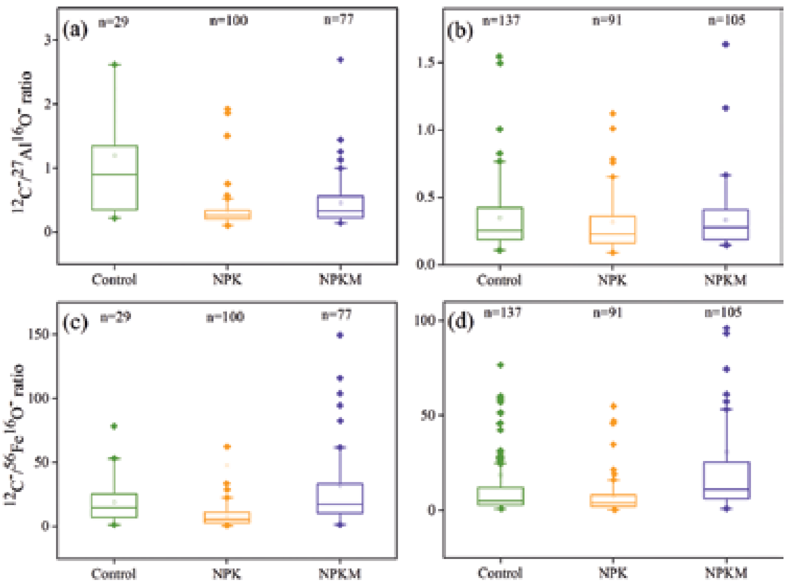

**Figure 17.** Box plots of $^{12}C^-/^{27}Al^{16}O^-$ (a, b) and $^{12}C^-/^{56}Fe^{16}O^-$ (c, d) ratios reflecting the $^{12}C^-$ rich ROIs (a, c) and $^{12}C^-$ less rich ROIs (b, d) of the soil colloids from three contrasting long-term (1990–2014) fertilization treatments using NanoSIMS (for all spots). [29] Control, no fertilization; NPK, chemical nitrogen, phosphorus, and potassium fertilization; NPKM, chemical NPK plus swine manure fertilization. The $^{12}C^-$ rich ROIs include the areas above 90 pixels, and the $^{12}C^-$ less rich ROIs include the areas in the range of 90–40 pixels under Control and NPK, which were above 50 pixels, and in the range of 50–30 pixels under NPKM. The number *n* in figures represents the number of the selected ROIs. The line in the middle of the box is the median value and the square in the box is the mean value. The lines that protrude out of the boxes represent the 25th and 75th population percentiles. Outliers are shown as diamonds.

To address the specific C components preserved by reactive minerals, synchrotron-based C 1s near-edge X-ray fine structure (NEXAFS) spectroscopy was used to identify C composition. Compared to NPK treatment, NPKM and M treatments markedly increased carboxylic groups (288.4–289.1 eV) from 24.2 to 33.2% and increased both the aromatic (283.0–286.1 eV) and phenolic (286.2–287.5 eV) groups by greater than 2.8-fold (**Figure 18** and **Table 7**). In conclusion, organic fertilization treatments (NPKM and M) enhanced the retention of carboxylic and aromatic C by reactive minerals in soils.

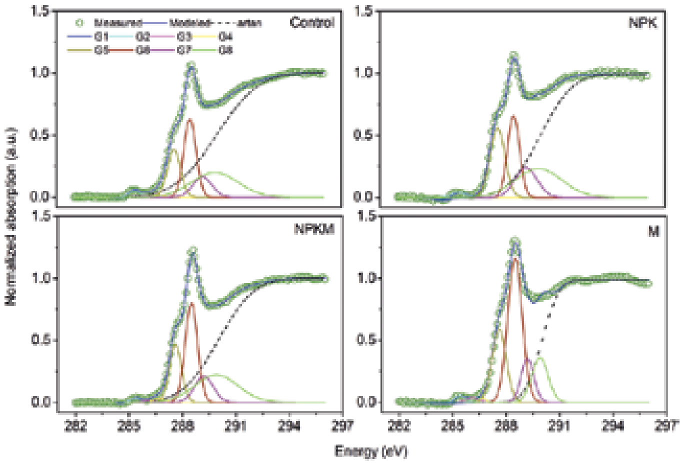

**Figure 18.** Organic C composition in the soil colloids from the various long-term fertilization treatments. [6] (a) Control, no fertilization; (b) NPK, chemical fertilization; (c) NPKM, chemical plus swine manure fertilization; (d) M, swine manure fertilization.

| **Treatment** | **Proportion of absorption regions (%)** | | | | | |
|---|---|---|---|---|---|---|
| | **Aromatic C** | **Phenolic C** | **Alkyl C** | **Carboxylic C** | **O-alkyl C** | **Carbonyl C** |
| | **(283–286.1 eV)** | **(286.2–287.5 eV)** | **(287.6–288.3 eV)** | **(288.4–289.1 eV)** | **(289.2–289.8 eV)** | **(289.9–290.2 eV)** |
| Control | 2.6 | 0.7 | 19.4 | 29.6 | 12.1 | 35.6 |
| NPK | 0.5 | 0.1 | 25.7 | 24.2 | 16.3 | 33.2 |
| NPKM | 1.4 | 1.1 | 18.9 | 33.2 | 13.2 | 32.2 |
| M | 1.8 | 0.5 | 22.9 | 46.6 | 12.3 | 15.9 |

Control, no fertilization; NPK, chemical fertilization; NPKM, chemical plus swine manure fertilization; M, swine manure fertilization.

**Table 7.** Deconvolution results for using C 1s NEXAFS on soil colloids from the various long-term fertilization treatments [6]

## 5. Conclusion

Composting is an inexpensive and sustainable treatment for solid organic wastes. The composting industry has been growing rapidly because of a boom in the animal industry in China over the past decades. In this chapter, we introduce composting process and status in China, especially in Jiangsu Province. Meanwhile, the developed novel spectroscopy techniques (i.e., NIRS-PLS and EEM-PARAFAC) are also introduced, which are more suitable for assessment of compost maturity than the conventional techniques in view of ease of sample preparation, rapid spectrum acquisition, nondestructive nature of the analysis, and the portability of the technology. In addition, organic fertilizer amendments can not only improve soil fertility but also offset chemical fertilizers' nanoscale changes. Recently, investigators have shown that organic fertilizer amendments could enhance the production of highly reactive minerals, for example, allophane, imogolite, and ferrihydrite, which further benefit for soil carbon storage and soil fertility improvement.

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## Chapter 2

# Organic Fertilizers in Alabama: Composition, Transformations, and Crop Response in Selected Soils of the Southeast United States

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Additional information is available at the end of the chapter

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### Abstract

Rapid growth in organic production in the past 20 years is due to consumer concerns about the impacts of conventional agriculture on the environment, food safety, and quality. There are considerable variations in nutrient concentration and the rate of mineralization among organic fertilizers. Some organic fertilizers and application rates are specific to soil types, which affect the nutrient potential. Two organic fertilizers produced in Alabama and added to soils are the chicken or poultry litter (1.8 million Mg annually) and the hydrolyzed liquid fish protein. The under- or overestimation of the total N content of the litter may result in its over- or underapplication with potential environmental consequences to surface waters. The overestimation of the total N may result in its inadequate application. The inorganic forms (ammonium, $NH_4^+$–N; nitrate, $NO_3^-$–N; and nitrite, $NO_2^-$–N) are found in small but sometimes significant amounts especially when broiler litter is stored under environmental conditions favorable to nitrification. Limited information is available on the usefulness of the various modifications of the regular Kjeldahl method in poultry litter analysis and transformations when added to soils. This chapter provides information and our experiences on the sources of organic fertilizers produced in the southeastern United States (Alabama).

**Keywords:** organic amendments, nitrogen, mineralization, organic carbon, sustainable agriculture

## 1. Introduction

An organic fertilizer is a soil amendment produced from plant materials and/or animal manures containing low levels of nitrogen (N), phosphorus (P), potassium (K), and some residues of micronutrients compared with synthetic chemical fertilizers. These plant byproducts include alfalfa meal or pellets (also used as animal feed), corn gluten meal (with allelophatic properties), cottonseed meal, and soybean meal (also used as animal feed). Animal byproducts include bat guano, blood meal (slaughterhouse waste product), bone meal, feather meal, enzymatically digested hydrolyzed liquid fish, fish emulsion, fish meal, and fish powder. In addition to these byproducts, compost of organic materials (mixture of leaves, food waste, and/or animal manures) and seaweed (valued for its micronutrient contents) are used as organic fertilizers. Contrary to synthetic chemical fertilizers for which nutrient contents are regulated, the term organic fertilizer is not regulated; theses organic fertilizers act as nutrients for plants and soil conditioners that feed soil microorganisms. Biosolids are another type of organic amendment or fertilizer used in agriculture. The United States Environmental Management Agency (USEPA) defines the term biosolids as treated sewage sludge that meets the USEPA pollutant and pathogen requirements for land application as well as surface disposal. As stated early, the term organic fertilizer is not regulated and therefore should not be confused with selected organic substances approved by the United States Department of Agriculture (USDA) for its National Organic Program (NOP) for use in organic production. An organic substance to be allowed in certified organic production must be approved by the Organic Materials Review Institute (OMRI) and the Washington Department of Agriculture.

In the southeastern states of the United States, the disposal of vast amounts of organic fertilizers in the form of animal waste can otherwise be used as organic fertilizer. More than 65% of US broiler production is concentrated in the southeastern states. In 2012, Alabama ranks second in the United States in broiler production and produced over 1 billion birds [1]. The litter that results annually from this broiler production averages 15 million metric tons, and its disposal represents a growing problem for the poultry industry.

In 2010, cash receipts in Alabama from poultry operations made up 68% of the total cash receipts for all commodities [2]. Mineralization of C, N, P, and S in poultry litter added to soil is the main cause of groundwater contamination in areas where mineral fertilizer application is limited [3].

In Alabama, there are only a few companies that transform raw poultry litter into organic fertilizers. MigthyGrow, Inc., produces OMRI approved organic fertilizers (4–3–4) as well as an AgBlend all-purpose fertilizer (3–3–3). Denali Organics, LLC, uses catfish byproducts with a proprietary digestive enzyme to produce an organic liquid fertilizer that can be top-dressed, banded with seeds, or foliar sprayed. Gulf Coast Organic is a distributor of liquid fertilizers such as Gator Perform SRN (30–0–0) for lawns, turf, and golf courses, and food crops, Primera one green fee super (15–0–0), Turf balance RSN (12–0–12), Medina has a Gro plant (6–12–6), and Medina hasta Gro lawn (12–4–8). It also distributes granular fertilizers such as Primera 3–3–3 crumbles and Primera 4–3–4 crumbles.

Concentrations of 15 trace and nontrace elements (silver, Ag; arsenic, As; barium, Ba; beryllium, Be; cadmium, Cd; chromium, Cr; copper, Cu; mercury, Hg; manganese, Mn; molybdenum, Mo; nickel, Ni; lead, Pb; antimony, Sb; selenium, Se; and zinc, Zn) were investigated by the United States Environmental Protection Agency [4] because of their potential toxicity Properties of chicken litter generated in Alabama have been investigated (**Table 1**).

| **Property**[a] | **Range** | **Median** | **Mean** |
|---|---|---|---|
| Litter age, month | 3.00–18.0 | 9.0 | 8.9 |
| Moisture, % | 7.9–28.5 | 12.7 | 13.7 |
| pH | 7.4–8.6 | 8 | 8.0 |
| Organic C, g/kg | 229–396 | 360 | 347 |
| C/N ratio | 6.86–11.4 | 8.35 | 8.56 |

[a] $n$ = 33; pH was determined by a combination glass electrode (broiler litter/water ratio, 1:2.5), organic C by the method of Mebius (1960) (adapted from Kpomblekou A et al., 2002).

**Table 1.** Selected properties of chicken litter generated in Alabama.

## 2. Metal contents of chicken litter

Variations in trace element contents of chicken litter have been reported. These variations are attributed to trace elements contained in ingredients fed to chicks (feedstuffs, drugs, feed spillage, and drinking water). Compounds and elements added to chicken diets to stimulate growth and feed efficiency include arsenilic acid, copper sulfate in addition to argon, cadmium, calcium, chlorine, cobalt, cerium, dysprosium, iron, lanthanum, manganese, samarium, selenium, titanium, uranium, vanadium, and zinc [5–8]. Moreover, for disease resistance, more than 20 antibiotics are often added to animal diets. All these elements and compounds have been found at elevated concentrations in chicken litter because those are not completely metabolized in their digestion system. Investigation of 33 chicken litter samples from 12 Alabama counties showed large variations in barium (0.014–0.038 g/kg), calcium (18.9–40.2 g/kg), magnesium (4.8–10.0 g/kg), potassium (18.1–36.5 g/kg), and sodium (3.6–9.2 g/kg). Means of these elements [9] are shown in **Table 2**. Although these means are similar to those previously reported for 106 broiler litter samples from Alabama, USA [9], they are not comparable to those published for Georgia, USA [10] where 86 samples were analyzed. Differences in these results could be attributed to variations in chicken diets in Alabama and Georgia. Other investigators [8, 10–14] confirmed that concentrations of trace elements in animal waste depend on animal diets. The concentration of arsenic in the Alabama samples varied considerably (<2.0–70.4 mg/kg) with a median of 19.1 mg/kg and a mean of 20.6 mg/kg (**Table 2**). However, at a detection limit of 2.0 mg/L, no arsenic was found in four samples reported in Alabama.

| **Total elemental content**[a] | **Range** | **Median** | **Mean** |
|---|---|---|---|
| Nontrace element | g/kg | | |
| Aluminum | 0.4–8.4 | 1.4 | 2.2 |
| Barium | 0.014–0.038 | 0.025 | 0.024 |
| Calcium | 18.9–40.2 | 27.3 | 26.6 |
| Magnesium | 4.8–10.0 | 6.1 | 6.3 |
| Potassium | 18.1-36.5 | 25.9 | 25.5 |
| Sodium | 3.6–9.2 | 7.1 | 6.9 |
| Trace element/micronutrient | mg/kg | | |
| Arsenic | <2.0–70.4 | 19.1 | 20.6 |
| Cadmium | <2.0–1.7 | <0.2 | 0.3 |
| Cobalt | <2.0–2.3 | <0.2 | 0.4 |
| Chromium | <2.0–17.6 | 3.2 | 3.7 |
| Cupper | 211–840 | 410 | 450 |
| Iron | 718–6691 | 1596 | 2073 |
| Manganese | 254–720 | 356 | 388 |
| Molybdenum | <2.0–4.9 | 0.2 | 0.9 |
| Nickel | <2.0–25.1 | 1.0 | 5.2 |
| Selenium | <2.0–24.3 | <2.0 | 5.5 |
| Zinc | 224–706 | 371 | 399 |

[a] *n* = 33; samples digested using the microwave-assisted acid digestion EPA 3052 method (adapted from Kpomblekou A et al., 2002).

**Table 2.** Range, median, and mean of trace and nontrace elements in chicken litter samples generated in Alabama.

Exposure of Cd to animals and humans at relatively high levels in food, water, or air has caused harmful effects. Almost all the samples tested did not contain or contained only trace amounts of Cd and varied from <0.2 to 1.7 mg/kg. Other researchers reported concentrations of 1 mg Cd/kg [15] and 6 mg Cd/kg [8] in single samples of chicken litter. Similarly, only trace amounts of Co were detected in the samples with a mean of 0.4 mg/kg (Table 2). The presence of Cr in the ecosystem must be carefully monitored. Chromium concentrations in tested samples in Alabama range from <2 to 17.6 mg Cr/kg with a median of 3.2 mg Cr/kg. Concentrations of other trace elements (Cu, Fe, Mn, Mo, Ni, Se, and Zn) are shown in Table 2 as well. The most abundant elements found in chicken litter are Cu (211–840 mg/kg), iron (718–6691 mg/kg), and manganese (254–720 mg/kg). Copper, Fe, and Zn are the most widely studied trace metals in chicken litter, probably because of their possible toxic effects on crops. In soils receiving excessive applications of chicken litter with high P content, there exists a possible P-induced

Zn deficiency. At detection limits of 0.2 and 2.0 mg/L, no measurable amounts of Ag and Pb, respectively, were found in chicken litter.

The use of chicken litter in agriculture as an organic fertilizer is not without challenges. The trace elements in the litter can accumulate in topsoil over time, can become a source of surface water pollution via water runoffs following storm events and depending on soil characteristics, and the elements may contaminate groundwater and may become bioavailable and phytotoxic. Soil profile samples taken in selected Alabama soils demonstrate that depending on soil types the trace elements can move through soil profiles. Differences in Cr concentrations in Alabama amended and nonamended soils with chicken litter exceed 40 mg/kg at 45–60 cm and 60 mg/kg at 60–75 cm. This strongly suggests that Cr is fairly mobile in these soils. For example, in Fuquay and Madison soils, the increased Cr concentration in the amended soils over the nonamended soils at 90 cm depth exceeded 10 and 30 mg/kg, respectively (**Figure 1**). Just like Cu and Ni, chromic ($Cr^{3+}$) forms of Cr are strongly complex with soil organic matter as well as chemisorbs on sexquioxides and therefore immobile in soils. Chromate ($CrO_4^{2-}$) although more toxic than $Cr^{3+}$ is less adsorbed and displays more mobility in soils under favorable environmental conditions. A complete different picture was obtained for cadmium in the soil profiles (**Figure 2**). A background concentration of Cd above 0.5 µg/g soil has been attributed to anthropogenic activities [16]. The cadmium concentration in the soils was relatively low (<5.63 mg/kg) with the exception of Madison soil where the Cd concentration achieved 10.2 mg/kg at 45 cm depth. The addition of poultry litter to Orangeburg soil did not change the Cd concentration it its profile. Mobility of trace elements in soil has been associated with presence of organic matter [17] For the mobility of other trace elements in Alabama soils, the reader is referred to Cadet et al. [18].

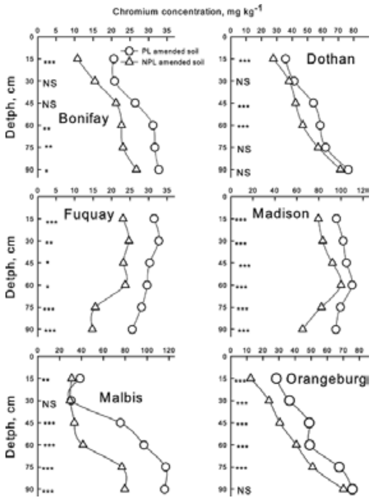

**Figure 1.** Mobility of Cr in benchmark Alabama soils after long-term poultry litter addition. *, **, *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively. NS: not significant at depth specified. From Cadet et al., 2012 [18].

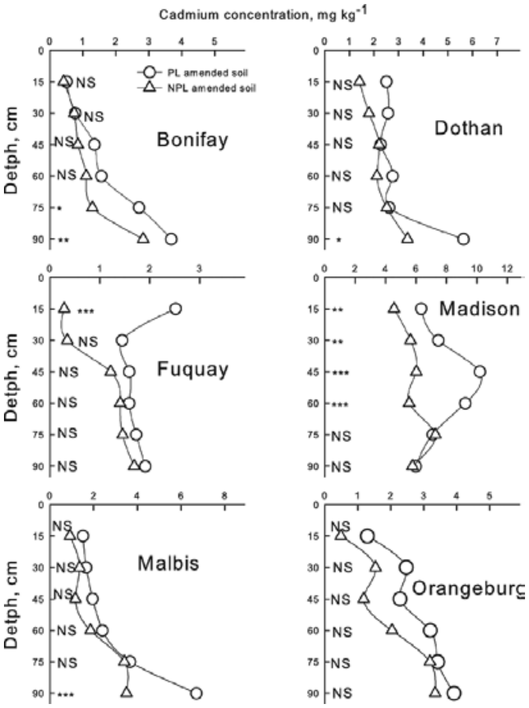

**Figure 2.** Mobility of Cd in benchmark Alabama soils after long-term poultry litter addition. *, **, *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively. NS: not significant at depth specified. From Cadet et al., 2012 [18].

## 3. Nitrogen contents of chicken litter

As organic fertilizer, chicken litter is valued because it contains macro- and micronutrients. A large majority of nitrogen in the litter (a mixture of chicken manure and bedding materials) exists in organic forms. Ammonium, $NH_4^+$–N; nitrate, $NO_3^-$–N; and nitrite, $NO_2^-$–N representing the inorganic form are found in small but sometimes significant amounts. This is especially true when chicken litter is stockpiled under environmental conditions conducive to nitrification (oxidation of $NH_4^+$–N to $NO_3^-$–N via $NO_2^-$–N). Failure to take this increase in N into account will lead to an underestimation of the total N in chicken litter.

### 3.1. Total nitrogen contents of chicken litter

The $NO_3^-$–N and $NO_2^-$–N contained in environmental samples are not recovered quantitatively by the regular Kjeldahl digestion procedure. A set of modifications of the regular Kjeldahl procedure have been developed to include $NO_2^-$–N and/or $NO_3^-$–N in soil and plant materials. As pretreatment, before Kjeldahl digestion, Asboth [19] reacted benzoic acid with nitric acid. Following this first attempt, several pretreatments of samples containing $NO_3^-$–N have been suggested: phenolsulfuric acid [19], ferrosulfate [20], NaOH solution, and Devarda's alloy to reduce $NO_3^-$- and $NO_2^-$–N to $NH_4^+$–N with its subsequent distillation into a receiving flask containing concentrated $H_2SO_4$ [21], ferrum reductum [21], whereas $KMnO_4$ was used successfully to oxidize $NO_2^-$–N to $NO_3^-$–N and then ferrum reductum to reduce $NO_3^-$–N to $NH_4^+$–N, which was then followed by the Kjeldahl digestion [22]. The most widely used modifications today include the salicylic acid–thiosulfate modification method [23], the alkaline reduction modification method [24], and the permanganate-reduced iron modification method [25]. There are serious doubts about the ability of the salicylic acid–thiosulfate method to recover $NO_2^-$–N quantitatively, especially in undried soils [26]. None of the modifications has shown satisfactory results in recoveries of $NO_3^-$–N and $NO_2^-$–N across a wide range of soils and plant materials. The permanganate-reduced iron modification does not give satisfactory results for samples containing organic matter that resists complete digestion [27]. The underestimation of the total N content of chicken litter may result in its overapplication with potential environmental consequences of surface water eutrophication. On the other hand, an overestimation of the total N may result in its inadequate application.

Statistics of the total N contents of chicken litter samples collected in Alabama and analyzed by various methods are shown in **Table 3**. The most unusual results obtained are those by the Leco-combustion method with an average total N content of 3.78%. This represents an underestimation of the total N by 13.5% as compared with the Devarda's alloy method. This could be attributed to partial oxidation and/or an ineffective oxidation of the samples. As compared with the regular Kjeldahl method, the Leco-combustion method underestimated all the chicken litter samples. The mean of samples by the regular Kjeldahl method was 41 g/kg, whereas that of the Leco-combustion was 37.7 g/kg [28]. One of the consequences of underestimation of the total N in chicken litter is its overapplication that would lead to the accumulation of nutrients such as nitrates and phosphates in topsoil. When not absorbed by plant roots, these nutrients may find their way into surface waters or groundwaters by percolation through soil profile. Reports [29] also indicated that Leco FP-428 Nitrogen Determinator underestimated N when compared with other methods (the regular Kjeldahl method, the phenyl acetate method, the salicylic acid method, and the $NO_3^-$–N prereduction method). The Leco FP-428 gave lower results of the total N for all the samples (Stockton soil, Copay soil, and in-house liver tissue standard) tested. A study showed that Leco FT-428 and CHN-600 provided slightly higher total N levels than the regular Kjeldahl methods [30]. Dry combustion methods have been widely used in laboratories because the procedures are automated and rapid (analysis time for C, H, and N <5 min/sample by the Leco CHN-600); as many as 80 samples can be performed in 24 h [31]. Seven pretreatments (salicylic acid–$Na_2S_2O_3$, aqueous $Na_2S_2O_3$, Devarda's alloy, Zn–$CrK(SO_4)_2$, $H_2O_2$–Fe, NaOCl–Fe, and $KMnO_4$–Fe) compared with the regular Kjeldahl method showed no significant difference between the N content in fresh manures [32]. The study, however, showed a significant difference between the modified methods and the regular Kjeldahl method in the case of composted poultry manure. It is well known that storage conditions may significantly affect the proportion of ammonium and nitrate in environmental samples. It is important to point out that the samples studied contained between 0.07 and 7.63 g $NO_3^-$–N/kg for poultry manures and the composted poultry manure with wood chips, respectively. Ratios of the total N determined by the $KMnO_4$ method over the regular, the salicylic acid, the Devarda's alloy, or the Leco-combustion method have been published [28]. The $KMnO_4$:Devarda's alloy method mean ratio was 1.01 and implies that these two methods are also similar. Extremely high concentrations of ammonium were reported in the Delaware samples and implies that large portions of the organic N were converted into $NH_4^+$–N with potential to be oxidized to $NO_3^-$–N during storage. If not taking into account, one may expect an overapplication of the chicken litter to topsoil with serious environmental consequences.

| **Total N determination method** | **Range (%)** | **Median (%)** | **Mean (%)** |
|---|---|---|---|
| Regular Kjeldahl | 2.75–5.49 | 4.22 | 4.10 |
| Potassium permanganate-reduced iron | 2.93–5.71 | 4.42 | 4.35 |
| Salicylic acid | 3.02–5.24 | 4.11 | 4.09 |
| Devarda's alloy | 3.11–5.52 | 4.30 | 4.37 |
| Leco-combustion | 2.57–5.01 | 3.83 | 3.78 |

[a] *n* = 33 collected in 12 counties; litter age varied from 4 months to 18 months; bedding materials include: pine shavings, peanut hulls, pine chips, and sawdust (adapted from Kpomblekou A, 2006).

**Table 3.** Range, median, and mean of total N in chicken litter samples[a] generated in Alabama and digested by total N determination methods.

### 3.2. Inorganic nitrogen contents of chicken litter

Inorganic N found in chicken litter is small and could be extracted with 2 M KCl solution (litter/solution ratio, 1:20). Following the filtration and centrifugation of the mixture, ammonium–N and ($NO_3^-$ + $NO_2^-$)–N in the filtrate could be determined by steam distillation [33] whereas $NO_2^-$–N could be determined by a modified Griess-Ilosvay colorimetric method [34]. Inorganic N contents of the chicken litter can vary significantly and may not be related to chicken litter age or bedding material types [28]. Ammonium is the most dominant inorganic N in chicken litter. The mean $NH_4^+$–N concentrations tested for samples from Alabama ranged from 1.61 to 5.39 g/kg (**Table 4**).

In general, $NH_4^+$–N contents of the samples were higher than those of ($NO_3^-$ + $NO_2^-$)–N, which varied from 0.19 to 5.56 g/kg. Under the storage conditions ($4 \pm 1$°C) nitrification was effectively reduced. However, ($NO_3^-$ + $NO_2^-$)–N contents of animal waste may be greater than those of $NH_4^+$–N [28]. Nitrite does not usually accumulate in animal waste because it is rapidly oxidized to $NO_3^-$ unless its oxidation is inhibited by environmental conditions. Nitrite concentrations could vary from 0 to 0.58 g/kg in a sample, therefore one should not be very much concerned about the recovery of $NO_2^-$–N in chicken litter analysis since its concentration is negligible. Ammonium concentration may vary from 3.49 to 16.4% for total Kjeldahl-N and could totally be recovered in samples by almost all total N determination methods. On the other hand, the ($NO_3^-$ + $NO_2^-$)–N content cannot be ignored. It represents 0.44–11.4% of the total organic N in chicken litter. A range of 60–97% and 3–40% of the total N in animal manures were reported present as organic and inorganic N, respectively [32]. The authors also reported that most of the inorganic N was in the form of $NH_4^+$–N (77–89%) with only a small fraction present in $NO_3^-$–N (6–12%) and $NO_2^-$–N (0.2–2%). Bedding materials seem to influence the inorganic N content of the litter. The mean values of the total N (53.2 g/kg), $NH_4^+$–N (20.6 g/kg), and $NO_3^-$–N (308 mg/kg) were reported in 20 poultry manures collected from stockpiled manure and poultry houses in Delaware [35]. The following trend has been reported for Alabama bedding materials: pine chips (6.29 g N/kg) > pine shaving (5.34 g N/kg) > sawdust (4.53 g N/kg) > peanut hulls (4.32 g N/kg) > mixture pine shavings–sawdust (3.41 g N/kg) [28].

| **Inorganic N[b]** | **Range** | **Median** | **Mean** |
|---|---|---|---|
| | g/kg | | |
| Ammonium, $NH_4^+$–N | 1.61–5.39 | 2.91 | 3.03 |
| Nitrate + nitrite, $(NO_3^- + NO_2^-)$–N | 0.19–5.56 | 1.45 | 1.57 |
| Nitrite, $NO_2^-$–N | 0–0.58 | 0.01 | 0.06 |
| Percentage inorganic N of total Kjeldahl N | % | | |
| $NH_4^+$–N | 3.49–16.4 | 6.57 | 7.51 |
| $(NO_3^- + NO_2^-)$–N | 0.44–11.4 | 3.48 | 3.99 |
| $NO_2^-$–N | – | – | – |
| Relative proportion of specified N of total inorganic N | % | | |
| $NH_4^+$–N | 41.1–92.2 | 68.9 | 68.4 |
| $(NO_3^- + NO_2^-)$–N | 6.28–58.3 | 30.6 | 30.3 |
| $NO_2^-$–N | 0.0–13.8 | 0.31 | 1.28 |

[a] *n* = 33 collected in 12 counties; litter age varied from 4 months to 18, and bedding includes: pine shavings, peanut hulls, pine chips, and sawdust.

[b] Ammonium–N and nitrate–N were determined in 2 M KCl filtrate by steam distillation (Keeney and Nelson, 1982) whereas nitrite–N was determined by a modified Griess-Ilosvay colorimetric method (Barnes and Folkard, 1951) (adapted from Kpomblekou A, 2006).

**Table 4.** Range, median, and mean of inorganic N in chicken litter samples[a] generated in Alabama.

### 3.3. Mineralization of organic nitrogen in chicken litter

Organic N to become available for plant uptake must be mineralized. The mineralization of organic N depends on several factors: soil types and litter bedding materials that could significantly alter N transformation in soils. Organic N mineralization in the 10 soils (amended or not) tested was best described by first-order kinetics, but the decomposition rates and half-life of remaining N vary significantly indicating that fractions of organic N in the chicken litter samples differ (**Table 5**). **Table 5** also shows that the decomposition was also affected by soil types.

| **Soil series** | **Broiler litter sample ID** | **Decomposition rate (week$^{-1}$)**[a] | | **Percentage of N mineralized at each phase** | | **Half-life of N remaining (weeks)** |
|---|---|---|---|---|---|---|
| | | $k_1$ | $k_2$ | $D_1$ | $D_2$ | |
| Appling | None | 0.00132 | 0.0003 | 6.73 | 1.38 | 75 |
| Cecil | None | 0.0127 | 0.0019 | 3.54 | 2.01 | 52 |
| Colbert | None | 0.004 | 0.002 | 2.27 | 1.34 | 50 |
| Decatur | None | 0.003 | 0.001 | 6.03 | 1.57 | 33 |
| Dothan | None | 0.006 | 0.002 | 3.80 | 1.78 | 50 |
| Hartsells | None | 0.0015 | 0.0006 | 1.85 | 0.78 | 66 |
| Linker | None | 0.007 | 0.003 | 5.10 | 0.37 | 33 |
| Maytag | None | 0.0105 | 0.0016 | 4.15 | 1.43 | 62 |
| Sucarnoochee | None | 0.012 | 0.001 | 4.68 | 2.31 | 38 |
| Troup | None | 0.0025 | 0.0026 | 2.49 | 0.95 | 40 |
| Appling | 1 | 0.0065 | 0.0023 | 18.8 | 1.10 | 15 |
| Cecil | 1 | 0.0026 | 0.0014 | 17.7 | 1.86 | 38 |
| Colbert | 1 | 0.0038 | 0.0025 | 18.2 | 3.32 | 26 |
| Decatur | 1 | 0.004 | 0.0018 | 11.3 | 2.40 | 25 |
| Dothan | 1 | 0.0028 | 0.0015 | 14.1 | 1.25 | 35 |
| Hartsells | 1 | 0.003 | 0.0014 | 20.7 | 2.29 | 33 |
| Linker | 1 | 0.0036 | 0.0012 | 20.4 | 2.32 | 28 |
| Maytag | 1 | 0.0044 | 0.0015 | 19.7 | 2.43 | 23 |
| Sucarnoochee | 1 | 0.003 | 0.0029 | 17.5 | – | 33 |
| Troup | 1 | 0.0023 | 0.00115 | 5.55 | 1.37 | 43 |
| Appling | 2 | 0.0039 | 0.0012 | 51.1 | 1.49 | 25 |
| Cecil | 2 | 0.0028 | 0.0014 | 29.9 | – | 35 |
| Colbert | 2 | 0.0031 | 0.0021 | 19.7 | 3.49 | 32 |
| Decatur | 2 | 0.0045 | 0.002 | 17.8 | 3.41 | 22 |
| Dothan | 2 | 0.0029 | 0.0018 | 33.7 | 1.56 | 34 |
| Hartsells | 2 | 0.0043 | 0.0021 | 28.6 | 2.69 | 23 |
| Linker | 2 | 0.0035 | 0.0014 | 26.6 | 2.58 | 28 |
| Maytag | 2 | 0.0044 | 0.0013 | 35.3 | 2.36 | 23 |
| Sucarnoochee | 2 | 0.0049 | 0.0018 | 28.7 | 2.93 | 20 |
| Troup | 2 | 0.0025 | 0.00095 | 18.5 | 1.29 | 40 |

[a] $k_1$ and $k_2$ were calculated from graphs prepared by plotting organic N remaining after each incubation time against time. No second phase was identified in Sucarnoochee and Cecil soils amended with broiler litter 2. From Kpomblekou-A and Genus, 2012.

**Table 5.** First-order rate constants for decomposition of organic N in soil alone and broiler titter-amended soils.

### 3.4. Total and inorganic phosphorus contents of chicken litter

Broilers are typically fed corn–soybean blend mix fortified diets with vitamins and minerals. Corn and soybean meal contain on average 1.88 and 3.88 g/kg phytate-P, corresponding to 71.6 and 59.9% of the total P in their grains, respectively. Because broilers in their digestive system lack phytase, an enzyme that splits P from the phytate molecule, P of the grain is not absorbed by the birds and therefore released into chicken manure. The reduction of nonphytate P and utilization of phytase enzymes to hydrolyze phytic acid in corn grains fed to poultry birds enabled a significant decrease in the total P in litters by 3.4–8.8 g/kg relative to normal diets [35]. The hydrolysis of phytate by addition of phytase to animal feeds increases endogenous P availability. Phytase addition not only increases P absorption and promotes healthy broiler growth, but also saves money that could have been spent on supplement P in broiler diets. Although enzymes have been successful in catalyzing the hydrolytic degradation of phytic acid and its salts, their high anticipated production costs have not convinced producers of their use as a suitable profitable alternative.

Thus, a major portion of phosphorus (P) in chicken litter originates from phytic acid and its phytate salts. The total P content of chicken litter ($n = 33$) sampled in Alabama varied between 1.58 and 3.20%. Fractions of the P removed by sequential extraction showed that they contain 29.5, 32.5, and 38.0% of organic, inorganic, and residual P, respectively (**Figure 3**). The organic fraction (**Figure 4**) contains sodium bicarbonate soluble-$P_o$ (44.6%), microbial-$P_o$ (27.2%), and sodium hydroxide soluble-$P_o$ (28.2%). The inorganic P (**Figure 5**) is made of water soluble-$P_i$ (40.2%), sodium bicarbonate soluble-$P_i$ (10.1%), microbial-$P_i$ (2.85%), sodium hydroxide soluble-$P_i$ (2.83%), and hydrochloric soluble-$P_i$ (44.0%). Although broiler litter is an excellent soil amendment that improves soil fertility of farmers' fields, its high P content puts broiler litter amended soils at risk and susceptible to P accumulation. Applications of chicken litter have resulted in accumulation of P in topsoil and its movement to depths. Results of studies conducted on six soils of southern Alabama showed a significant increase in the total P (**Figure 6**). The total P concentrations are higher in the chicken litter-amended soils than in their nonamended counterparts. In Bonifay soil, however, throughout the profile the total P concentration was higher in the nonamended soil than the broiler litter-amended soil. In many cases, the observed differences were statistically ($P < 0.05$) significant. In Fuquay soil, the application of chicken litter increased the total P concentrations from 263 to 835, 165 to 805, 121 to 244, and 153 to 1555 in the 15–30, 30–45, 45–60, and the 60–75 cm depths, respectively. Madison soil showed that the total P concentrations in the chicken litter amended soils were significantly different from the nonamended soils throughout the soil profile. The Bray 1-P concentrations (**Figure 7**) were ≦10 mg/kg for Madison and Malbis soils, <40 mg/kg in Orangeburg soils, and >75 mg/kg in the Bonifay, Dothan, and Fuquay soils. The Bray-1 extractable P accumulated in the 0–15 cm depth of each of the six soils following broiler litter application. Bonifay, Fuquay, Malbis, and Orangeburg soils showed accumulation throughout the profile and decreased as depth increases. Dothan and Madison soils showed an accumulation only in the 0–15 cm depth, but the accumulation was not significant in Madison topsoil. Bonifay and Orangeburg soil showed considerable accumulation down to 45 cm, but the accumulation was significant ($P < 0.05$) only at the 0–15 and 15–30 cm depths. Fuquay soil showed significant accumulation throughout the soil profile, and Malbis and Orangeburg soils showed similar trends. Elevated Bray 1 soil test P levels following a long-term application of manures and wastes have been reported [36]. The study found that several Oklahoma soils receiving a long-term application of broiler litter reported Bray 1 soil test several P levels up to 279 mg/kg.

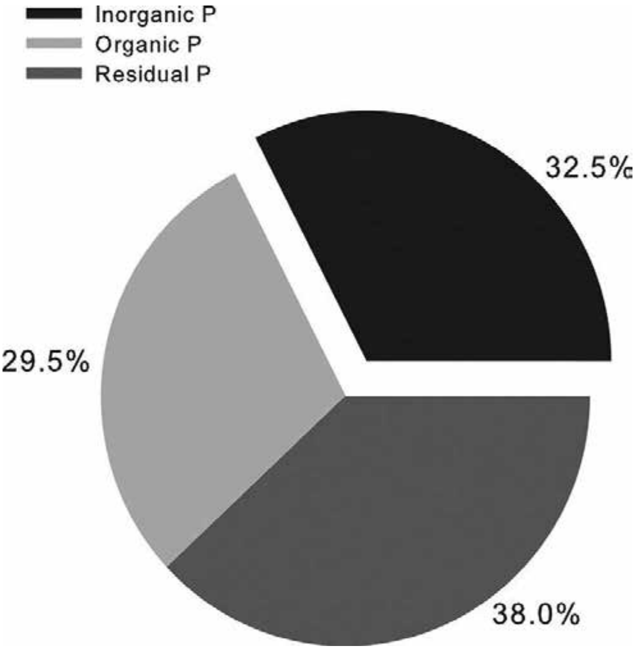

Figure 3. Average P fractions removed by sequential extraction from broiler litter.

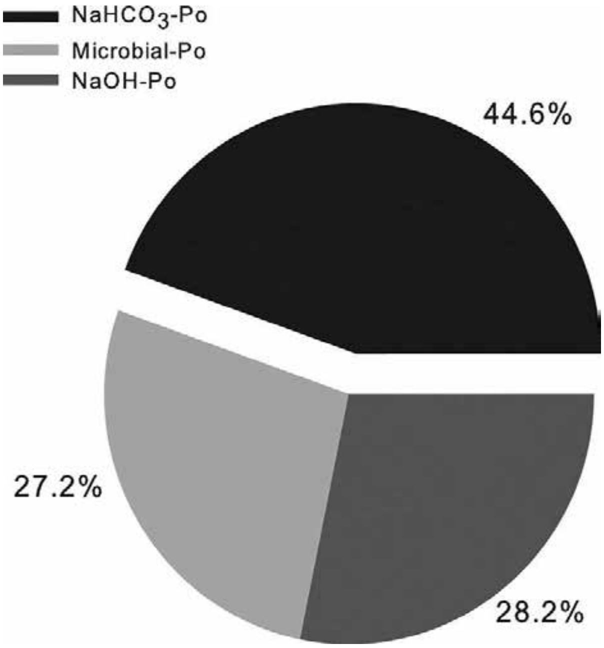

Figure 4. Average organic P fractions removed by sequential extraction from broiler litter.

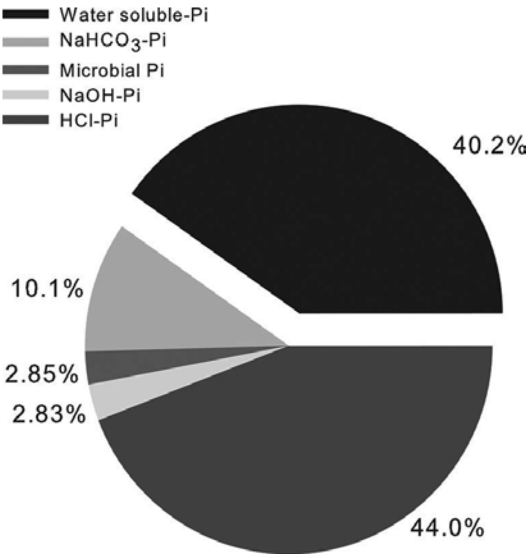

**Figure 5.** Average inorganic P fractions removed by sequential extraction from broiler litter.

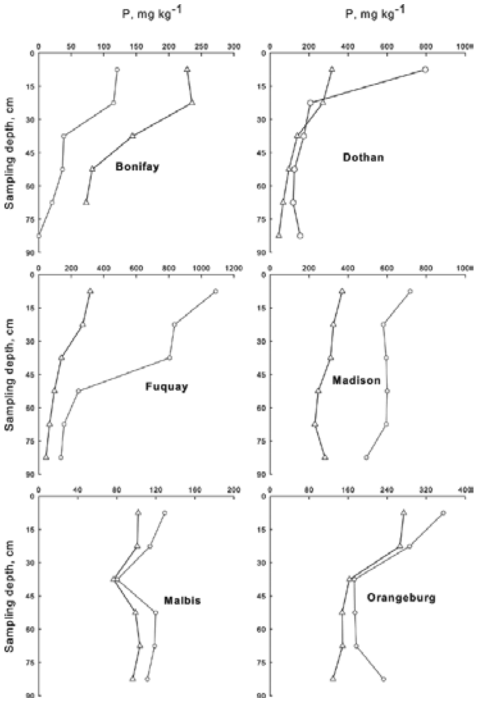

**Figure 6.** Distribution of total P in poultry littered (open circle) and nonlittered (open triangle) soils. Adapted from Dotson, 2000.

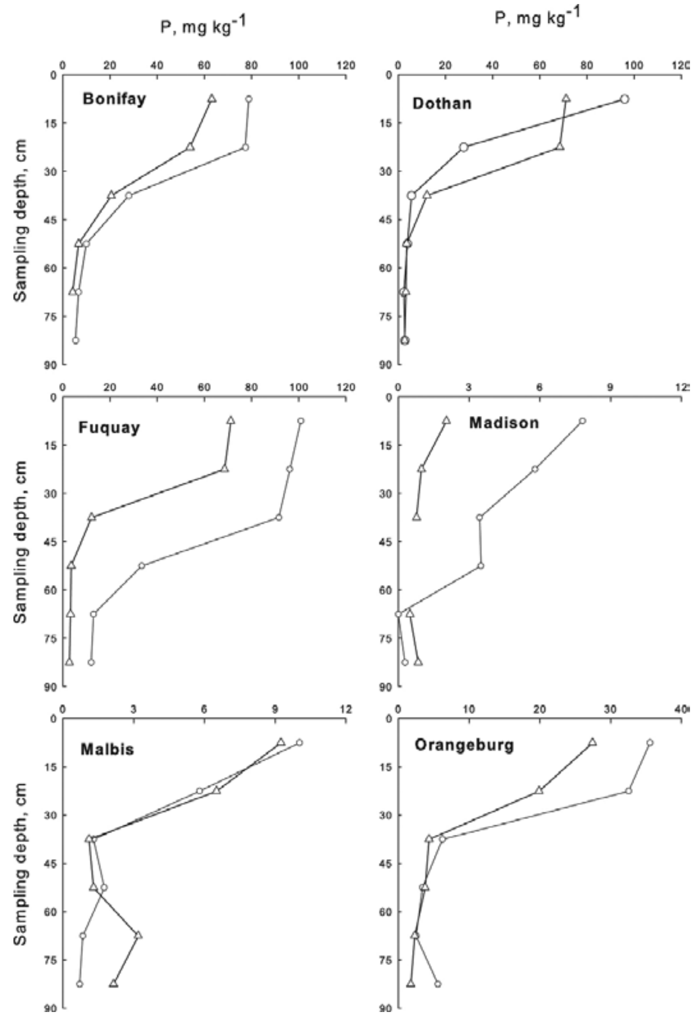

**Figure 7.** Distribution of Bray 1-P in poultry littered (open circle) and nonlittered (open triangle) soils. Adapted from Dotson, 2000.

The USEPA required Concentrated Animal Feeding Operations (CAFO) to develop and implement Best Management Practices that minimize phosphorus and nitrogen transport from fields to surface waters. The standard requires soil phosphorus not to exceed 200 ppm by 2018 and soil with greater than 400 ppm not to receive any poultry litter applications. It is in light of these requirements of the CAFO regulations that emerged the need to develop a chemical method that would reduce phosphorus content of poultry litter before its application to agricultural land in order to avoid a long-term builds up of phosphorus in soil. An extraction procedure was developed at Tuskegee University and includes steps of equilibrating an amount of chicken litter with an extracting solution [37]. After a contact time, the solution removes a significant amount of the phosphorus from the chicken litter. The chicken litter is then separated from the solution to obtain phosphorus-depleted chicken litter. Phosphorus contained in the phosphorus-rich solution can be precipitated and recovered to fertilize soils that are phosphorus-depleted. The extracting solutions proposed removes excess quantities of phosphorus (about 90%) in the chicken litter while retaining other essential elements (e.g., carbon, nitrogen, and sulfur) needed for plant growth and development.

## 4. Crop responses to organic fertilizers

Conventional farming has evoked fears of pesticide residues in food and declining energy resources [38] and organic fertilizers such as poultry litter and fish emulsions improve crop vigor and yields, increase disease and insect resistance, extend shelf life of produce and enhance microbial activity and soil nutrients [39]. Organic nutrients such as manure or cover crops provide balanced nutrient combinations over a longer period because they are slowly released based on microbial transformation in the soil [40]. Studies on the influence of poultry litter and hydrolyzed fish fertilizer on various vegetable crops to determine impact on yield and quality, phytochemical contents and on soil microbial community, and chemical properties in the rhizospheres were conducted. These crops included Vegetable Amaranth (*Amaranthus hybridus*), a leafy vegetable similar to spinach [41], Celosia (*Celosia argentea*) commonly known as lagos spinach, quail grass, soko, celosia, or feather [42], and Gboma eggplant (*Solanum macrocarpon*) is grown for its fruit production as well as its leaves [43]. West African Okra (*Abelmoschus caillei*), a multipurpose herb grown either as an annual [44], Long Bean (*Vigna unguiculata*) popular in Asian countries, African Eggplant (*Solanum aethiopicum*) is high yielding, adaptable and can be grown and harvested in a wide range of climates [44], and sweetpotato [Ipomoea batatas (L.) Lam.]. (45, 46).

### 4.1. Materials and methods

Experiments were conducted on the Small Model Research Farm at the George Washington Carver Agricultural Experiment Station, Tuskegee University, on Norfolk sandy loam (fine, siliceous, thermic Typic, Paleudults) with a pH of 5.9 and organic matter content of less than 1%. The field was prepared conventionally and soil samples were collected for elemental analysis according to the method of [47] at 15 cm depths in a zig-zag pattern using an auger. The cores were composited and analyzed by the Plant and Soil Testing Laboratory at Auburn University, Alabama, USA, for mineral constituents (Ca, Mg, P, K, and pH).

Seeds for all species (or 15 cm long stem cuttings for sweetpotato) were sown in polystyrene trays filled with moistened Jiffy mix (Ferry Morse Fulton, Kentucky, USA) in a greenhouse. One seed was placed into each cell and covered with approximately 0.6 cm of medium. Trays were watered as needed and fertilized once per week with Peters 20–20–20 at the rate of 15 g per 3.78 L of water. Temperature in the greenhouse averaged about 36°C, and relative humidity and photosynthetic photon flux (PPF) were 40% and 1159 μmol/m$^2$ s, respectively.

### 4.2. Treatment calculations and planting

Treatment rates for each of the six species were based on soil test recommendations. Sources of nutrients were ammonium nitrate (34% N), triple superphosphate (46% P), muriate of potash (60% K), poultry litter (54% N), and Megabloom (2% N), a fish protein fertilizer. Organic amendments were calculated based on the total N content.

Poultry litter, unlike commercial fertilizers, is quite variable and according to ref. [48] can vary up to 50% based on animal sources. The available values of litter nutrients using data from Fulhage and Pfost [47] were total N of 54 lb/ton, comprised of 48 lb/ton organic·N and 6 lb/ton $NH_4N$, 59 lb/ton $P_2O_5$, and 38 lb/ton $K_2O$. In addition, the amount of organic N available was based on days from collection to incorporation, which is 20% beyond 7 days. The calculations were based on the following equation:

$$\frac{\text{crop N - residual N}}{\text{available } NH_4N + \text{available organic N}}$$

Ten plants from each species were transplanted into three-row plots 1.2 m × 6 m at the recommended within- and between-row spacing for each species and drip irrigation applied. Fertilizer treatments for each species were based on soil test recommendations and were applied in single bands approximately 15–20 cm away from the plants. Six plants of each species from the middle row only were harvested. Physiological measurements were performed once per week, starting approximately one week after planting. These included stem diameter, plant height, and leaf area. Plant height and stem diameter were measured on each species starting at 2 cm above the soil stem interface to the terminals (for the former) and at the widest section for the latter, and recorded as cumulative growth over time. Leaf area was determined from leaf samples collected at each harvest every two weeks, using a LICOR-1800 leaf area meter (LI-COR, Lincoln, Nebraska, USA). All species were harvested periodically throughout the growing season (succulent stems of Amaranth and Celosia of approximately 15 cm length were harvested every two weeks) and once over at the end of the season. Fresh weights of harvested samples were recorded and subsamples collected for nutritional analysis. Samples were dried in ovens at 65°C for 72 h and the dry weights recorded. These data were used to estimate fresh and dry biomass yield per unit area.

The sweetpotato study was conducted as a randomized complete block design with a 4 × 4 factorial treatment arrangement in three replications. The treatment factors were conventional NPK fertilizer, poultry litter, Megabloom (fish fertilizer; FSH), and an untreated check (O). The sweetpotato cultivars were J6/66, NCC-58, TU Purple, and Whatley-Loretan. Treatments were split-applied at the rate of 134–67–67 kg/ha NPK equivalent based on soil tests recommendations one and four weeks after planting as single bands 15 cm from the plants.

Triplicate rhizosphere soil samples from each plot were taken at harvest, composited and analyzed for pH, organic carbon (SOC), and enzyme activity. pH was determined using 1:2.5 soil/water and SOC using the wet oxidation method [49]. Phosphomonoesterases activity was determined by the method of ref. [50]; β-glucosidase and *N*-acetyl-β-glucosaminidase activity by assay [51, 52]; and whole DNA by Power Soil Extraction Kit and quantified using spectrophotometer. Pooled DNA samples were tested for PCR optimization and pyrosequencing analysis (Research and Testing Labs, Lubbock, TX, USA).

## 5. Results and discussion

Organic amendments had no significant influence on fresh and dry biomass production, while species exerted a greater impact, and there were no significant interaction between organic amendments and species for any biomass variable (data not shown). Plants treated with Megabloom produced greater fresh fruit biomass (774, 572, 345 kg/ha, for Megabloom, NPK, and poultry litter, respectively), whereas NPK-treated plants produced greater dry biomass (302, 297, and 226 kg/ha, for NPK, Megabloom, and poultry litter, respectively). Although Amaranth, Celosia, and Okra produced greater total biomass, it was not statistically different from that of either Gboma or Longbean. Gboma plants had larger leaves than Amaranth and Celosia but similar to Longbean, Okra, and Eggplant. Amaranth and Okra plants were taller than the other species, with Amaranth having the greatest stem diameter but Okra having the highest total number of fruits than the other species (**Table 6**).

| Species | Fresh (kg/ha) | Dry (kg/ha) | Fruit (kg/ha) | Leaf area (cm$^2$) | Plant height (cm) | Stem diameter (cm) |
|---|---|---|---|---|---|---|
| Amaranth | 2015ab | 357ab | – | 36b | 52b | 1a |
| Celosia | 2094ab | 455a | – | 35b | 42b | 1a |
| Gboma | 911b | 111c | – | 53a | 8d | 0.5d |
| Longbean | 2471a | 1078a | 1354a | 48a | 8d | 0.6d |
| Okra | 1490ab | 269bc | 898b | 46ab | 55a | 0.7cd |

[a]Mean separation within columns followed by the same letter are not significant based on LSD, 5% level.

**Table 6.** Main effect of species on the total biomass yield, leaf area, plant height, and the stem diameter of vegetables[a].

There were significant interactions between organic amendment and the different species for contents of vitamin C, betacarotene, total phhenolics, and total antioxidant capacity (**Table 7**). Effects of the interaction between Amaranth and fertilizer amendments on vitamin C showed the highest content among plants receiving NPK compared to the other two treatments. The betacarotene content was similar among plants receiving both Megabloom and poultry litter and substantially greater than plants receiving NPK (**Table 7**). The total phenolic content was higher with NPK whereas plants receiving Megabloom had higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity. Results of the interaction between Celosia and organic amendments on nutrient content show that NPK enhanced vitamin C content and, along with Megabloom, betacarotene content. There were greater total phenolics among plants receiving Megabloom followed by those receiving poultry litter, and DPPH activity was similar among plants receiving Megabloom or NPK. For Gboma, vitamin C content and DPPH activity were enhanced among plants treated with Megabloom, whereas NPK significantly increased the betacarotene content compared to those of the other treatments.

| ORAMD[a] | Vitamin C (mg/100g) | Betacarotene (mg/100g) | Total phenolics (mg/100 g) | antioxidant capacity[b] (μmol AAE/g) |
|---|---|---|---|---|
| Amaranth | | | | |
| Megabloom | 156 | 91.6 | 467 | 30.6 |
| NPK | 188 | 77.9 | 542 | 25.3 |
| Poultry | 133 | 93.5 | 362 | 24.5 |
| Significance | ***[c] | *** | *** | *** |
| Celosia | | | | |
| Megabloom | 163 | 81.1 | 400 | 30.7 |
| NPK | 197 | 87.9 | 420 | 31.8 |
| Poultry | 185 | 54.2 | 440 | 19.8 |
| Significance | *** | ** | *** | *** |
| Gboma | | | | |
| Megabloom | 160 | 189.4 | 200 | 77.2 |
| NPK | 102 | 200.2 | 304 | 68.6 |
| Poultry | 102 | 157.8 | 453 | 57.2 |
| Significance | *** | *** | *** | *** |
| Longbean | | | | |
| Megabloom | – | – | 556 | 47.1 |
| NPK | – | – | 592 | 37.1 |
| Poultry | – | – | 401 | 39.8 |
| Significance | – | – | *** | *** |

[a] ORAMD, organic amendments (poultry litter, Megabloom-fish protein-based).
[b] 2,2-Diphenyl-1-picrylhydrazyl (DPPH) % radical scavenging quenched.
[c] Significant at $P = 0.01$ (*), $P = 0.001$ (**), $P = 0.0001$ (***).

**Table 7.** Effect of interaction between species and fertilizer amendments on nutrient content of vegetables.

Interaction between Longbean and fertilizer amendments on nutrient content was not determined for vitamin C and betacarotene due to sample size. The total phenolic content was similar among plants receiving megabloom and NPK, whereas DPPH activity was significantly greater among plants receiving Megabloom.

These results indicated that species exerted a stronger influence on yield than organic amendments. Longbean had a 44% greater fresh biomass than okra or eggplant and a 24% greater fresh fruit yield. However, okra produced 52% greater total fruit number than longbean or eggplant. Eggplant had greater leaf area and stem diameter than the other fruit-bearing species whereas okra plants were taller. Among the leafy greens, Amaranth and Celosia produced a 39% greater fresh and dry biomass yield than Gboma that had a 19% greater leaf area. Amaranth and Celosia were taller than Gboma and had thicker stems. Although organic amendments had no significant impact on biomass, there were trends toward a positive response by the plants. For example, plants receiving poultry litter produced 10% greater fresh and dry biomass. Similarly, plants receiving Megabloom had 23% greater fresh fruit biomass than those treated with NPK, whereas the total fruit number and leaf area and NPK-treated plants were 15% higher (Table 6).

Nutrients in organic fertilizers are released through mineralization by soil microorganisms [53]. Depending on soil conditions such as pH and moisture content, mineralization rates can be impacted. It is probable that the lack of response to organic amendments in this study could be due in part to slow mineralization rates resulting in fewer nutrients available for plant uptake [53]. In fact, Whitmore [54] reported that 40% of the total N from composted chicken litter was available in the first year and the remainder at the rate of 6–12% per year thereafter because of the slow mineralization rates, and researchers have recommended applying 50% more organic fertilizer 14–20 days earlier than normal to compensate for slow mineralization rates.

Nutrient content of the vegetables varied with species. NPK enhanced vitamin C and total phenolics in Amaranth but not betacarotene or DPPH activity. These results are inconsistent with those of Wheeler et al. [55] and Muso and Ogaddiyo (in kale and hibiscus) [56] who reported lower vitamin C with increased nitrogen fertilization. The increase in vitamin C could be due in part to a decrease in protein production and an increase in carbohydrate production [54]. High vitamin C in the leaves may make plants more tolerant of stress since reducing vitamin C increases susceptibility to stresses [57].

All three amendments enhanced betacarotene content. Megabloom and poultry litter amendments produced similar levels in Amaranth similar to Megabloom and NPK in Celosia and Gboma. This increase is probably due to increased chlorophyll from nitrogen and or light-absorbing pigments including carotenoids that are critical in photosystems I and II of the photosynthesis process [54]. Indeed, research has shown that light enhances the biosynthesis of phenolics in the chloroplasts of the cells and thus tends to accumulate in high amounts in the vacuoles or deposits in secondary cell walls as lignin [58].

Betacarotene content of Amaranth, Celosia, and Gboma increased with time for all species up to 51 days after transplanting except for Amaranth and Gboma plants receiving NPK. The betacarotene content of Amaranth plants receiving NPK appeared to decline with time whereas Celosia plants receiving NPK increases substantially with time (data not shown).

Sweetpotato results showed an interaction between the fertilizer amendments and cultivar for rhizosphere pH that varied depending on cultivar and cultivar response varied with pH (data not shown). The pH was lowest in rhizospheres of Whatley/Loretan and NCC-58 receiving Megabloom, and generally, pH ranged from 6.1 to 6.8. Thus, fertilizer amendments lowered rhizosphere pH values with TU Purple plots receiving PL and Whatley/Loretan and NCC-58 plots receiving Megabloom, having the lowest values, respectively. SOC was similar among amendments but was highest for TU Purple and J6/66 and ranged from 0.63 for Whatley/Loretan to 1.07 for J6/66 (data not shown). Storage root yield was similar regardless of the amendment applied ranging from a low of 12.0, 10, 21.1 t/ha for control and plants receiving NPK, respectively (**Table 8**).

| **Fertilizer amendments** | **Root yield (t/ha)** | **ACP (μg *p*-nitrophenol (per g soil/h))** | **ALKP (μg *p*-nitrophenol (per g soil/h))** | **βNAG (μg *p*-nitrophenol (per g soil/h))** | **βGLU (μg *p*-nitrophenol (per g soil/h))** |
|---|---|---|---|---|---|
| Con | 12.0 | $172.89^{a}$ | 2.21a | 16.56 | 13.05 |
| PL | 19.7 | $287.71^{b}$ | 7.06b | 31.39 | 45.86 |
| FSH | 18.1 | $329.99^{b}$ | 7.41b | 23.14 | 48.25 |
| NPK | 21.1 | $308.66^{b}$ | 7.69b | 29.40 | 49.47 |
| Significance | NS | * | ** | *** | ** |

[a] ACP, acid phosphatase; ALKP, alkaline phosphatase; βGLU, β-glucosidase; βNAG, β-glucosaminidase.
*, **, ***significant at 0.05, 0.01, 0.001 levels of probability.

**Table 8.** Main effect of fertilizer amendments on storage root yield soil enzyme activity[a].

Therefore, the addition of organic amendments increased both soil enzyme and microbial activity, which is consistent with the findings of others [59–61]. NPK-treated plots had higher enzyme activity compared to the controls and, the organic amendments as a nutrient source did not adversely affect enzyme activity relative to NPK treated plots.

In general, the addition of fertilizer and organic amendments had a significant impact on bacteria at every taxonomical level while TU Purple and Whatley/Loretan impacted the *Gemmatimonadetes* at every taxonomical level (**Table 9**).

| **Class** | **Con** | **BL** | **NPK** | **FSH** |
|---|---|---|---|---|
| Actinobacteria | 6.69b | 6.60b | 7.78ab | 9.29a |
| Chloroflexi | 1.83a | 1.55a | 1.50a | 0.59b |
| Cytophagia | 1.56a | 1.36a | 1.36a | 0.75b |
| Δproteobacteria | 4.80a | 4.34ab | 4.43a | 3.06b |
| Rubrobacteria | 1.82b | 1.73b | 2.26ab | 3.03a |
| | Cultivars | | | |
| | TU Purple | J6/66 | NCC-58 | Whatley/Loretan |
| *α*-Proteobacteria | 3.62b | 4.17b | 3.50b | 5.34a |
| Gemmatimonadetes | 3.10bc | 3.44b | 2.57c | 4.34a |

Means with same letters in rows are not significantly different Tukey's (0.05).

**Table 9.** Effect of fertilizer, organic amendments, and cultivars on class bacterial composition of sweetpotato rhizosphere.

The results indicated that *Proteobacteria* was the most dominant phylum and class identified, of which three of its classes (*alphaproteobacteria, betaproteobacteria*, and *gammaproteobacteria*), as well as the class *actinobacteria*, were the most prevalent in the class groups. This observation is consistent with the literature that proteobacteria are ubiquitous in the soil ecosystem. TU Purple and Whatley/Lortan significantly impacted *Gemmatimonadetes* at every taxonomical level suggesting that these cultivars produce exudates that may attract these bacteria. Bacteria belonging to phylum *Gemmatimonadetes* are frequently detected in a variety of environments and are noted as one of the nine most commonly found phyla in 16S rRNA genelibraries from soil [62, 63]. Further, *emmatimondetes* play a role in P cycling by improving P removal in wastewater and could play a similar role in soil.

## 6. Conclusions

The impact of organic amendments on biomass production varied based on the species that were grown as plant species exerted a stronger influence on biomass production than the organic amendments. Vitamin C content was enhanced by NPK fertilizers for Amaranth and Celosia and by Megabloom in Gboma, similar to what others reported [64]. Gboma also showed higher radical scavenging (DPPH) than the other species. DPPH values according to reference [65] showed that the normal values are 37.7–89.5 with Gboma and Longbean in range or surpassing these values. Overall, these results show that organic amendments exerted inconsistent influences on phytochemicals. Thus, based on yield and phytochemical content, these vegetables can potentially be produced successfully on these sandy loam soils in South Central Alabama using organic amendments, but more conclusive data through additional studies are required. For sweetpotato, the results show that bacteria associated with C and N cycling under aerobic conditions can dominate in their rhizosphere.

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Chapter 3

# Green Manures and Crop Residues as Source of Nutrients in Tropical Environment

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Additional information is available at the end of the chapter

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**Abstract**

Tropical areas have prevalence of soils with low fertility, which makes the management of soil fertility a necessary practice to maintain a farming system economically and environmentally sustainable. The purpose of this chapter is to demonstrate the importance of green manure and the use of crop residues as management for soil fertility. We highlight the potential of these practices to increase/sustain productivity by providing nutrients. First, we made a short review on the main factors influencing the decomposition and mineralization processes. Subsequently, we discuss green manure techniques, presenting the main green manures, criteria for choosing, managements, potential for nutrient accumulation, and advantages and disadvantages of this practice. Finally, we use some examples to demonstrate the potential nutrient supply of crop residues from the main crops grown in the tropics. The difficulties and limitations involved are also discussed.

**Keywords:** cover crops, legumes, biological nitrogen fixation, fresh organic matter, mineralization

## 1. Introduction

The global demand for food will grow considerably in the coming years due to the increasing global population that is supposed to reach 9 billion people by 2050. The agriculture practiced in the tropics has key importance on food supply for much of the current global population and may become even more important for future generations.

The soils in most of these tropical environments have high acidity and aluminum toxicity, and are rich in oxides and poor in nutrients. Therefore, the use of lime and fertilizers accounts for a large part of the agricultural production cost. Thus, to increase the environmental and economical sustainability of these environments, it is important to make rational use of fertilizers and find viable alternatives to maintain a good physical, chemical, and biological soil characteristics.

We highlight the use of green manures and crop residues as practices that can help maintain or increase the productivity capacity of the soils, since they act as conditioners of the physical, chemical, and biological characteristics. Since the ancient Greeks, Romans, and Chinese, the humanity wisely used fresh organic matter as green manures in order to maintain the land productivity, and even today, this practice has been used with the same purpose. Meanwhile, crop residues were "a problem" for many years in agriculture. The removal and/or burning of residues were common practiced used in order to accelerate its degradation in conventional tillage. The expansion of no-till system, from the 1970s, presented several benefits by conserving and managing residues of annual crops, especially at medium and long term. The cultivation of perennial crops also presented major recent changes, such as management of weeds between plants, not using fire in renewing and especially the use of processed residues.

In this chapter, we present some of the benefits of management of green manure and crop residues, mainly the nutrient supply potential for crops of economic interest. Initially, we discuss the factors that most influence the fresh organic matter decomposition and nutrient mineralization in tropical areas. Subsequently, we discuss the concept of green manure, its management, the amount of nutrients potentially accumulated and their advantages and disadvantages. In the last part, we present examples from the main crops grown in the tropics. Regarding the annual crops, we focused on legumes with greater economic impact, since they are the first crops to be planted in rotation or succession managements. Regarding perennial crops, we present the contribution of the main crop residues and the processing of sugarcane, coffee, and eucalyptus.

## 2. Main factors influencing the decomposition of crop residues and green manures in tropical environments

The decomposition of crop residues or green manures in the soil is a complex process, which is the result of the interaction between different factors (biotic and abiotic) specific of each environment. However, the main abiotic factors that drive this process are related to their influence on soil organisms, since the decomposition is essentially a biological process [1].

Initially, the decomposition consists in the physical fragmentation process of the organic residues into smaller particles, which is a process performed by components of the soil macro-, meso-, and microfauna. Physical fragmentation of residues provides an increase in surface area, facilitating microbial colonization and subsequent hydrolysis by microbial extracellular enzymes. Thus, complex polymers are degraded into monomeric compounds and ions, which can be absorbed by microbial cells or plants.

The factors that can affect the direction and magnitude of the decomposition process are the nutrient content and biochemical composition of the crop residues or green manure added to the soil, the nature and abundance of the present microbial communities, Soil moisture, temperature, aeration, pH, the carbon/nutrient ratios of the soil organic matter (SOM) [2, 3], and the presence or absence of inhibitor substances.

In this part, we discuss the main factors involved in decomposition and its peculiarities in tropical environments.

### 2.1. C/N, C/P, and C/S ratios in crop residues and green manures

The use of green manures and crop residues provides various conditioning effects to the soil; however, the main objectives of this practice in low-fertile tropical soils are increasing soil cation exchange capacity (CEC) and provide nutrients for the plants. Thus, the nutrient contents present in these plants (mainly N, P, and S) are one of the first characteristics to be observed.

However, N, P, and S contents in the residue do not necessarily mean that they will be released synchronously with the plant needs during the decomposition process. After the decomposition, monomeric compounds and ions can be absorbed by microorganisms present in the soil, which use them as energy supply or metabolic precursors. After these requirements are satisfied, excess ions may be released into the soil solution and be available for the plants.

### 2.2. Mineralization and immobilization

Extracellular enzymes released by the fauna and soil microorganisms during the decomposition release part of the P, S and N initially linked to organic compounds in the fresh organic matter (crop residues or green manures). Extracellular phosphatases are responsible for the mineralization of P in organic compounds (C–O–P links) to phosphate ions, $HPO_4^{2-}$, and $H_2PO_4^-$ (prevalent in tropical soils). Sulfatases are responsible for breaking estersulphates (C–O–S links) to $SO_4^{2-}$ ions, and urease converts urea into $NH_4^+$.

After organic compounds fragmentation, amino acids containing C–N and C–S bonds, amino sugars, and nucleic acids are absorbed by microorganisms to attend their energy, C, N and S demand. Then, intracellular hydrolases convert C-N and C-S bonds into $NH_4^+$ and $SO_4^{2-}$ for subsequent internal use. Mineralization is the sequence of reactions that converts the nutrient from organic to inorganic form, resulting from microbial decomposition [4]. However, in practice, mineralization is when nutrients are released in the soil solution during the decomposition process. Thus, we say that nutrients were immobilized in microbial biomass and are unavailable for absorption by the plants when the N and S are mineralized into the microbial cell and/or when the $NH_4^+$, $SO_4^{2-}$ and $H_2PO_4^-$ ions from soil solution are carried to microbial cytoplasm and subsequently used as precursors for synthesis of other compounds.

The microbial cell demand for energy, nutrients, and C does not occur at the same proportion and is different for each microbial community. The C/N ratio of bacteria range between 4 and 5, while for fungal, it is about 15. But, since fungal biomass is often twofold larger than bacterial in most soils, it is assumed that the C/N ratio of the total biomass of the soil is approximately 8 [5].

Each microbial community in the soil has a different C/N, C/P, and C/S demand, as well as C utilization efficiency. Thus, an average C/N, C/P and C/S are also assumed to the fresh organic matter (crop residues or green manures), from which the nutrient mineralization or immobilization will be defined. The average C/N in plant residues is 20–30, when the C/N > 30, the N is immobilized in the microbial biomass and when the C/N < 20, the N mineralization is favored. The average C/P in the plant matter is approximately 200–300. When C/P>300, P immobilization is greater than mineralization, the opposite occurring when C/P<200. The average C/S in plant residues is 200, with C/S>200 promoting greater S immobilization and C/S<200 favoring S mineralization.

The C/N, C/P, and C/S of the crop residues and green manures added to the soil must be known, since they present major influence on the mineralization/immobilization processes. Legumes have higher levels of N in their tissues compared to non-legume species, due to the biological nitrogen ($N_2$) fixation (BNF). Studies have shown the effectiveness of the use of these species as green manures to meet N plant demands [6, 7]. However, this is not the case for P demand, which is barely attended by use of such cover crops [8–10].

The immobilization and mineralization process does not depend only on the C/N, C/P, and C/S in the residues, since they are also greatly influenced by these ratios in the SOM compounds. Organic compounds from decomposed residues interact with the SOM during the decomposition process. SOM decomposition may increase when organic residues are added to the soil. A theory for this fact is that the soil microorganisms degrade easily degradable organic compounds present in SOM to acquire energy, C or N (and possibly other nutrients) co-metabolically with the residues added to the soil [11–13]. This effect is known as "priming effect" [14] and can be positive when it accelerates SOM decomposition or negative when it slows down SOM decomposition. Therefore, N, P and S contents in the residues and SOM are important, as well as the availability of these nutrients in the soil solution, which influences mineralization and immobilization processes, being an important tool in the management of residue decomposition.

However, the decomposition of residues and SOM would release other nutrients like Ca, Mg, K and trace elements, which are subjected to the same principles of the mineralization and immobilization explained above. K is a very abundant element in plant tissues, however it is not part of biomolecules, being released very easily and at high rates [15]. Studies have shown that legumes used as green manures have been effective in supplying K for plants [6, 7]. Besides N, they also show higher levels of K due to their branched and deep root systems, allowing nutrient cycling [16].

Tropical soils are generally acid and with low natural fertility, presenting restrictions to the crops because their high $Al^{3+}$ availability in the soil solution and the low P availability for plants, due to the formation of irreversible Tropical soils are generally acid and with low natural fertility, presenting restrictions to the crops because their high $Al^{3+}$ content and low P availability for plants, especially due to the formation of irreversible bindings with Fe and Al oxyhydroxides. In this context, the high production of organic acids by the decomposition of residues and green manures causes a competition for P adsorption sites on the soil, promoting a greater P availability in the soil solution [17]. This process occurs due to: *i*) $H^+$ and $Al^{3+}$ sorption on the surface of the organic material; *ii*) $Al^{3+}$ complexation with organic acids; *iii*) competition with phosphate by binding sites, decreasing P adsorption. Moreover, in tropical environments, the P organic forms from residues are essential for P availability to plants [18]

## 2.3. Other factors influencing the decomposition

### 2.3.1. Biochemical composition of green manures and crop residues

Biochemical composition influences plant residue decomposition and microbial communities in the soil. Plant residues consist basically of the same components, but the proportions can vary between species, plants of the same species, organs of the same plant, and crop conditions [19].

Green manure residues quality is dependent on the species used ($N_2$-fixing species have usually lower C/N compared with non-legume species), nutrient content and the age of the crop used as green manure, which affect the size, fiber content, lignin content and C/N ratio [20].

In general, the compounds present in the plant cell cytoplasm and walls are waxes and pigments (1%), amino acids, nucleotides and sugars (5%), starch (2–20%), proteins (5–7%), hemicellulose (15–20%) celulose (4–50%), lignin (8–20%) and secondary compounds (2–30%) [21]. Most of these components are present in the primary and secondary cell walls of plant cells. The primary wall is formed basically by cellulose and hemicellulose. After the primary wall growth cease, the secondary walls begin to form, which has the lignin as the main component and gives resistance to the cell wall [22]. Phenolic compounds are secondary metabolites such as polyphenol and lignin, which have no direct function in the plant growth and development [22]. Additionally, non-structural carbohydrates, such as free sugars, starch, and arabinose, may affect the decomposition of materials in the soil [23].

Some studies report that soluble carbohydrate materials are readily decomposed, as well as the components rich in N, establishing the initial decomposition rate of the crop residue [23–25].

The decomposition of some organic compounds of wild pine trees (*Pinus sylvestris*), for example, can be explained through a system comprising two phases [26]. In phase 1, nutrients found in higher concentrations such as N, P and S favor the mass loss of non-lignified organic compounds. In general, organic compounds from simple structures (labile) tend to be used more efficiently compared to those more complex (polymerized) or associated with other compounds (e.g., lignin–cellulose) [27–29]. In phase 2, lignin content increases during residue decomposition [30, 31], remaining most of the more lignified material. Plant degradation is determined by the reduction of the lignin concentration, which is negatively affected by high N concentrations and positively affected by high cellulose concentrations.

The negative influence of high N concentrations on lignin degradation may be due to the ligninolytics enzymes suppression at high levels of $NH_4^+$ and N organic compounds of low molecular weight. This repression can be explained by: *i*) the N can change the decomposing microorganisms competition, including those able to degrade lignin [32]; *ii*) high $NH_4^+$ levels reduce the production of ligninolytics enzymes [33, 34]; *iii*) the amino compounds condensate with polyphenols, forming toxic compounds or inhibitors [35, 36].

On the other hand, the positive effect of lignin degradation by high cellulose content occurs because lignin has very stable bonds, which require energy to break. Thus, a co-metabolism with more labile (easy degraded) compounds is necessary and positively influences the residue decomposition [37]. The SOM also influences this process by providing nutrients and more labile compounds, which can supply the most immediate forms of energy to the microorganisms, enabling them to degrade some recalcitrant compounds of the residues, which direct affect the energy supply to the microorganisms along the decomposition.

Consequently, the values of some biochemical fractions of the residues can serve as decomposition rate indicators. The fractions commonly used are the water-soluble extractives and extractives soluble in neutral organic solvents, which are secondary components and are not part of the cell structure. The holocellulose fraction, which consists of cellulose and hemicellulose, is constituent of structural components as well as the lignin fraction. Thus, the proportion of these fractions in crop residues and green manures, as well as the C/N, lignin/N and polyphenols/N, directly affect the decomposition of crop residues [38, 39]. This information can help managing the residues in order to synchronize nutrients release to plants.

### *2.3.2. Temperature and humidity*

Temperature and humidity are factors that directly affect the microbial activity, more precisely the microbial enzyme complex [40, 41]. Temperature and humidity are factors that directly affect the microbial activity, more precisely the microbial enzyme complex [40, 41]. Residue decomposition rate correlates positively with temperature and water availability within a broad range [42].The enzymatic activity increases with an increase temperature or humidity up to a plateau, from which temperature and humidity can limit decomposition. Thus, the weather strongly affects the residue decomposition rate [43].

Moist tropical soils, which have Moist tropical soils, which have high average temperature and humidity throughout the year, have faster decomposition rates than temperate soils. Thus, in the tropical environments, the temperature and humidity present less restriction to decomposition, which depends primarily on the quality of the residues and the SOM [44].

It has been shown that chemically complex litterfall have stronger responses to temperature compared to less chemically complex litterfall [45]. Moisture is essential for the reactions that occur in the soil, and water is required for all hydrolytic reactions, affecting the extracellular enzymes activity and the diffusion coefficients [46]. Soil water content of 60% of its total porous space is assumed to be the optimum for the decomposition rate of aerobic soil microorganisms [47].

Natural cycles of wetting and drying in the soil are important modulators of decomposition rates [48]. Some agricultural practices can assist water management in order to achieve higher rates of decomposition, such as: (*i*) irrigation; (*ii*) drainage furrows installation in the field to remove excess water; (*iii*) maintenance of residues on the soil surface to increase water infiltration and decrease evaporation and *iv*) synchronization of residue incorporation with the rainy season based on historical and forecast rainfall [49].

Excess moisture can also cause anaerobic conditions that will negatively influence plant residues decomposition rates. Anaerobic environments are not favorable to fungi and actinomycetes, and only some bacteria are able to perform anaerobic digestion, decreasing decomposition rates.

### *2.3.3. Microbial communities in the soil*

Different organisms work in the residues within the soil. The soil microbial community is diverse and shows an uneven distribution along the soil profile and throughout the microenvironments [50].

There is a change in the biochemical composition of the residues during the decomposition process, what drives a microbial succession. Simpler compounds are used as a growth substrate for a large number of microorganisms that have short life-span, which are called r-strategists or copiotrophs. In the later, degradation stages occur the metabolism of more complex compounds, in which some microorganisms break components more slowly and are called oligotrophic or k-strategists [4].

Among the r-strategist and k-strategist microorganisms there are autotrophic and heterotrophic bacteria, fungi, and actinomycetes. Moreover, studies have reported that the presence of N influences the microbial community diversity during decomposition.

Changes in the microbial community are reported to happen within150 days of incubation of eucalyptus residues due to changes in the residue biochemical composition [51]. Moreover, these same authors found that, with the application of N, the change in the microbial community occurred within the 25 days of incubation, remaining constant to the end of the experiment, suggesting that the chemical differences of residues can be minimized when the C/N of the residues are closer to the C/N of the soil microorganisms [52, 53].

High bacteria and low fungi biomasses were observed for 8 years in soils with eucalyptus receiving applications of N [54]. Moreover, an increase in the Gram+/Gram-bacteria ratio was observed in an experiment with incubation of residues (leaves) of *P. massoniana* and *M. macclurei* in coniferous forest soils [55]. A study of *Pinus sylvestris* L. forests grown for 50 years in northern Sweden, report that, during two decades with nitrogen fertilization, there were reductions in the ectomycorrhizal fungi community, showing that the effects of N go beyond simple enzymatic removal of fungi and may comprise factors that are still unclear regarding the decrease in residue decomposition with presence of N [52].

Another important factor during residue decomposition is the soil pH, which directly affects the type, density, and activity of bacteria, fungi, and actinomycetes. The residue decomposition rate is higher in soils with neutral pH than in more acidic soils such as tropical soils. However, liming acidic soils promotes accelerated decomposition of the residues.

The construction and maintenance of microbial diversity in the soil favor higher rates of decomposition [49]. Some agronomic practices are recommended for this purpose, such as: (*i*) regular application of organic residues or the use of biochemically complex green manures associated with those biochemically simple and easy decomposing, which supports the greater diversity of microbial communities in the soil; (*ii*) maintenance of soil cover, which promotes energy (via root exudates) for free-living and symbiotic microorganisms and produces extracellular enzymes in addition to the enzymes released by plant roots [56].

## 3. Green manures

Green manure can be defined as the practice of plant cultivation until the flowering stage or until the incomplete development of seeds, with subsequent cutting and/or incorporation of its biomass into soil [57, 58]. The basic purpose of this technique is to improve chemical, physical, and biological soil characteristics in order to increase or stabilize the production of one or more crops in an area [57, 58]. Famers usually use legumes as green manure because of the high biomass yield, biological nitrogen fixation (BNF), and cycling of nutrients from deeper soil layers [57, 58].

After Green Revolution, the practice has gained importance, especially in organic production systems [58]. However, the species used as green manure are not restricted to these systems. They are also used, for example, to control soil degradation in minimum tillage, no-till (NT) and in integrative systems [58–60]. In some situations, these species are called "cover crops," since the main purpose may be to conserve the soil. Species from the Poaceae family are the most common used cover crops [58]. A better soil cover and diversification of species in an area may also be used to suppress the development of weeds and also the emergence of pests and diseases [58, 61]. From an environmental stand point, the use of these plants can be a form of sequestering $CO_2$ from the atmosphere [58]. This practice can also improve the use of mineral fertilizers, especially for those nutrients more susceptible to losses by leaching or sorption [58].

The main species used as green manure, their benefits, limitations, and managements are discussed in the following topics. More information, you can find in the references [58, 62, 63].

### 3.1. Plants used as green manure

Tropical environments allow the cultivation of a wide variety of green manure species, with plants from the families Fabaceae, Brassicaceae, Asteraceae, and Poaceae, among others. Some of the main species used in tropical environments, their characteristics of dry matter yield, amount of fixed nitrogen, and mineral composition of macronutrient and micronutrients is presented in **Table 1**.

| Common name | Specific name | Dry matter | Fixed N | N | P | K | Ca | Mg | S | B | Cu | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Preferred sowing in spring–summer (September to March)** | | | | | | | | | | | |
| | | **t/ha** | **kg/ha** | **g/kg of dry matter** | | | | | | **mg/kg of dry matter** | | | |
| Brachiaria | *Brachiaria decumbens* | 4–20 | – | 12–20 | 0.8–3.0 | 12–25 | 2–6 | 1.5–4.0 | 0.8–2.5 | 10–25 | 4–12 | 40–250 | 20–50 |
| Sunnhep | *Crotalaria breviflora* | 3–5 | 67 | 32.9 | 1.4 | 28.4 | 9.1 | 2.5 | – | – | 17 | 81 | 31 |
| Sunnhep | *Crotalaria juncea* | 10–17.6 | 150–450 | 11.3–44.0 | 0.9–3.7 | 5.7–33.7 | 3.3–23.1 | 2.5–8.0 | 1.2–2.7 | 15–25 | 5.5–14 | 23–179 | 16–44 |
| Sunnhep | *Crotalaria paulina* | 5.4–15 | 100–170 | 12.4–14.8 | 0.9–1.1 | 4.2–9.5 | 6.3–8.0 | 2.4–4.1 | 0.8–3.6 | 17 | 5,0 | 34 | 17 |
| Sunnhep | *Crotalaria spectabilis* | 4–14.9 | 60–120 | 19.7–33.0 | 0.7–2.5 | 7.9–17.8 | 4.3–18.5 | 3.7–5.0 | 1.5–1.6 | 34–41 | 8–9.3 | 53–126 | 23–30 |
| Jack bean | *Canavalia ensiformis* | 3.4–8 | 49–v190 | 22.2–33.9 | 1.2–5.7 | 11.1–56.2 | 16.4–25.8 | 2.4–6.3 | 1.1–2.0 | 24–33 | 9–17 | 15.7–106 | 13–62 |
| Canavalia | *Canavalia brasiliensis* | 3–6.5 | 142–173 | 22.7–27.1 | 1.1–1.5 | 15.8–18.1 | 2.0–15.9 | 1.6–2.1 | – | 25–28 | 4–24 | 17–35 | 12–18 |
| Sunflower | *Helianthus annuus* | 2–12 | – | 10.2–18.0 | 1.5–2.4 | 24.0–27.8 | 15.5 | 6.2 | – | – | 18 | 96 | 31 |
| Pigeonpea | *Cajanus cajan* | 8–15.6 | 37–280 | 13.2–33.5 | 0.9–2.5 | 4.7–28.4 | 5.7–17.9 | 1.9–4.9 | 1.9–2.1 | 22–25 | 5–12 | 26–99 | 15–66 |
| Labe-labe | *Dolichos lab lab* | 5–11 | 66–180 | 13.6–50.0 | 1.3–11.5 | 5.3–27.7 | 11.6–16.5 | 2.7–6.6 | 1.1–3.5 | 26 | 5–10 | 48–143 | 16–33 |
| Leucaena | *Leucaena leucocephala* | 5.5–16 | 400–600 | 31.8–44.3 | 1.7–2.8 | 10.0–19.4 | 6.9–8.6 | 5.0–5.6 | – | – | – | – | – |
| Millet | *Pennisetum glaucum* | 8–15 | – | 3.4–34.0 | 2.0–4.0 | 17.0–35.0 | 2.5–8.0 | 1.5–5.0 | 1.5–3.0 | 7–35 | 5–16 | 27–101 | 24–98 |
| Dwarf velvet bean | *Mucuna deeringiana* | 2–4 | 50–100 | 27.5–35.2 | 1.6–5.3 | 15.7–48.4 | 19.4–23.7 | 4.6–6.5 | 2.6–2.9 | 35 | 9–27.6 | 179–358 | 61–85 |
| Velvet bean | *Mucuna nívea* | 5–8 | 120–210 | 15.6–26.5 | 1.5–5.7 | 10.0–15.5 | 11.0 | 2.7 | – | – | 16 | 183 | 28 |
| Velvet bean | *Mucuna aterrina* | 6–13 | 120–210 | 19.7–30.8 | 1.1–6.1 | 7.8–20.5 | 8.7–12.8 | 2.7–3.5 | 1.2–2.8 | 27 | 15–26 | 133–174 | 10–29 |
| Soybean | *Glycine max* | 2–4 | 60–180 | 13.5–40.0 | 2.1–2.5 | 10.8–17.6 | 10.0–20.0 | 4.0–6.0 | 2.0–2.5 | 21–55 | 10–30 | 20–100 | 20–50 |
| Perennial soybean | *Neonotonia wightii* | 4–6 | 40–100 | 24.4–28.5 | 1.7–3.0 | 22.4–24.5 | 9.9 | 3.5 | – | – | 8 | 102 | 32 |
| Forage sorghum | *Sorghum bicolor* | 10–20 | – | 5.0–11.0 | 1.0–3.0 | 14.0–22.0 | 3.0–4.0 | 2.5–5.0 | 0.1–0.2 | 4–20 | 4–20 | 10–190 | 14–50 |
| | | Preferred sowing in autumn–winter (April to August) | | | | | | | | | | | |
| White oat | *Avena sativa* | 2.5–7.0 | – | 8.1 | 0.6 | 24.0 | 2.4 | 1.7 | 1.5–4.0 | 5–20 | 6 | 138 | 9 |
| Black oat | *Avena strigosa* | 2.5–11 | – | 7.0–16.8 | 1.0–4.2 | 10.8–30.8 | 2.5–3.6 | 1.7–2.0 | – | 21–22 | 5–7 | 41–102 | 11–22 |
| Ryegrass | *Lollium multiflorum* | 2–6 | – | 11.6–13.4 | 0.7–1.0 | 21.2–26.0 | 4.1–4.4 | 2.2 | – | – | 9 | 214 | 23 |
| Rye | *Secale cereale* | 2–8 | – | 5.8–12.2 | 0.8–2.9 | 7.5–14.5 | 1.8 | 1.4 | 1.5–5.0 | 5–20 | 5–25 | 14–150 | 15–70 |
| Grasspea | *Lathyrus sativus* | 2–6 | 80 | 22.0–32.5 | 1.0–2.6 | 29.0–30.0 | 3.9–7.9 | 1.9–4.3 | – | – | 11–29 | 52–70 | 11–22 |
| Pea | *Psium sativum* | 2.5–7 | 60–90 | 20.9–28.9 | 1.2–2.0 | 15.0–21.7 | 7.0–8.5 | 2.0–3.2 | – | – | 22 | 102 | 8 |
| Vetch | *Vicia sativa* | 2–10 | 90–180 | 2.0–46 | 1.3–3.8 | 21.0–25.6 | 8.6–10.5 | 2.7 | – | – | 9–24 | 69–87 | 22–30 |
| Forage turnip | *Raphanus sativus* | 2–9 | – | 9.2–29.6 | 1.8–3.3 | 20.2–49.0 | 10.0–21.5 | 2.3–9.5 | 4.5–5.2 | 22–23 | 8–15 | 18–84 | 14–49 |
| Lupine | *Lupinus albus* | 2–5 | 128–268 | 12.2–19.7 | 0.9–2.9 | 10.0–26.6 | 4.6–5.9 | 3.9 | – | – | 12 | 330 | 57 |
| Triticale | *Triticosecale wittmack* | 2–8 | – | 13.7 | 1.1 | 25 | 3.8 | 2.7 | – | – | 12 | 53 | 19 |

Source: Wutke E.B., Trani P.E., Ambrosano E.J., Drugowich M.I. Adubação verde no Estado de São Paulo. Campinas: Coordenadoria de Assistência Técnica Integral-CATI; 2009. 89 p.

**Table 1.** Dry matter yield, amount of fixed nitrogen, and mineral composition of macro- and micronutrients in the shoot of species frequently used as green manure and cover crops in Southeastern Brazil.

The first thing to consider in the process of choosing a green manure is the purpose of the farmer in using it. When the farmer objective is nutrient supply, plants with high biomass production and capable of associating with N-fixing bacteria can be suggested (**Table 1**). Among these plants are species from the genus *Crotalaria*, *Canavalia*, *Cajanus*, *Leucaena*, and *Acacia*. We recommend inoculation of seeds with *Rhizobium* in order to increase the symbiosis efficiency and consequently the biological nitrogen fixation. On the other hand, when the farmer objective is erosion control, species with higher C/N ratio, fasciculate root system, greater biomass production and lower shoot/root ratio can be suggested, such as some species of genus *Pennisetum* and Brachiaria. (**Table 1**).

Another important point to consider for choosing the correct green manures is the phytosanitary aspect. Some species can control plant pathogens, such as species from the *Crotalaria* genus, which are effective in controlling certain nematode species that cause root-knot [58]. However, others are hosts of pathogen species and pests of commercial crops. An example of this problem is the cultivation of common beans (*Phaseolus vulgaris*) in succession or rotation with jack beans (*Canavalia ensiformis*). This green manure hosts the whitefly (*Bemisia tabaci*), an insect that transmits the bean golden mosaic virus [57]. Therefore, the use of species from different phylogenetic groups is recommended. Moreover, the use of species from different families is convenient, since they have different patterns of nutrient accumulation and root architecture, enabling the exploitation of different soil layers.

Many green manure species are better adapted to the climatic conditions of spring and summer while others to the autumn and winter (**Table 1**). Sunnhep (*Crotalaria* sp.) and pigeonpea (*Cajanus cajan*), for example, have greater biomass and nutrient accumulation when grown between the spring and summer due to their water requirements, photoperiod, and/or thermoperiod [63]. In general, sunnhep and pigeonpea plants require short days to flowering, so they have low biomass production when sown in late summer [63]. These plants should be selected for pre-cultivation management of commercial crop. Other species are more versatile, like the millet (*Pennisetum glaucum*), canavalia (*Canavalia brasiliensis*), and jack bean (*Canavalia ensiformis*) [62], which have little or no sensitivity to photoperiod and good drought tolerance [63]. Such species may have satisfactory dry matter yield in semi-arid conditions; however, they have to be sown, preferably, when there is still moisture in the soil for germination, such as the period after the summer harvest [63]. On the other hand, the genres *Avena*, *Lollium*, *Lathyrus*, and *Vicia* have to be cultivated preferably between the autumn and winter, and some of them are better adapted to subtropical conditions.

Therefore, the success in using the green manures technic depends on its adaptability to the region soil, climate conditions, cultivation system, and on their phytosanitary aspects. Experiments determining which species have greater biomass and nutrient accumulation are recommended when some of this information is unavailable.

### 3.2. Management of green manures in tropical conditions

Green manure plants can be used in minimum tillage, no-till or in systems with plowing and harrowing. These plants can be managed as soil cover in fallow, intercropping with perennial crops or in rotation or succession plans with annual crops. In general, when the plants reach the reproductive stage, when approximately 50% of the flowers are opened, a cut must be carried out with or without the plant matter incorporation into the soil. Some species might have longer cycles, but have to be cut before the seeds become viable. The black oat (*Avena strigosa*), for example, should be cut when grain maturation reaches the milky grain stage [63]. Cutting these plants before this stage allows a regrowth, and after this stage the grains may become viable [63]. Both cases may result in economic losses due to competition between the black oat plants and the main crop [63].

The toppling of plants in conservation systems can be obtained using mechanical or manual mowing, a roll-knife or herbicides for desiccation [62]. In conventional systems, set of harrows are commonly used to incorporate the green manure into the soil or a rotary hoe is used in some cases [62].

**Figure 1.** Coffee seedlings with pigeonpea tipped over between planting lines. Photos by Lucas de Ávila-Silva.

Plants from the Fabaceae family with C/N ratio around 20 (Table 2) are rapidly decomposed in tropical conditions. Therefore, the commercial crop planting in rotation/succession to this type of plant should be performed few days (less than two weeks) after the green manure tipping over and/or incorporation, since much of the N is mineralized in the first 60 days [58]. Adopting species with higher C/N rates, around or above 40, such as species from the Poaceae family, enable to wait longer to the commercial crop planting. A 2-week interval between the grass cutting and the commercial crop planting is recommended, because of the low availability of N and possible allelopathic effects. Regarding the interim crops (**Figure 1**), the use of green manure species with climber habits should be avoided, since they may use commercial species as tutors. A solution to this problem is the use of more than one green manure species, a technique known as cocktail, using species with climbing and erect growth habit, so that the latter will serve as tutor for the first. It is also important an adequate inter-line plant density, in order to avoid competition for growth resources between the green manure and the commercial crop.

A study carried out in Brazil evaluated different green manures in rotation with maize under two managements, minimum or conventional tillage [64]. The green manures used were as follows: *Crotalaria juncea*, *Canavalia ensiformes*, *Rafhanus sativus*, mixed species (cocktail) with *Crotalaria juncea*, *Canavalia ensiformes* and *Rafhanus sativus*, and weeds (control) [64]. The green manures treatments had maize yield higher than the control with weeds, with average of 2.8 t/ha of peeled green ears [64]. Another study, in the same region, evaluated the effect of N topdressing absence, pre-cultivation of *C. juncea* and topdressing with ammonium sulfate or urea on maize yield produced for silage [65]. The use of *C. juncea* incorporated 12 days before the maize planting resulted in a yield increase of 14 t/ha compared with the control treatment without nitrogen topdressing [65]. However, the treatment with green manure did not surpass the treatments with ammonium sulfate or urea topdressing, which increased silage productivity by 33 and 27 t/ha compared to the control without topdressing, respectively [65].

Therefore, in order to maintain a competitive agricultural system, the green manure practice must be managed together with chemical fertilizers. Amado et al., for example, proposed to consider the green manure contribution to define the nitrogen fertilizer dose, which is especially important in no-till and integrative systems. These authors considered the following criteria for setting the dose: the soil organic matter content; the commercial crop expected yield; and the previous cover crop (green manure) residue nature and amount [66].The previous cover crop contribution was considered in three situations: legumes, grasses in monocrop, and consortia (cocktails) [66].They considered that the higher the green manure dry matter yield, higher the N supply to the commercial crop in succession to legumes [66]. The contribution of grasses for the N supply was estimated to be very small or non-existent compared with uncultivated areas and may even reduce the availability of N due to its short term immobilization [66]. Regarding the consortia system (cocktails), the contribution is estimated according to the percentage of legume biomass in total biomass of green manure. In some conditions, fertilizer savings can reach up to 80 kg/ha of N [66].

An example of the green manure potential intercropped with perennials plants is a study with soursop (*Annona muricata*) in southeastern Brazil, in which the author evaluated three green manures species (*Gliricidia sepium*, *Crotalaria juncea*, and *Cajanus cajan*) [67]. The greater amount of biologically fixed nitrogen was found with *G. sepium* (80% of the accumulated N) and with *C. juncea* (64.5% of the accumulated N). During the 2 years of experiment, the *C. juncea* added to the soil, in two cuts, about 149.5 kg/ha of N, from which 96.5 kg/ha came from biological nitrogen fixation (BNF) [67]. The *G. sepium*, managed with three annual pruning, added 113–160 kg/ha of N, with 90–128 kg/ha derived from BNF. Variations in natural $^{15}N$ amounts indicated that green manure with *C. juncea* and *G. sepium* contributed to N supply for the soursop, transferring about 22.5 and 40% of the fixed N, respectively [67]. They conclude that the use of these two green manure legumes contribute as organic fertilizer, supplying nutrients, mainly N [67].

The use of fresh organic matter from green manures brings more than just nutrients, affecting physical-chemical and biological characteristics of the soil. Pegoraro et al. [68] showed the effect of the use of green manure (*Acacia mangium*) grown after the *Eucalyptus* cut in short rotation system (6–7 years of growth). The authors noted that the use of a legume in succession enabled the increased in stocks of total C and N, C and N in humic substances and in microorganisms compared to crops without the legume succession [68]. Vegetable residues with lower C/N ratio accelerate the recycling of residues from commercial crops (ex.: *Eucalyptus*), which reduces the microbial attack on soil organic matter (SOM) and increases the levels of stabilized organic matter [68].

The effect on the soil organic matter supply makes the green manure a promising practice for recovery degraded areas and help to restore the A horizon of "beheaded" soil profile. Alves et al. studied green manures as a component of strategies for recovery degraded areas [69]. Positive effects were noted 365 days after the experiment implementation, such as soil compaction reduction and water infiltration time reduction [69]. Kitamura et al. reported, in another paper concerning the same research, that the treatments also provided results related to the soil macroorganism population [70].

### 3.3. Advantages and disadvantages of green manures

The main advantages and disadvantages of green manures to tropical weathered soils [58, 62, 63] are listed below.

#### *3.3.1. Advantages*

##### *3.3.1.1. Chemical aspects*

1. Nitrogen input to the soil because of the green manure association with nitrogen-fixing bacteria;
2. Green manures with deep root systems allow cycling of nutrients that have been leached to deep layers;
3. Increased cation exchange capacity (CEC) due to an increase in soil organic matter content;
4. Green manures make possible to increase or stabilize the content of soil organic matter;
5. The release of organic acids allows the solubilization of more stable forms of phosphorus.

##### *3.3.1.2. Biological aspects*

1. They favor the microflora and macroflora and fauna through carbon supply;
2. Some species control nematodes population;
3. They can serve for attracting insect pests and stop disease cycles;
4. They release compounds with allelopathic effect on weeds;
5. They compete for growth resources with weeds.

##### *3.3.1.3. Physical aspects*

1. They promote protection against erosion by covering the soil;
2. They enhance stability of aggregates and porosity by adding organic matter and growth and death of roots;
3. They increase water retention by cover the soil and by add organic matter;
4. They allow natural decompression of the soil, when using species with deep root system;
5. They reduce the thermal soil amplitude.

### *3.3.2. Disadvantages*

1. Inadequacy of some green manure species to the production system or the soil and weather conditions;
2. Lack of interest from consultants and farmers in this technology, which adopt immediate postures;
3. Sometimes, green manure involves costs with no direct financial return;
4. Low development of breeding technologies of green manure species;
5. Some green manures can host diseases and pests that attack the commercial crop;
6. Possibility of negative allelopathic effect of green manure residues on the commercial crop;
7. Possibility of competition between green manure plants and the commercial crop by inadequate management of the technology in intercropping systems;
8. Some green manures have incompatible decomposition rates with the nutrient requirements of crops;
9. Uneven seed germination of some species of green manure;
10. Difficulty of obtaining seeds for sowing;
11. Lack of functional decomposition models to predict nutrient release.

## 4. Crop residues

Crop residues can be defined as any part of the plant without direct economic value produced in the field (derived from the harvest) or after processing on the farm. The crop residues value in agriculture coincided with the success of no-till system, until then, soil revolving or even fire was commonly used to accelerate residue decomposition. More recently, the crop-livestock-forestry integrated system is also an example of crop residues conservation. The no-tillage system implementation is based on three pillars: (*i*) no soil tillage, (*ii*) crop rotation, and (*iii*) permanent soil cover. Although essential, the soil cover maintenance in tropical environment is not a simple task, mainly due to the high rate of straw decomposition from leguminous plants, and is difficult to grow plants in succession (second crop) because adversities such as the rainfall patterns.

The maintenance of residues benefits the agricultural system by conserving the soil free from erosion. The homogeneous distribution of residues in the area with great amount of straw generates a slower runoff and less water and soil loss [71], consequently avoiding topsoil losses, which normally has a great amount of nutrients. Another important feature of crop residues is the maintenance of higher water content in the soil, a problematic factor in the tropical agriculture.

The crop residue maintenance also contributes to a decrease in the soil surface temperature, ensuring a better condition for the plants and other soil organisms. Another relevant factor is the ratio between the straw production, weed control, and soil compaction reduction capabilities, which is a recurring problem in poorly managed conservation systems.

The longer the time the crop residues are in the soil surface, the greater the positive effects. The residue permanence time in the soil depends on several factors, such as the: (*i*) fragmentation level, (*ii*) amount, (*iii*) chemical composition, (*iv*) contact level with the soil, (*v*) weather conditions, (*vi*) microbial community, and (*vii*) soil type. In addition to the factors discussed in the first part, it is also known that residues with greater contact surface with the soil accelerate the decomposition rate.

Green manures residues can be choosing from plants with high mass production, chemical composition with high C/N ratio and lignin, favoring the permanence of straw, the use of equipment which allows less fragmentation, such as the roll-knife.

Weeds and green manures (or cover plants) can be managed between rows of perennial crops; in this case, we can also use the roll-knife, although normally brush cutters and grinders are used.

Heavy equipment may be required in some situations, such as the scarifier or harrow, for example, in excessive amount of plant residues, rigid materials, or seeder inefficiency.

The residues management options for residues from commercial crops are less flexible, since the fragmentation is performed according to the harvest type, the amount is dependent on the yield and the material chemical composition is usually consequence of the best-adapted material. The legume residues have high decomposition rates, often cooperating little to the soil cover. The cultivation of legumes is usually performed first to leave a N balance for the sequential culture.

In specific cases of succession after grasses cultivation, an increase in N doses or even an anticipate application of nitrogen fertilizer is suggested before sowing, basically due to the N immobilization because the high C/N ratio of grasses.

After reminding here, the crucial importance of crop residues to the soil cover and the main factors affecting its decomposition, we can discuss a second advantage of residues from commercial crops: the potential supply of nutrients. The management of residues from a preceding crop (annual) or residues from the crop itself (perennial) can complement the nutrient supply.

Regarding the green manure, nutrient mineralization from the crop residues depends on the residue quality, soil moisture and temperature, as well as specific soil factors such as texture, mineralogy and acidity, biological activity, and the presence of other nutrients [72]. Most of the nutrients are exported in the harvest, remaining just a portion in the residues. In this part, we are going to see yield increases by the nutrient supply potential of the residues from the main crops in tropical environments.

### 4.1. Annual crops residues

The nutrient net mineralization from the annual crop residues that precede other crop must be considered in nutritional management. We focus here on annual crops that associate with $N_2$ fixing bacteria, because they are the major contributor to the nutrient supply (nitrogen) for crops in succession or rotation. Subsequently, we will see the most used crops in tropical environments that leave nutrient to the system.

#### 4.1.1. Soybean

The soybean (*Glycine max*) represents 50% of the global area of leguminous crops and 68% of global production of this family. The annual input of N fixed is 16.4 Tg, which represents 77% of N fixed by legume crops [73]. A crucial fact to these crop success was the priority given to association with *Bradyrhizobium* and BNF in breeding programs [74].

Most of the soybean crop in Brazil is carried out in no-tillage system, and this management provides more root nodulation by the bacteria in the soil, best nodulation (nodulation deep in the soil profile), higher rates of BNF and yields compared to conventional tillage [75, 76]. These factors, combined with the efficient association with *Bradyrhizobium*, allow the non-use of nitrogen fertilizers in this crop.

Approximately 80–83 kg of N is required for each 1000 kg of soybeans produced, from which 51–65 kg are allocated in the seeds and 15–32 kg in the roots, stems, and leaves [74, 77]. The $N_2$ fixation potential of the soybean can be as high as 360–450 kg/ha [78, 79].

In southern Brazil, Paraguay, Uruguay, and northern Argentina (subtropical) is recurrent to get higher wheat yields (winter planting) when soybean is the preceding crop in the summer compared to the maize, precisely because of the remainder N [80]. The maize, in rotation with soybeans, may have its nitrogen fertilizer rate reduced by 20% in this environment, considering the effect of rotation after a soybean crop with adequate productivity [81]. In a similar environment, some producers in various states of the Midwestern United States reduce 45 kg/ha of the nitrogen fertilizer dose, when maize is planted following soybeans compared to sequential crops of maize [82].

The Cerrado biome (Brazilian savanna) has no winter crops due to insufficient rainfall, and thus the soybean is the main summer crop and, after the harvest, in late summer, usually maize or sorghum is planted ("second crop"). A contribution of 20 kg/ha of N is assumed as a practical parameter in the region for these crops after soybean [83].

The first part of this chapter showed that the C/N ratio and biochemical constitution of the residues are important indicators to predict the nutrient mineralization rate. Regarding the soybean grown in tropical environments, different C/N ratios are observed in the roots (31.6), stems (22.5) and leaves (10.7), as well as larger amounts of lignin–suberin. These data explain the great decomposition rates of shoot residues in the first 20 days after harvest, with N mineralization, especially from leaf residues, under controlled conditions [84].

Unfortunately, there are few long-term field experiments in typically tropical regions, which hinder an accurate estimate of the decomposition rate of soybean residues and mineralization

**Figure 2.** Residue deposition and homogeneous distribution after the harvest. Photos by Lucas de Ávila-Silva.

of nutrients. A field experiment carried out for 12 years in Brazil (Parana State, Brazil), estimated the residue decomposition percentage according to the time and management, with $y = 93.819^{e-0.0031x}$ ($R^2 = 0.91$) to the no-till and $y = 90.061^{e-0.0054x}$ ($R^2 = 0.92$) to the conventional tillage [85]. The residue decomposition time in that work was autumn–winter, a less rainy period that the summer. Moreover, that is a climate transition region (Cfa subtropical climate in the Köppen classification) with low temperatures and a distinct pluviometric regime compared to Brazilian Midwest (**Figure 2**).

### *4.1.2. Beans*

The genus *Phaseolus* has more than 200 species described [86], but those that have the greatest economic impact are the *Phaseolus vulgaris*, *Phaseolus coccineus*, *Phaseolus lunatus*, *Phaseolus acutifolius*, and *Phaseolus semierectus*, mainly the first. Different from the soybean, common beans have low symbiotic efficiency [87]. The nitrogen fertilizers use is indicated in some situations to achieve good yields, even though they may affect negativity the BNF efficiency. Mean values of 35% and maximum of 70% of N derived from the atmosphere were observed at the plant biomass considering six field experiments in tropical countries and Austria [88]. Some progress is occurring with the best selection of strains, adapted to the conditions of each site [89–92].

Different C/N ratios were observed in stem fractions (79), straw pods (66), and senescent leaves (24) in four varieties of beans [93]. A shorter leaf half-life was observed, although the straw pods also showed low half-life value (both of approximately 70 days). Those authors observed N and P release and half-life following the residue decomposition rate, while the K release was faster (average half-life of 18 days) and showed low difference regarding the material quality. In this case, N and P could be better used by the subsequent crop, while K can be reused by the same crop. The yield of 1350 kg/ha presented a potential for cycling 31.5 kg/ha N and 2.37 kg/ha P from the bean residues [93].

The species *Vigna mungo*, *Vigna radiata*, and *Vigna unguiculata* stand out in the *Vigna* genus, the latter being the most cultivated. These species have short cycle, low water requirement, and good development in low fertility soils; in addition, the BNF is capable of supplying more than 100 kg/ha of N [94, 95]. An increase in millet productivity of 9–24% was observed in an experiment carried out in three different locations in Niger, when cowpea was previous cultivated compared to successive cultivation of millet [96].

A C/N ratio of 15.8 was found in cowpea residues grown in a field experiment evaluating N mineralization depending on the phosphorus content in tissues [97]. In the same experiment, the authors found an increasing in N mineralization (25, 32, and 34%) with increasing P concentration in tissues (1.0, 1.2, and 2 g/kg, respectively) in 8 weeks. This fact demonstrates the close relationship between nutrients interfering in the mineralization process, emphasizing the importance of fertilization management together with residues management. A potential mineralization from 6.8 to 9.2 g of N per kilogram of dry matter of cowpea residues is considered as a practical reference.

### *4.1.3. Groundnut (peanut)*

India is largest producer of peanuts (*Arachis hypogaea*), which usually uses 10–20 kg/ha of N from ammonium sulfate. A large number of farmers in that country use crop rotation with groundnut to take advantage of its ability to improve soil fertility and increase the subsequent crop yield, due to the BNF [98].

The peanut cultivation in Brazil is also carried out with the same goal, but most in rotation with sugarcane. A C/N ratio of 15 and 24, and an addition of 70 and 38% of N from BNF in plant tissues, was, respectively, observed in the IAC-Caiapó and IAC-Tatu varieties, in acid soils [99]. These authors emphasized that the N values from BNF for IAC-Tatu were low because the sampling, which was performed 120 days after sowing, when the plant was in an advanced stage of pods maturation and much of the N had translocated to the grains. About 90% of N was from the shoot in the average for these cultivars. In this same experiment, there was a yield increase of 12.2 (IAC-Caiapó) and 15.5 t/ha (IAC-Tatu) in the sugarcane crop compared with the control. Bagayoko et al. [96] also reported an increase of approximately 39% in average yield of sorghum after groundnut cultivation compared to subsequent sorghum crops; during 3 years of experiment in Kouaré (Burkina Faso), they found N mineral increment available for sorghum.

The groundnut may also be infected by arbuscular mycorrhizal fungi [99], which favors the next culture infection [96] and improves the P cycling.

#### 4.1.4. Chickpea

The chickpea (*Cicer arietinum*) is the second more cultivated legume in the world to obtain grains. Most of the chickpeas production and consumption occur in developing countries (95%). A BNF evaluation by the $^{15}N$ natural abundance method in different areas of Punjab showed that 58–86% of N (an average of 78%) could be derived from symbiosis, fixing 87–186 kg/ha of N. The average balance of N left by chickpea was 28 kg/ha N in that experiment, the yield ranged from 0.6 to 2.0 t/ha and the N in the soil increased in 38%, on average. The yield increase in the wheat planted in the sequence ranged from 19 to 73%, even with shoot residues removed [100]. Around 90 kg/ha of N fixed was found in a cultivation field in Australia using similar methods [101]. Turpin et al. [102] suggest that chickpeas can fix 146–214 kg/ha of N and contribute with 80–135 kg/ha of N, including the roots.

The ICRISAT work in Kenya reported that chickpea residues contributed from 30 to 35 kg/ha of N to the subsequent wheat crop [103], while the TSBF reported contributions of about 40 kg/ha of N for maize [104].

#### 4.1.5. Difficulties

The absorption and mineralization must be synchronized for an effective N supply by a leguminous species [105]. According Palm, organic residues release about 80% of their nutrients during decomposition, but <20% is absorbed by the crops, often because the lack of synchronization between the release and absorption [106]. There are several studies reporting the decomposition rate and mineralization depending on soil and climatic characteristics, but there is still a lack of field data in some major producing regions and data to create models to predict the nutrients mineralization and availability.

The contribution of N mineralization arising from the root system is noteworthy, for example, Evans et al. [107] and Larson et al. [108] reported negative contribution of N to some tropical legumes when considering only the N from the shoot, with different results found when they accounted the N contained in the nodulated roots. Another factors to consider in the experiments are the biochemical characteristics and C/N ratios of the residues. We must pay attention to the fact that how higher residue decomposition rate, lower will be the vegetation cover time.

### 4.2. Perennial crop residues

Different from annual crops, nutrients from perennial crop residues are normally used in the same culture, but similarly, they take advantage of nutrients not exported at harvest. A perennial crops peculiarity is the use of residues from processing in the farm. Once seen as an environmental impasse, such residues are currently good sources of nutrients.

#### 4.2.1. Sugarcane

The estimated BNF in sugarcane may reach 0.5 Tg per year [109]. Although the N added by BNF generate savings for the system, the straw C/N ratio is around 97–149 [110, 111] and the stem around 118 [112], promoting an initial immobilization of N. Straw is the main residue left in field, which has high C/N ratio and high lignin content, about 21% [111]. It is estimated that each ton of sugarcane produces 150 kg of sugar, 140 kg of dry bagasse and 140 kg dry matter of straw [113], approximately 17 t/ha of straw [110].

In an experiment with $^{15}N$ labeled straw, Gava et al. [110] found that the N use efficiency by ratoon cane was 9% (68 kg/ha N) and that the main contribution of N from straw was to maintain or increase the organic N in the soil. Furthermore, this N became available in the second half of the cycle.

Another effective contribution of the sector is the vinasse return to the crops. Each liter of ethanol produced in distillery produces between 12 and 14 L of vinasse, which has 0.5–1.0% of soluble carbon and high levels of K (typically 12 g/L), and contain considerable amounts of other nutrients [114]. Gava et al. [110] used vinasse with the following contents (kg/m$^3$): 0.41 of N, 0.07 of $P_2O_5$, 2.72 of $K_2O$, 0.91 of CaO, 0.38 of MgO, and pH (water) of 4.9. Typically, the dose applied is around 80–100 m$^3$/ha.

Resende et al. observed a sugarcane yield increase of 12–13% after application of vinasse in a period of 16 years [115]. However, in some situations, vinasse is recurrently applied near of the distillery due to the cost of transportation, occurring concentrated applications with ground water salinization and/or contamination potential.

The filter cake is also a residue from the sugarcane processing used in the field. The sugarcane processing in Thailand produces 3.4% of filter cake and 25–30% of bagasse from the sugarcane fresh material [116]. Each ton of sugarcane crushed generates around 40 kg of filter cake [117], which has variable composition, but with high levels of organic matter (OM), P, N, Ca, substantial amounts of K and Mg [118], as well as Fe, Mn, Zn, and Cu [119].

Two works illustrate the variability of nutrient content in the filter cake. In Brazil, Fravet et al. [120] found pH of 4.5, C/N ratio of 20.9, C/P ratio of 17.65, OM of 20.1%, humidity of 71.4%; Ca of 2.43%, Mg of 0.26%, S of 0.39%, P ($H_2O$) of 0.33%, P (CNA + $H_2O$) of 0.40%, P (citric acid) of 0.40%; $P_2O_5$ (total) of 0.98% and K of 0.25%. In Thailand, Meunchang et al. [116] found pH (water 1:5) of 7.7, C/N ratio of 14, OM of 48%, P (total) of 0.96%, K of 0.39%, Ca of 7.1%, Mg of 0.4%, Cu of 1.9 mg/kg, Zn of 51 mg/kg, Mn of 257 mg/kg, and Fe of 803 mg/kg.

The filter cake can be applied in the entire area at pre-planting in the furrow or planting lines. According to Nunes Júnior [118], 20 t/ha of filter cake on wet base or 5 t/ha on dry base can provide up to 100% of the N required by the plants and 50% of the P, 15% of K, 100% of Ca, and 50% of Mg. In ratoon cane, the application of 70 t/ha of filter cake provided the greatest productivity of sugarcane stalks, regardless of application mode [120].

### *4.2.2. Coffee*

Coffee is the second most traded commodity in the world, behind oil. The coffee husk is a common residue of the processing on farms, accounting for about 50% of the dry fruit harvested [121]. This residue can return to coffee plants for nutrients release, providing around 29 g/kg of N (mostly in the form of nitrate) and 45 g/kg of K [122].Values of 23 g/kg of K-total (7.4 g/kg K-soluble), 14.8 g/kg of N, C/N ratio of 30, and 21 g/kg of lignin have also been found in coffee husks naturally dry. The K release from husks is high (above 90%) and regardless of the coffee bean constitution, decomposition rate, or processing type [123].

Another processing variation is the pulping done by the wet method, which withdraws just the husks, and the pulp and grain are placed to dry along with the parchment. Afterward, the parchment is removed from the coffee bean, which constitutes 12% of the dry fruit harvested. The pulp and parchment have 3.65 and 0.38% of K and 1.85 and 0.59% of N, respectively [124]. The C/N ratio in the pulp is around 24 while in the parchment is 63. In the husk in the same processing, 38.9 g/kg K-total (17.7 g/kg K-soluble), 26.7 g/kg of N, C/N ratio of 16 and 20.9 g/kg of lignin were found [123].

The variation in nutrient content within the same post-harvest processing must be taking into account, but mainly when they are from different processing (**Figure 3**). Composting is also a good option for husk use [125, 126].

**Figure 3.** Coffee pods stack after processing. This residue returns to farming by providing mainly K and N. Photo by Lucas de Ávila-Silva.

### 4.2.3. *Eucalyptus*

Eucalyptus crops with ever shorter cycles (6–7 years) are extremely dependent on the biogeochemical cycling of nutrients from the residues that are added to the soil over the cycle and also at harvest. A eucalypt forest produces an average of 75% of commercial wood, 1.5–3% of leaves, 4–6% of branches, 6–19% of barks, and 10–12% of roots, considering the total biomass, after 7 years [127].

Studies have shown that eucalyptus planted forests can deposit 7–84 tons of dry matter to the soil during 7 years, from old dead branches and dry fruits (25–30%), barks (10–15%) and leaves (55–65%) [128].

The treetops begin to close between the 1st and 2nd year after planting, and the competition causes the disposal of branches and lower leaves that are gradually deposited on the ground. The trees are taller and with small treetops from the 3rd to the 4th year, occurring the deposition of barks. However, the quantities of residue deposited to the ground depend on the eucalyptus species, climate, and evapotranspiration. The components of the eucalyptus forest deposited to the ground are called litterfall, which has great influence on the nutrients availability to the eucalypt [127].

Thus, considering that the estimated content of nutrients accumulated (in relation to the total accumulated in the plant) in the tree tops and eucalyptus bark after 6.5 years has on average 65% of N, 70% of P, 64% of K, 79% of Ca, and 79% of Mg [128], considerable amounts of nutrients are deposited to the ground and are considered in the fertilization management. In addition, the harvest of trees leaves in the area large amounts of residues such as leaves, branches, tree tops, and small trees discarded during harvest. The trees can be pruning and strips in the area or at companies, depending on the harvesting modules used. Some companies separate the so-called woody debris (thick branches, tree tops and small trees) and sell as wood or transformed into wood chips to produce biomass fuel for the company itself, depending on the demand.

However, we must emphasize here the importance of the retention of crop residues in the area because during the harvest the accumulated litterfall on the soil surface has order values of 8–14 t/ha [129, 130]. Studies have been shown that lower amounts of nutrients are required in fertilization when the bark is left in the field at harvest [131–133]. Moreover, the roots also remain in the area, since that the currently practice of stump removal is increasingly scarce due to the large impact on the soil.

The crop residues have nutrient availability potential remaining in the area and can reduce the impact on the soil due to the heavy-machinery used [134, 135]. This is an important fact, since forestry operations can alter the physical and mechanical properties of the soil [136], increasing soil compaction. The productivity of eucalyptus forests may reduce with increasing soil compaction levels [137, 138], due to: (*i*) physical obstruction of developing roots; (*ii*) lower water and nutrients absorption; (*iii*) gas exchange reduction.

Many factors influence the residue decomposition rates in the soil, with later nutrients availability for plants as we mentioned in the item 2. Generally, the leaves have faster biodegradation, since they have C/N ratios of 25–45 and C/P of 250–300, while the branches and trunk have C/N of 350–500 and C/P of 500–700 and the barks have C/N of 150–250 and C/P of 300–450 [127]. However, the nutrients allocated in the residues will not be fully available for eucalyptus plants; thus, many companies conduct tests in their crop areas trying to estimate the decomposition rates of the plant compartments and the recovery rates of applied fertilizers. Many companies use these data to establish the complementary fertilization management, seeking to minimize costs and make a more sustainable system.

Works describing the decomposition rates of different compartments in different environments can be found in the literature [139–143], which can be used for fertilization estimates when perform the tests is not possible. Some modeling programs such as the Nutricalc-UFV, allows us to estimate the different rates that will assist in the fertilization management. The residues have great importance on the nutrient balance of a system, and we cannot ignore the benefits of the construction and permanence of soil organic matter.

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Chapter 4

# Use of Organic Fertilizers to Enhance Soil Fertility, Plant Growth, and Yield in a Tropical Environment

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Additional information is available at the end of the chapter

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## Abstract

Soils rarely have sufficient nutrient for crops to reach their potential yield. Applying organic fertilizers without prior knowledge of their properties may cause yield decline under low application or pollute the environment with excessive application. Understanding the nutrient variability and release pattern of organic fertilizers is crucial to supply plants with sufficient nutrients to achieve optimum productivity, while also rebuilding soil fertility and ensuring protection of environmental and natural resources. This chapter presents the authors' experiences with different organic amendments under Hawaii's tropical conditions, rather than an intensive literature review. For meat and bone meal by-products (tankage), batch-to-batch variability, nutrient content/ release pattern and quality, and plant growth response to the liquid fertilizer produced from tankage were evaluated. For animal livestock, dairy manure (DM) and chicken manure (CM) quality, changes in soil properties, and crop biomass production and root distributions were evaluated. For seaweed, an established bio-security protocol, nutrient, especially potassium (K) variability, and plant growth and yield response were evaluated in different tropical soils.

**Keywords:** organic fertilizers, tropical soils, nutrient variability, mineralization, plant growth, yield

## 1. Introduction

Sustainable and organic agriculture practices apply management ideals that include a diverse assembly of farming methods, usually with a reduced reliance on purchased inputs [1], this is especially for new farmers with limited resources [2]. As commercial fertilizer/shipping costs increase, a wide range of food producers in the Hawaii and the Pacific region have realized the need for locally available fertilizers from organic sources to improve soil fertility, crop health, and productivity. In addition to concerns surrounding availability of affordable soil amendments, interest in sustainability and organically produced crops has risen among American consumers in the past few decades. Increased tourism has further amplified the need for fresh local fruits and vegetables, especially "locally grown" labeled goods. Shifting from conventional farming to organic farming has many benefits to the human's well-being, protecting the environment (soil, water, and air), rebuilding soil fertility through improving its physical, chemical, and biological characteristics, and improving the quality of produced crops [3]. However, producing crops organically may come with higher production costs (i.e., lower yield and higher labor costs). Recycling, composting, and using local inputs may decrease the production cost [4]. In general, soils rarely have sufficient nutrients available for crops to reach their potential yield. Therefore, farmers tend to apply soil amendments (synthetic or organic amendments) that are rich in nutrient, i.e., N, P, and K to enhance soil fertility and increase crop productivity [5]. However, most growers apply fertilizers based on the general recommendations for each crop [6], without prior knowledge of the soil fertility status and nutrient mineralization and release pattern from the fertilizers [7]. In addition, Hawaii farmers face the continuous challenge of declining soil organic matter (SOM) and fertility [8] due to the optimum environmental condition (e.g., temperature and rainfall) for SOM decomposition [9]. These losses are more critical with the use of organic amendments, where nutrients have to be converted from organic to inorganic forms in order to be available for plant uptake [10]. Also, rebuilding/restoring soil fertility and improving the physical, chemical, and biological function of soils are critical to support optimal plant growth, yield, and quality [11]. Sustainable health of the soil relies on carbon-rich amendments that will feed the biological processes that are the core foundation of a healthy soil [12]. Short-term needs must also be met with fertilizers that rapidly become available to plants, so that nutrients are available in synchrony with plant needs [13]. In Hawaii, there are many locally available resources to meet both long- and short-term crop nutrient and soil function needs when used properly [14]. Improving farmers' knowledge and their capacity to determine the quality of different fertilizers and soil and crop's needs are essential elements in organic agriculture [15]. This chapter focuses on the authors' experiences with certain organic fertilizers that are available in Hawaii rather than being an extensive review of them.

## 2. Meat and bone meal by-products (tankage)

Tankage is the solid by-product of animal waste rendering (**Figure 1**). The nutrient content of tankage varies with feedstock and storage time, but the product available in Hawaii has been fairly consistent on average 9.5, 2.5, and 0.75% of N, P, and K, respectively, With a Carbon/Nitrogen (C/N) ratio of 5:1 [16, 17]. The Hawaii material is derived from fish scraps (~50%), waste meat, carcasses, and other mixed materials (~45%) and offal (~5%). The current and only running plant in Hawaii is producing about 25 tons/month. Often called meat and bone meal, tankage is a valuable agricultural input used as fertilizer in Hawaii for at least 20 years [18, 19]. The material is National Organic Program (NOP) compliant and listed as an approved generic material by OMRI. The primary agricultural use of tankage is as a supplemental N source [20], especially for, but not limited to, certified organic growers. Nitrogen (N) mineralization rates for tankage have always been assumed to be high given its low C/N ratio and high N content, but actual mineralization rates in Hawaii soils have not been readily available. Other gaps in our knowledge of this material include batch-to-batch variability in the material and N loss during storage.

**Figure 1.** Meat and bone meal by-products (tankage).

## 2.1. Nutrient content and nitrogen release pattern

### *2.1.1. Nutrient content variability among tankage batches*

Batch-to-batch evaluation was carried out for 2 years by collecting tankage samples (every 3 months) from Island Commodities Co., on Oahu. Initial subsamples were submitted to the University of Hawaii's Agricultural Diagnostic Services Center (ADSC) for total nutrient content analysis. The results showed that tankage can provide fairly good amount of the macro- and micro-nutrient, except potassium (K), which was fairly low. N content in tankage initial samples varied between 8.7% and 12.1% with an average of 9.8%, and C/N ratio varied between 3.5 and 5.3:1 with an average of 4.7:1 (**Table 1**). Periodical analysis of N content in the stored initial tankage samples under lab condition showed a significant continuous decline in N content from 10% to 30% of the initial N (date not presented). N volatilization in the form of ammonia ($NH_3$) is a major source of N loss to the atmosphere. Soil acidification is caused by an increase in $H^+$ resulting from the deposition of the $NH_3$ into the soil. Under field/farm condition (higher temperature, humidity, rainfall, etc.), N loss is expected to be higher and faster because climate is the major factor leading to increased N loss [21].

| **Collection date** | % | | | | | | | | μg/g | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **C** | **C/N** | **P** | **K** | **Ca** | **Mg** | **Na** | **Fe** | **Mn** | **Zn** | **Cu** | **B** |
| May 2012 | 10.6 | 45.57 | 4.3 | 3.21 | 0.92 | 6.06 | 0.18 | 0.78 | 1069 | 12 | 97 | 4 | 5 |
| Aug 2012 | 10.3 | 45.50 | 4.4 | 3.54 | 0.83 | 6.22 | 0.17 | 0.77 | 788 | 9 | 96 | 4 | 2 |
| Nov 2012 | 12.1 | 42.26 | 3.5 | 3.22 | 1.07 | 5.81 | 0.19 | 0.92 | 662 | 12 | 106 | 4 | 4 |
| Feb 2013 | 9.8 | 47.10 | 4.7 | 3.50 | 0.73 | 6.18 | 0.17 | 0.72 | 730 | 8 | 91 | 5 | 3 |
| May 2013 | 8.7 | 45.93 | 5.1 | 3.16 | 0.74 | 5.70 | 0.17 | 0.65 | 745 | 10 | 85 | 1 | 3 |
| Aug 2013 | 8.9 | 46.81 | 5.3 | 3.23 | 0.83 | 5.88 | 0.18 | 0.83 | 728 | 8 | 91 | 3 | 4 |
| Nov 2013 | 8.8 | 46.13 | 5.3 | 3.07 | 0.81 | 5.55 | 0.17 | 0.72 | 738 | 10 | 85 | 1 | 3 |
| Feb 2014 | 9.3 | 45.81 | 4.9 | 3.09 | 0.81 | 5.43 | 0.17 | 0.69 | 667 | 9 | 75 | 2 | 3 |
| May 2014 | 9.4 | 45.93 | 4.9 | 3.48 | 0.85 | 6.05 | 0.17 | 0.72 | 725 | 9 | 88 | 4 | 3 |
| **Mean** | **9.8** | **45.6** | **4.7** | **3.27** | **0.84** | **5.87** | **0.17** | **0.75** | **761** | **9.7** | **90** | **3.1** | **3.3** |
| **SD** | **1.09** | **1.38** | **0.58** | **0.18** | **0.10** | **0.28** | **0.01** | **0.08** | **121** | **1.5** | **8.8** | **1.5** | **0.8** |
| **CV** | **0.11** | **0.03** | **0.12** | **0.06** | **0.12** | **0.05** | **0.04** | **0.11** | **0.16** | **0.16** | **0.1** | **0.5** | **0.2** |

* Each value is a mean of three replicates.

**Table 1.** Tankage macro- and micro-nutrient content (μg/g), C/N ratio, standard deviation, and CV of samples collected over a 2-year period.

### *2.1.2. Nitrogen release pattern*

To determine the N release pattern from tankage, a leachate column incubation experiment was conducted using tankage applied at four application rates (0, 100, 200, and 400 kg N/ha) with two soils [Wahiawa series (Oxisol) and Waialua series (Mollisol)] with three replicates for each application rate. A total of 24 PVC leachate columns (30 cm long and 10 cm diameter) were used. The columns were set up from top to bottom with 10 cm soil and tankage mixed layer, 15 cm soil layer, 2 cm gravel layer, and plastic fine mesh to prevent soil passing through. Incubation started with adding half-pore volume of deionized water for each column. At each collection time (weekly), half-pore volume of deionized water was added, and leachate subsamples were collected with glass beaker up to 3 months. Leachate subsamples were analyzed for nitrate ($NO_3$-N) and ammonium ($NH_4$-N) using a Vernier meter and electrodes. Results showed that $NO_3$-N concentration in the leachate solutions followed the application rate (**Figure 2A** and **2B**), and $NH_4$-N concentration in the leachate samples was very negligible (0.1–2.7 ppm). The mineralization rate in a 3-month period was between 50% and 75%. Under field conditions, actual mineralization is expected to be at or above the higher end of this range. The N release pattern under the two soils was the same. However, the $NO_3$-N values were higher under the Oxisol soil (Wahiawa series), which might be related to the fertility level and structural differences between the two soil types [22].

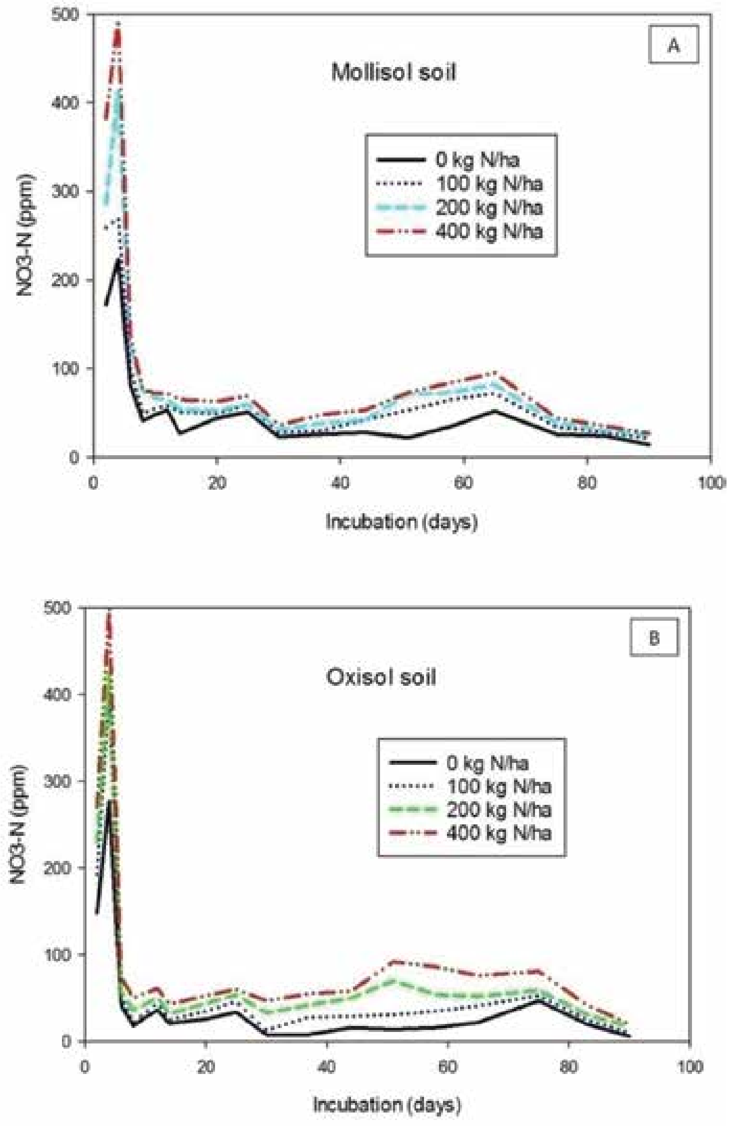

**Figure 2.** $NO_3$-N (ppm) release in a leachate column study from tankage applied at 0, 100, 200, and 400 lbs N/acre over 90-day periods under Mollisol (A) and Oxisol (B) soils.

## 2.2. Liquid fertilizer from tankage for fertigation purposes

Fertigation (fertilizer + irrigation) is a practice when both water and nutrient are supplied together through drip irrigation [23]. The practice is very beneficial for long-term crops, to meet the demand of crops for nutrient, integrated with the use of mulching, and to reduce nutrient losses [24]. Through a Western Sustainable Agriculture Research and Education grant, producing liquid fertilizer with high-N content from tankage was carried out at the University of Hawaii at Manoa (**Figure 3**).

**Figure 3.** Flask contains 1 g tankage and 50 ml water. Each treatment was replicated three times.

### *2.2.1. Factors studied and final recipe*

| Treatment factor | Level |
|---|---|
| Incubation time | 0, 4, 8, 24, and 48 hours |
| Incubation temperature | 24°C and 35°C (75°F and 95°F) |
| Cover/lid | Covered and open/uncovered |
| Inoculants/accelerators | Baking soda, soil, sugar, and vermicompost |

On the basis of the results from the previous study (**Figure 4**), different factors in various combinations were evaluated for the N release from tankage. Based on these results, a suggested recipe was developed for greenhouse and on-farm trials:

- Add 1 kg (2.2 lbs) of tankage into 60 l (~15 gallon) water.
- Add about 40 g (1.5 oz) of vermicompost.
- Air (brew) for 12–24 hours.
- Strain and apply with drip irrigation (fertigation).

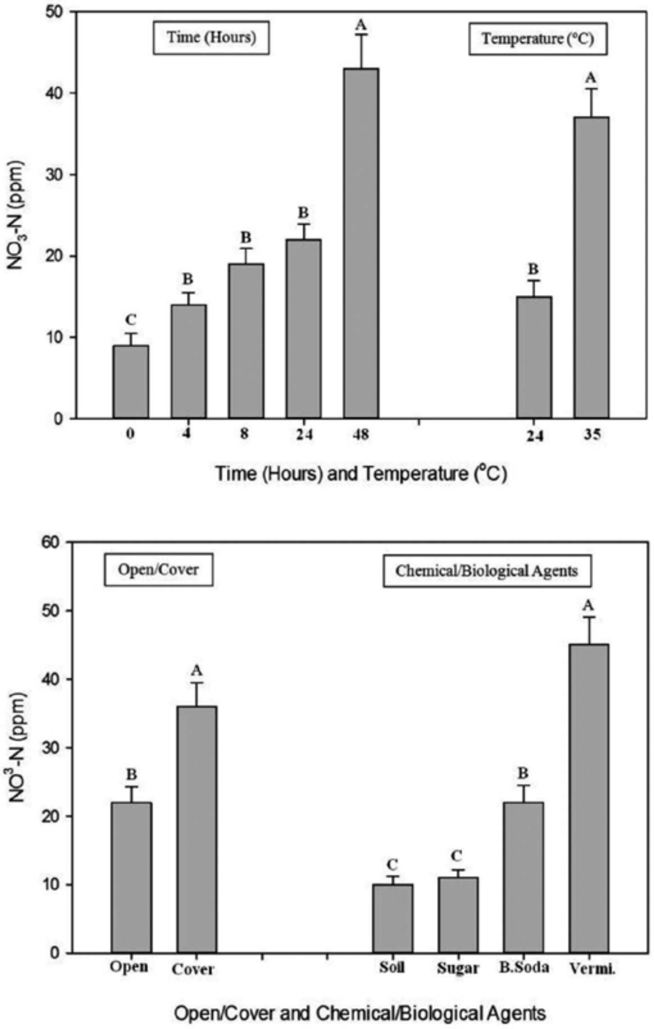

**Figure 4.** Nitrate release (ppm) from tankage under the effect of time (hours); temperature (°C); open or covered conditions, and chemical and biological agents. Bars with different letters, within each factor, are significantly different under 5% probability.

We found that the use of fresh tankage and vermicompost resulted in a higher N concentration in the liquid fertilizer. In addition, the use of thick cotton un-dyed bag to mix the tankage and vermicompost prior to brewing helped significantly reduce the need to strain the liquid fertilizer before fertigation.

### *2.2.2. On-farm and field trials*

The above recipe was provided to a local farmer in Hawaii. The farmer used the recipe to grow watermelon on a 1-acre field with a Oxisol soil (Molokai series). The liquid fertilizer recipe was applied weekly till 2 weeks prior to harvest. The experiment was not fully replicated, but the results were consistent throughout the field. Randomly selected watermelon subsamples were taken, and the average weight and total soluble solid (TSS) contents were taken (**Table 2**). The TSS values were within the excellent range (10.2–13.0) for watermelon [25]. Also, the average weight and watermelon flesh color were representative of the overall crop quality. The yield and high TSS value suggested that the liquid fertilizer provided good amount of nutrient to the watermelon to grow well and accumulate the high-sugar content. As the on-farm field trial was not fully replicated, we conducted a field trial on Oahu Island at Poamoho Research Station on an Oxisol (Wahiawa series) soil. The objectives of the trial were to evaluate the effect of two liquid fertilizers (organic and synthetic) on the yield of different vegetable crops. The experiment was conducted on a 21 × 18 m area for three consecutive harvests of lettuce (*Lactuca sativa*), pak-choi (*Brassica rapa*, Chinensis Group), and daikon (*Raphanus sativus*) in a randomized complete block design (RCBD) with three replicates. Lettuce and pak-choi seedlings were transplanted after 2 weeks of seeding into trays in the greenhouse. Daikon was directly seeded into the field. The liquid fertilizers (tankage- and synthetic-based) were injected into the drip irrigation weekly. To ensure a uniform distribution of the liquid fertilizer, the irrigation water was applied till the drip pipes were completely filled, and then the liquid fertilizers were injected. Lettuce, pak-choi, and daikon were harvested at 4, 5, and 9 weeks, respectively. Leaf chlorophyll content of five randomly selected plants from each replicate was measured weekly using SPAD Minolta 502 m. At harvest, five random plants from each replicate were measured for fresh weight and dry weight (samples were dried at 70°C for 72 hours). The analysis of variance results were consistent throughout the three consecutive harvests, where the results showed a significant effect of the liquid fertilizer treatments on the fresh and dry weights of the harvested crops and leaf chlorophyll content. The fresh and dry weight means from tankage-based liquid fertilizer treatments were significantly higher ($P < 0.01$) than the synthetic-based liquid fertilizer (**Figure 5A** and **5B**). Also, the leaf chlorophyll content values were significantly higher under tankage-based liquid fertilizer than synthetic-based liquid fertilizer (**Figure 6**).

| **Melon** | **Weight (lbs)** | **TSS/sample location** | | | | | **BRIX mean** | |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | | |
| 1 | 24 | 10.2 | 12.0 | 12.2 | 12.3 | 12.1 | **11.8** |  |
| 2 | 19 | 10.8 | 12.0 | 12.2 | 11.8 | 10.8 | **11.5** | |
| 3 | 18 | 11.2 | 13.0 | 13.0 | 13.0 | 12.0 | **12.4** | |
| **Mean** | **20.3** | **10.7** | **12.3** | **12.5** | **12.4** | **11.6** | | |

Data were taken from different locations in each watermelon fruit.

Data were collected by Alton Arakaki, an Extension Agent on Molokai Island.

**Table 2.** Average weight (lbs) and TSS of three watermelon fruit harvested randomly from the Molokai on-farm trial.

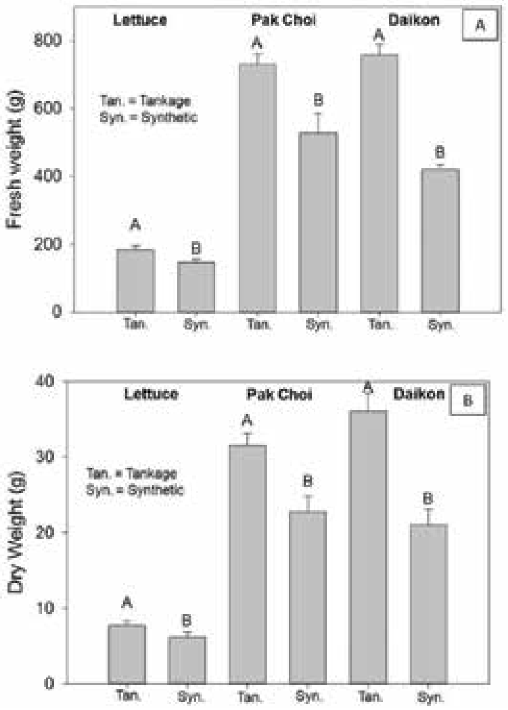

**Figure 5.** Fresh weight (A) and dry weight (B) for lettuce, pak-choi, and daikon under organic (tankage) and synthetic (30–10–10) liquid fertilizer application.

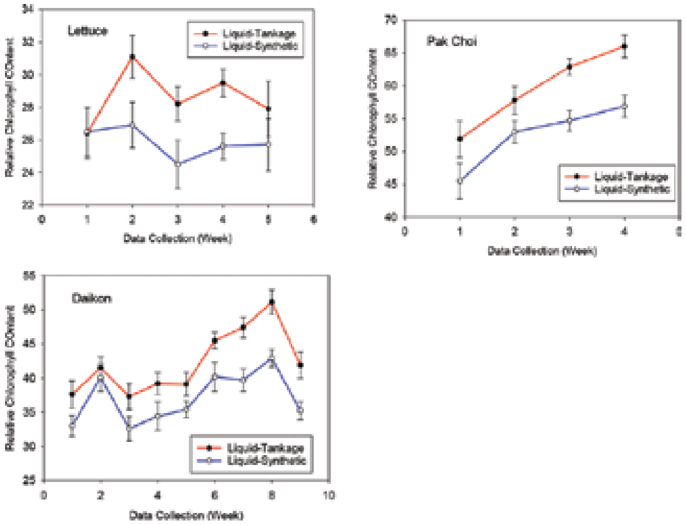

**Figure 6.** Weekly changes in leaf chlorophyll content under liquid-tankage and liquid-synthetic treatments for lettuce, pak-choi, and daikon crops, respectively, using Minolta Chlorophyll Meter.

## 3. Livestock manure

Using livestock on small-scale farms is beneficial for supplying small family needs for milk, eggs, meat, and other goods/products. Also, it can be a good source of organic fertilizer [26]. For example, on average, a 1000-pound cow may produce about 15 tons of manure annually. This 15-ton may contain about: 200 lbs of N, 190 lbs of phosphorus ($P_2O_5$), and 250 lbs of potassium ($K_2O$). Also, dairy manure (DM) contains the essential micro-nutrients [calcium (Ca), magnesium, sulfur, manganese, copper, zinc, chlorine, boron, iron, and molybdenum] [27]. Another example is chicken manure (CM), which contains all of the essential nutrients needed for healthy plant growth [28]. These include N, phosphorous, K, Ca, magnesium, sulfur, manganese, copper, zinc, chlorine, boron, iron, and molybdenum. Nutrient content and percentages vary based on the feed, supplement, medications, and water consumed by the animals. CM is known to provide a good portion, if not all of the nutrients required by plants [29]. Livestock manure is commonly applied in irrigated agriculture to improve soil fertility and crop yields [30, 31] and to improve the soil biology [32]. Soil physical properties, for example, bulk density and total soil porosity may change with agricultural management practices [33]. Manure amendments increase SOM, which may decrease soil bulk density and increase porosity of the amended soil [34]. Animal manures need to be well composted before application to benefit both soil and plants [6]. However, $NO_3$-N leaching can be a problem in organic and conventional farming. Under aerobic soil condition and with heavy application of manure, organically bound N is rapidly converted biologically into $NO_3$-N and that is highly leachable in soils or runoff and can lead to environmental and health issues. In a field study, we used CM applied to a Mollisol soil (Waialua series) under sweet corn crop, at a high-application rate (30 ton/ha); the $NO_3$-N concentration in soil water leached below the root zone

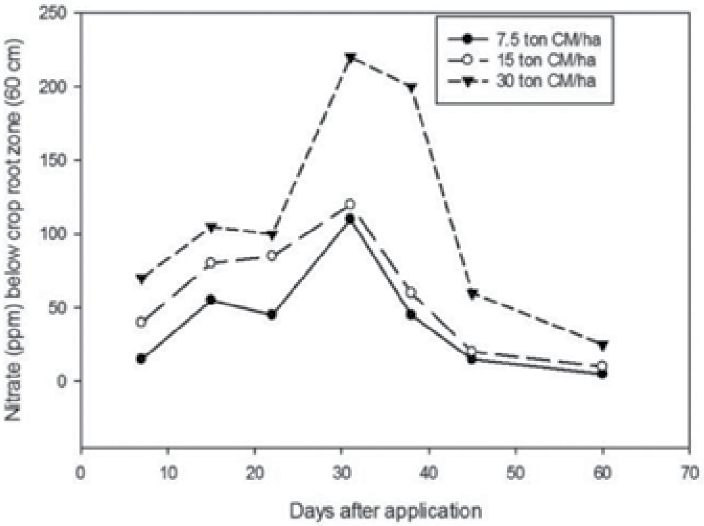

**Figure 7.** Nitrate (ppm) below the root zone of sweet corn under different application rates (ton/ha) of chicken manure (CM) applied to Mollisol soil (Waialua series) in Hawaii.

of corn was very high and could lead to potential groundwater contamination (**Figure 7**). Moderate applications (7.5 and 15 ton/ha) and timing of manure application to meet plant needs may reduce the environmental pollution risks [35].

## 3.1. Livestock manure effects on soil physical properties and root distribution

### *3.1.1. Soil physical properties*

Most commonly available animal manures in Hawaii are CM and DM. Macro- and micronutrient content and C/N ratio for the CM and DM used for field trials on Oahu, Hawaii, are presented in **Table 3**. Under the Wahiawa series soil using CM and DM applied at four application rates (0, 165, 335, and 670 kg N/ha), soil bulk density ($\rho_b$) and total porosity ($\theta_t$) were measured using soil core samples. Soil bulk density significantly decreased with increased manure type ($P < 0.01$) and application rate ($P < 0.05$). Soil bulk density values for manure type and application rate were significantly different from the control treatment (**Figure 8A**). The soil bulk density was lowest under CM/high-application rate (670 kg N/ha) as compared to all other treatments. Bulk density decreased by 4%, 8%, and 9% for the low, medium, and high application rates, respectively, compared to control. Total soil porosity significantly ($P < 0.05$) increased with manure type and application rate. Soil porosity increased by 3%, 5%, and 10% for low, medium, and high application rates, respectively, compared to control. However, there was no significant difference between manure type and application rate (**Figure 8B**). The changes in soil physical properties could be related to the increase in SOM depending upon the animal manure type and application rate, and as Ref. [36] suggested the organic matter (animal manure) application can have different effects on soil properties by adding "less dense" material or by changing soil aggregate.

| **Manure type** | **%** | | | | | | | **µg/g** | | | | | **C/N ratio** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **C** | **P** | **K** | **Ca** | **Mg** | **Na** | **Fe** | **Mn** | **Zn** | **Cu** | **B** | |
| **Chicken** | 3.0 | 21.5 | 1.5 | 1.9 | 14.0 | 0.7 | 0.4 | 209 | 967 | 397 | 43 | 30 | 7.1 |
| **Dairy** | 1.8 | 15.0 | 0.5 | 1.8 | 2.0 | 1.0 | 0.5 | 1317 | 330 | 123 | 191 | 44 | 8.2 |

**Table 3.** Macro- and micro-nutrient and C/N ratio in chicken and dairy manure used in the field trials on Oahu, Hawaii.

Changes in soil bulk density and total porosity under chicken manure (CM) and dairy manure (DM) applied at 0 (Con), 165 (L), 335 (M), and 670 (H) kg N/ha. Means followed with different letters are significantly different at 5% probability based on Duncan's multiple test.

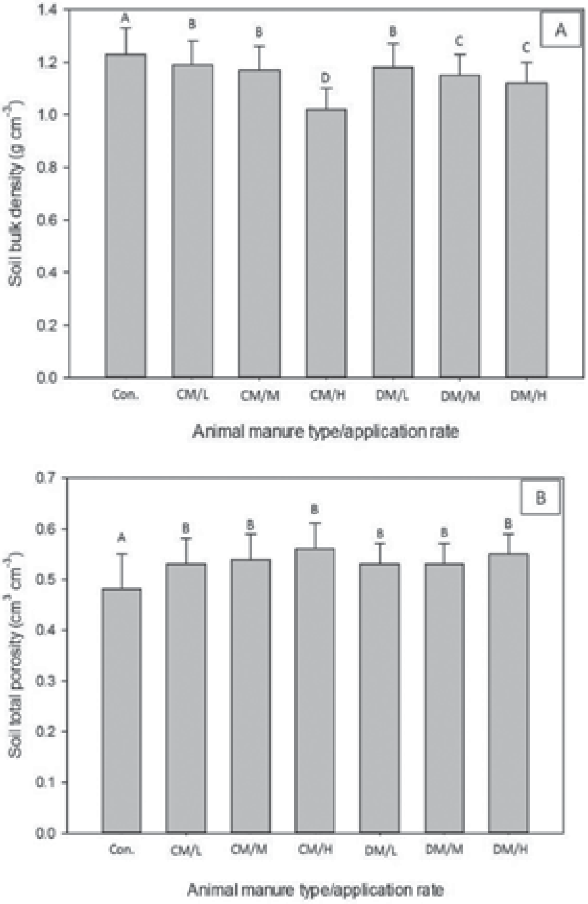

**Figure 8.** Changes in soil bulk density and total porosity under chicken manure (CM) and dairy manure (DM) applied at 0 (Con), 165 (L), 335 (M), and 670 (H) kg N/ha. Means followed with different letters are significantly different at 5% probability based on Duncan's multiple test.

### 3.1.2. *Sweet corn root distribution*

In the same field study above, sweet corn (*Zea mays* L. subsp. *mays*) percent roots at three depths (0–15, 15–30, and 30–45 cm depth) were evaluated for same animal manure types and application rates. The study included collecting soil cores from three locations, total of nine soil cores/plant, around the plant stem of five randomly selected plants in plots that measured 2 × 9 m each. The roots were collected from each soil core manually. As no visible roots were found in the cores collected from 30 to 45 cm depth, the results were considered for the top two depths (0–15 and 15–30 cm) only. The root percentage at the 0–15 cm depth under the CM and DM treatments was significantly ($P < 0.05$) higher than the control treatment (**Figure 9A**). However, the root percentage at the 15–30 cm depth showed the opposite pattern and root percentage under the CM and DM treatments was significantly ($P < 0.05$) lower than the control treatment (**Figure 10B**). The animal manure application rate increased significantly ($P < 0.05$) the root percentage at the top (0–15 cm depth) layer as compared to control treatment. The highest root percentage was for 670 kg N/ha (high) application rate, and the lowest mean was the control treatment. However, the reversed results were obtained from the lower (15–30 cm depth) layer, where the highest root percentage was the control treatment (**Figure 9B**). The increase in root biomass at 15–30 cm depth under the control treatment might be related to the expansion of roots' seeking water and nutrients beyond the top 15 cm (plowing layer) of soil as compared to the availability of nutrient (animal manure) within the top 15 cm soil layer under CM and DM treatments [37] or due to changes in soil bulk density as previously mentioned by Celik et al. [38].

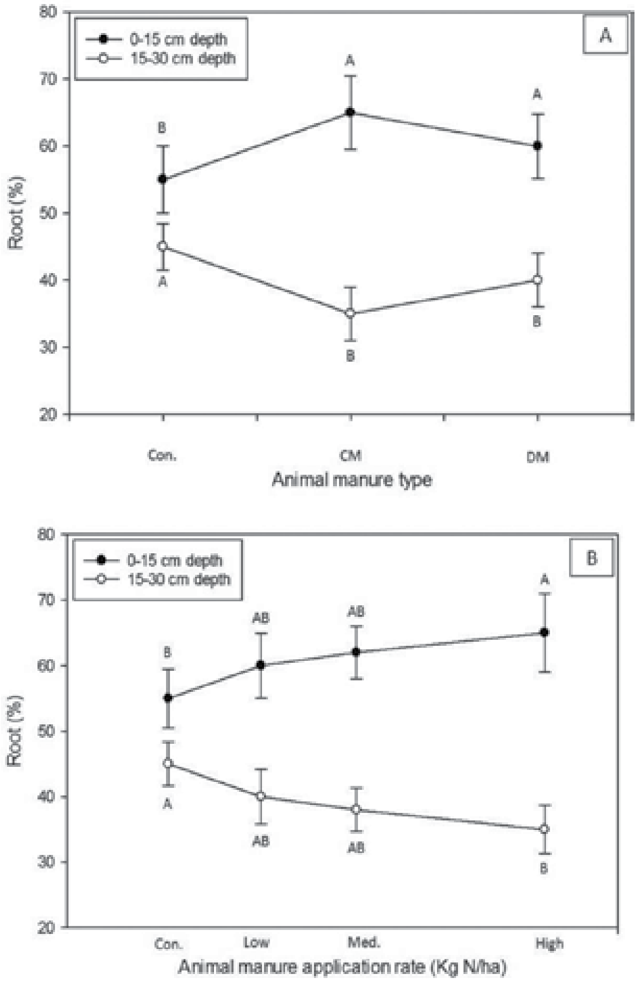

**Figure 9.** Sweet corn root distribution (%) at two depths (0–15 and 15–30 cm) under manure type (A) and application rate (B). Means followed with different letters are significantly different at 5% probability based on Duncan's multiple test.

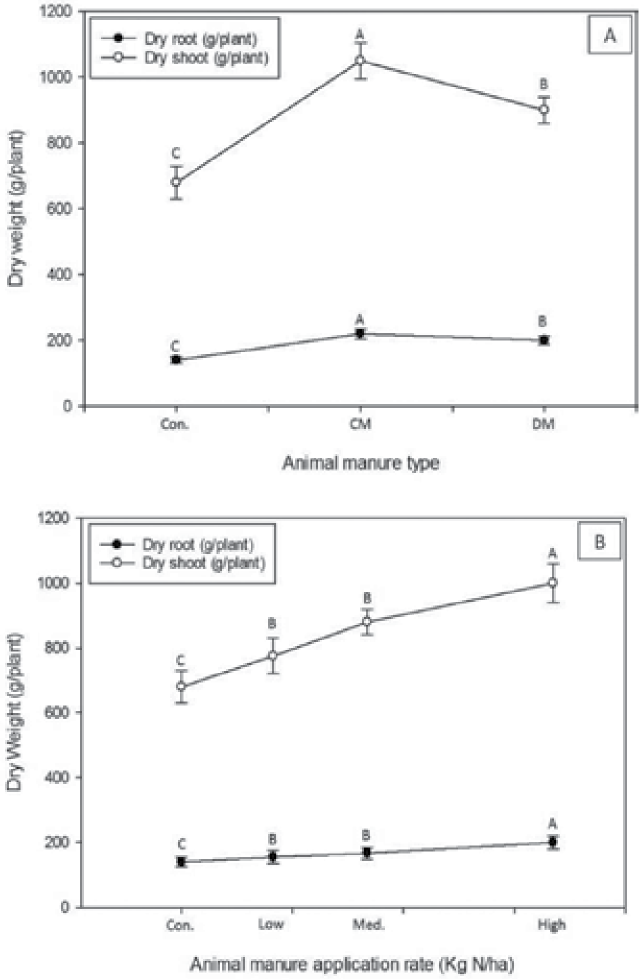

**Figure 10.** Sweet corn root and shoot dry weight (g/plant) under manure type (A) and application rate (B). Means followed with different letters are significantly different at 5% probability based on Duncan's multiple test.

### 3.2. Livestock manure effects on sweet corn biomass

In a field trial for two consecutive growing seasons, root and shoot biomass of sweet corn were evaluated under the application of CM and DM applied at 0 (Con), 165 (L), 335 (M), and 670 (H) kg N/ha. The analysis of variance showed a highly significant ($P < 0.01$) effect of both manure type and application rate on sweet corn root and shoot biomass. Sweet corn root biomass increased by 57% and 42% for the CM and DM treatments, respectively, compared to the control. Also, root biomass under CM was higher (10%) than DM treatment (**Figure 10A**). The shoot biomass increased by 54% and 32% for CM and DM treatments, respectively, compared to the control treatment. The shoot biomass under CM treatment was higher (17%) than DM treatment. Sweet corn root biomass increased by 42%, 20%, and 11% under high, med, and low application rates, respectively, compared to the control. Shoot biomass increased by 47%, 29%, and 13% under high, med, and low treatments, respectively, compared to the control (**Figure 10B**). The significant increase in root and shoot biomass might be related to increased nutrient availability and improved soil structure and SOM content [39]. The second-growing season data showed a similar pattern with higher means and no significant effect for the season on the studied parameters. The significant increase in sweet corn root and shoot biomass with the animal manure (type and rate) application is a good indicator of improved growth of sweet corn and forage produced under organic manure application, resulting in more feed for livestock and consequently more food for human consumption [28].

## 4. Algae species

Hawaii imports about 85% of the food consumed in the state, leaving it extremely vulnerable in terms of food safety and global events [40]. High level of goods imported and distributed throughout the state also poses a threat of introduced invasive plants (**Figure 11**) and animals [41, 42]. Marine non-native invasive seaweed has proven to be very costly to control in addition to developing a threat to the marine native ecosystem [43, 44]. The non-native seaweed species that have settled along the reefs of Hawaii grow and propagate more readily than the native seaweeds in Hawaii [45]. This is most likely because these seaweeds have less natural predators and herbivorous grazers since they are non-native to the area. Below is a description of the most common seaweed species found in Hawaii.

**Figure 11.** *Eucheuma* spp. sample collected on Oahu Island.

*Gracilaria salicornia*, also known as the Giant Ogo, is one of the most successful invasive seaweed species in Hawaii and is found mostly on Oahu and Hawaii Island. *G. salicornia* was first discovered in Hilo Bay on Hawaii Island and is believed to have originated somewhere throughout the Indian and Pacific Oceans [46]. This seaweed is much fitter than the native seaweeds and is more tolerant to light adjustments. It forms a thick mat that inhibits the growth of native seaweed species. This seaweed propagates both sexually and asexually by cloning through the fragmentation process [47].

*Kappaphycus* spp. (*K. striatum* and *K. alvarezii*) are coarse, spiny, and invasive seaweed and are usually dark green in color but may appear red if shaded. It was first introduced in Kaneohe Bay, Oahu, in 1979 for experimental aquaculture. This seaweed mostly resides in shallow subtidal reef flats in Kaneohe Bay on Oahu. Its fast vegetative growth increases with the environmental temperatures, allowing it to reproduce very rapidly [46] (http://www.botany.hawaii.edu/invasive).

*Eucheuma* spp. (*E. dentriculatum* and *E.* spp.) are much like *K.* spp. characteristics that make them difficult to distinguish between species. Rather, the term (clades) has been used to describe the physically different *E.* spp. without the use of molecular markers to distinguish between types. These types are commonly found on the east shores of Oahu Island as well as in the Waikiki area in Honolulu [48].

*Avrainvillea amadelpha*, also known as the mud weed, consists of wedge-shaped blades that are thin, diaphanous, 1–3 cm tall, and 1–4 cm wide. It has a dense cluster shape from attaching the blades by stalk to a compact basal holdfast. Blades are green to green-gray in color with smooth to lacerated edges. Clumps are muddy brown from being covered with silty sand. In Hawaii, *A. amadelpha* can be found in abundance on the shallow reef flats of Oahu's south shores, where it has disturbed and replaced native seaweed beds. It is expected to be a natural component of the deep-water community in Hawaii (http://www.botany.hawaii.edu/invasive).

*Acanthophora spicifera* seaweeds are abundantly found on calm, shallow reef flats, tide pools, and rocky intertidal benches. Often free floating, much of the success of these seaweeds is credited toward its brittle nature, allowing more widespread asexual distribution. The success of these seaweeds has contributed to the displacement of the native species of seaweeds. Evidence of its success in Hawaii is found in Maui, Molokai, Lanai, Kohoolawe, Oahu, and Kauai Islands (http://www.botany.hawaii.edu/invasive).

*Hypnea musciformis* is mostly recognized by its broad curls at the ends of some branches, allowing it to twine around other seaweeds. *H. musciformis* seaweeds are usually red in color but can also be yellow to brown in high-light environments or nutrient poor waters. During the bloom stage, it may be found free floating but is otherwise found on intertidal and shallow subtidal reef flats, tidepools, and rocky benches. It tends to grow on other large seaweeds and reproduces by fragmentation. These invasive seaweeds are destructive because they grow much faster than the native seaweed and shade out coral (http://www.botany.hawaii.edu/invasive).

The species that are currently targeted by cleanup efforts on Oahu Island are *G. salicornia*, *K.* spp., and *E.* spp. [49]. These species are predominantly found in Kaneohe Bay, reproduce asexually, and have not been observed to reproduce sexually in Hawaii. However, they are very capable of dominating the reefs with fragments of 0.5 cm and bigger [46, 50]. Some species of invasive seaweed have shown potential for use as an agricultural amendment due to its high K (13.5–18%) content (**Table 4**) and provide an opportunity to utilize an otherwise ecologically disruptive species.

| Species | Washed/unwashed | % | | | | | µg/g | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | C | P | K | Ca | Mg | Na | Fe | Mn | Zn | Cu | B |
| *G. salicornia* | Unwashed | 1.43 | 20.44 | 0.11 | **12.48** | 6.93 | 1.24 | 3.53 | 3564 | 553 | 22 | 11 | 316 |
| *G. salicornia* | Washed | 1.32 | 18.23 | 0.09 | **9.15** | 3.21 | 0.91 | 2.65 | 3204 | 482 | 19 | 9 | 286 |
| *E.* spp. | Unwashed | 1.01 | 21.14 | 0.07 | **18.02** | 1.08 | 0.63 | 4.81 | 123 | 18 | 18 | 3 | 196 |
| *E.* spp. | Washed | 0.78 | 17.78 | 0.06 | **16.94** | 0.37 | 0.61 | 3.71 | 45 | 9 | 14 | 2 | 166 |
| *K.* spp. | Unwashed | 1.39 | 22.10 | 0.07 | **14.81** | 0.47 | 0.53 | 4.71 | 83 | 8 | 14 | 5 | 139 |
| *K.* spp. | Washed | 1.21 | 21.78 | 0.06 | **14.11** | 0.28 | 0.52 | 4.43 | 67 | 7 | 12 | 3 | 135 |
| *A. amadelpha* | Unwashed | 0.67 | 12.21 | 0.05 | **0.36** | 30.13 | 2.21 | 1.81 | 9157 | 215 | 2 | 10 | 42 |
| *A. amadelpha* | Washed | 0.48 | 11.13 | 0.04 | **0.21** | 26.44 | 2.08 | 1.56 | 7853 | 197 | 2 | 6 | 42 |

Washing was by soaking each sample in a bucket of tap water for 3 minutes. Each value is a mean of three values.

**Table 4.** Seaweed species macro- and micro-nutrient content (with and without washing).

### 4.1. Nutrient variability and bio-security protocol for algae

#### *4.1.1. Nutrient variability among algae species*

Different batches of the four main species (*G. salicornia*, *Kappaphycus* spp., *Eucheuma* spp., and *A. amadelpha*) were collected from the Department of Land and Natural Resources (DLNR) "SuperSucker" team on Oahu at Kaneohe Bay. To reduce the salt content from the seaweeds and to evaluate the washing effect on nutrient content, the samples were split into two portions. One portion was washed with tap water, by soaking the sample in a bucket for 3 minutes. The other half was not washed. The two portions were dried at 95°C for 96 hours, and three subsamples of washed and not washed species of nutrient contents were determined. The results (**Table 4**) showed a high content of K in the *E.* spp. (18.02%), *K.* spp. (14.81%), and *G. salicornia* (12.4%). However, *A. amadelpha* was found to contain 0.36% K only, but a high content of Ca was 30.13%. Also, all species had a relatively good amount of N and other macro- and micro-nutrients beneficial for plant growth, yield, and rebuilding soil fertility [51]. Washing decreased the content of all macro- and micro-nutrients of all four species. However, the nutrient loss did not reach a significant level, and it is believed to significantly reduce the sodium content.

#### *4.1.2. Viability and bio-security protocol*

Viability and the spread of alien algae species into new shores and beaches across the Hawaiian Islands is a major concern and limitations to the use of these species as a major organic source of K fertilizer in agriculture, especially for direct application (without composting). A lab experiment was conducted to evaluate the effect of time and temperature on four seaweed species (*K.* spp., *E.* spp., *G. salicornia*, and *A. amadelpha*). The samples were dried in a conventional oven at ~90°C for 3 or 4 days (72 and 96 hours). Viability of dried samples was tested in a lab experiment with fresh (tap) and salt (ocean) water. Three random samples of 10 g from each species were placed in a 200 ml beaker with 100 ml of fresh or salt water. The lab experiment was repeated twice for 2 weeks each time. Monitoring changes on the seaweed species was performed over the 2-week test duration by taking pictures for each subsample. The results were identical for the repeated experiment. In both trials, the four seaweed species show no signs of growth or changes in volume, as a sign of water absorption during the first week. In the second week, the species showed decomposition signs (**Figure 12**). No differences were found between drying the samples for 72 or 96 hours and soaking the subsamples in fresh or salt water.

*A. amadelpha*: Decomposition

*E. spp.*: Decomposition

*K. spp.*: limited water absorbing

*G. salicornia*: No new growth

**Figure 12.** The four algae species showing signs of decomposition at the end of the second test experiment.

### 4.2. Direct application as organic source of potassium

Two field trials were conducted to evaluate the effect of different application rates of K on sweet potato growth and yield. K was applied at four application rates (0, 55, 110, and 220 kg K/ha) under two soil series (Wahiawa and Waialua). The experiment was under RCBD with three replicates. At harvest, the tuber fresh weight was recorded. Harvested tubers were cut down to pieces and dried at 75°C for 72 hours and then dry weight was recorded. The analysis of variance showed a highly ($P < 0.01$) significant effect of K application rates on the fresh and dry weights of sweet potato tubers. The highest means were at 220 kg K/ha, and the lowest was in the control (**Figure 13A** and **B**). The results were similar in pattern for both soils. However, the fresh and dry weights of tubers were higher in the Oxisol (Wahiawa series) soil than the Mollisol (Waialua series) soil, although the Mollisol is thought to have higher fertility than the Oxisol soil that might be related to the differences in structure between the two soils [22]. The initial K content in the two soils was higher than 300 ppm. However, the application showed a significant effect on the sweet potato growth and yield. This suggested that the soil K might not be available to the plant [52], and/or that the seaweed application improved the SOM, and/or the improvement in soil physical properties [53], allowing good tuber growth.

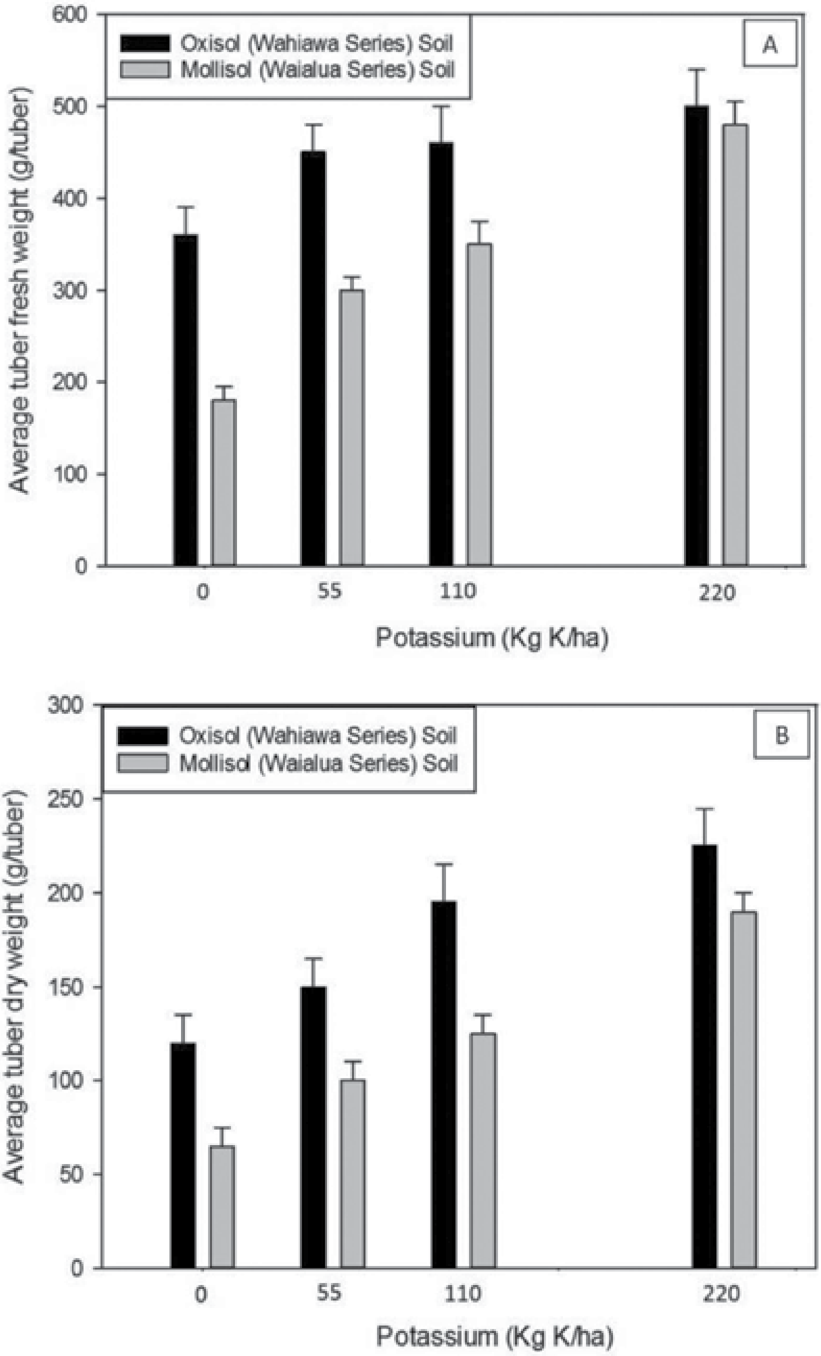

**Figure 13.** The effect of different potassium (K) application rates (kg K/ha) on average sweet potato tuber fresh (A) and dry (B) weight under Oxisol and Mollisol soils.

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## Chapter 5

# Bio-Organo-Phos: A Sustainable Approach for Managing Phosphorus Deficiency in Agricultural Soils

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Additional information is available at the end of the chapter

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### Abstract

Sustainable agriculture is essential for a positive relationship between supply and demand of food for the growing world population. This relationship was found to be affected by many environmental factors, including biotic and abiotic. From the point of view of crop nutrition, sustainability in the supply of essential nutrients particularly phosphorus is vital. Due to the energy crisis, the fluctuation in the prices of chemical fertilizers, environmental concerns, and cessation in the supply of high quality rock phosphate (RP) are hindering the use of chemical phosphatic fertilizers for sustainable crop production. Therefore, there is great need for a sustainable solution to this problem. It could be solved by employing a strategy to use native low quality RP. It is only possible by composting of organic material in the presence of RP and phosphate solubilizing microorganisms. During composting, most of organic P is mineralized. Due to release of organic acids, P availability to crop plants increases. In this chapter, the importance of economical and sustainable sources of P and comparative efficacy of the use of organic fertilizer containing RP for legumes is critically reviewed.

**Keywords:** Phosphorus, rock phosphate, sustainable crop nutrition, PSMs, legumes

## 1. Introduction

Agriculture plays a crucial role in the economic development and poverty alleviation in developing countries. Also, agricultural sustainability is vital for a sustainable agriculture. There must be a positive link between its supply and demand. Unfortunately, this link has been disturbed by many factors from which the deficiency of the nutrients especially that of phosphorus (P) has been a major one [1]. Moreover, its application to the soil in developing countries has been hindered due to alarming increase in its price.

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Adequate amount of phosphorus (P) is critical for normal plant growth and development as it is a vital component of plant energy system and genetic material in the form of adenosine triphosphate-ATP and deoxyribonucleic acid-DNA, respectively [2]. Moreover, it also is involved in many plant processes like photosynthesis, carbon metabolism, membrane formation, energy generation, nucleic acid synthesis, glycolysis, respiration, activation and inactivation of enzymes, and nitrogen fixation [3]. Root architecture, seed development, and crop maturity is also affected by P deficient conditions [4].

Leguminous crops engaged in symbiotic nitrogen ($N_2$) fixation generally require high amount of P due to high energy requirement which is mainly contributed through ATP [5]. Under P-sufficient conditions, nodules have a higher P concentration (up to 1.5% of the total plant P) as compared to that of the shoots and roots [6]. It is also needed for signal transduction, membrane biosynthesis, and nodule development and function [7]. Many studies concluded that P is the most limiting nutrient in many soil types for the production of crops especially the nitrogen fixing leguminous crops [8–10]. The direct and positive role of P in nodulation of red clover [11], peas [12], white clover [13], *Medicago truncatula* L. [14], and soybeans [15] has been reported. Under P deficient conditions, reduced root growth and photosynthetic carbohydrate supply to the nodules occurs [6, 16], which results in reduced nodule growth and function and ultimately reduced symbiotic $N_2$ fixation [17]. It has been found that N and P control the nodule growth and modulate the symbiotic processes of the legume and *Rhizobium* [11]. Phosphorus has its basic function in plant energy system and reduction of $N_2$ to $NH_3$ requires 16 ATP molecules. The availability of soil P is optimum at pH ranging between 6.5 and 7.0 for plant absorption, hence creating a soil environment that is more favorable for $N_2$-fixers like *Rhizobium*.

Rock phosphate (RP) is an economical source of P to the crop plants and 200–300 billion tons of RP are available throughout the world. For example in Pakistan, 20–30 million tons of different grade phosphate rock has been documented. The major issue with RP is having a low available P. Research work is needed to find out a way to enable farmers to utilize this RP as P-fertilizers to meet fertilizer demand and cope with the prices. A huge share of foreign exchange is utilized in importing phosphatic fertilizers which is not feasible for countries, especially the developing ones. Another constraint with chemical phosphatic fertilizers is that these are prepared from high quality RP which may be depleted by the year 2050 [7]. These problems have increased the necessity to find other measures and approaches so as to exploit indigenous RP resources in bioavailable form without compromising on yield.

From direct application of RP as a P source to the soils, it has been clearly found that this approach is feasible for the acidic soils having low pH and direct application of RP to the alkaline soils [18]. Another approach which could help solubilize the fixed P in RP could be to use the bio-inoculants, which through the release of organic acids (acetate, lactate, oxalate, tartarate, succinate, citrate, gluconate, ketogluconate, glycolate, etc.) reduce the pH of the micro-environment prevailing around these microbes [1, 19–21]. The plant growth promoting rhizobacteria (PGPR) include all the bacteria found in the rhizosphere which directly or indirectly enhance plant growth. Phosphate solubilizing microorganisms (PSMs) are the PGPR and can be utilized for enhancing the availability of P which has a direct effect on nodulation.

There are many reports about the use of RP along with PSMs as an alternative cheaper source of P [22, 23].

Scientists around the world have documented positive effects of organic fertilizers in improving the physical properties of soils, thereby increasing the availability of nutrients especially the least mobile ones like P [24–29]. The combined application of RP-EC with PSMs could be helpful in improving the nodulation, growth, and yield of crop plants. There are few reports about their combined application. Shahzad and his co-workers [30] found that combined application of rhizobacteria and P-enriched compost resulted in an increased growth and yield parameters compared to uninoculated control without compost. So PGPB along with SSP-enriched compost was found highly effective in improving growth, yield, and nodulation of chickpea as compared to their application alone. Saleem *et al*. [29] conducted a series of field and pot experiments to check the effectiveness of RP, compost, and PSMs in increasing the growth and yield of wheat. RP-enriched compost was used based on 25 and 50% P of the crop requirement and the balance amount of P was applied through chemical phosphatic fertilizers. From the results, it was found that application of 50% P from RP-EC with PSMs and 50% from the chemical fertilizers maximally increased most of the growth and yield parameters compared to the controlled (100% P from chemical Fertilizers).

Recently, we have conducted a series of pot and field experiments in comparing P nutrition of legumes through bio-organo-phos (a mixture of PSMs, RP, and compost) and found a significant improvement in growth, nodulation, and yield compared to the recommended chemical fertilizers [31]. This chapter will give an overall research progress in finding out the economical and sustainable source of P for the crops especially for the legumes and their future perspectives. It also addresses the pros and cons with their future perspectives and research needs.

## 2. Importance of phosphorus for symbiotic nitrogen fixation in legumes

Phosphorus is the most limiting nutrient for the production of crops especially the nitrogen fixing leguminous crops for adequate growth and nodulation. There are many reports about its direct and positive effect on nodulation of red clover, peas, white clover, *Lupinus* L., soybeans and many more [11–15]. Indirect effect on plant growth has also been reported, thereby increasing the nodulation and stimulating the nitrogenase activity [32]. Reduced root growth and photosynthetic carbohydrate supply to the nodules occurs under P deficient conditions [6, 16] which results in reduced nodule growth and function and ultimately reduced symbiotic $N_2$ fixation [17]. It has been found that N and P control the nodule growth and modulate the symbiotic processes of the legume and Rhizobium [11, 33]. Various morphological and physiological changes occur under P deficient conditions including increase in the root/shoot ratio, changes in root architecture [4, 34], development of root hairs [35], induction of the high-affinity phosphate transporter [36], and synthesis and exudation of the organic acids, phosphatases and ribonucleases (RNases) [37–39]. It has been reported that crop yield is seriously affected under P limited conditions especially during early stage of growth [40].

Phosphorus (P), being the second most important macronutrient after nitrogen, has a critical role in biological nitrogen fixation (BNF). It has been a renowned fact that legumes require more phosphorus (P) compared to non-leguminous crops as they perform the process of BNF through nodulation which is a characteristic property of legumes. Nodulation occurs in almost all the legumes. However, physiology and efficiency of nodules to conduct the process of nitrogen fixation is species specific. So for the process of BNF, P serves as an ultimate source of energy in the form of ATP [5, 41, 42]. It is also needed for signal transduction, membrane biosynthesis, and nodule development and function [8]. Moreover, nodules under P-sufficient conditions have a higher P concentration (up to 1.5% of the total plant P) as compared to shoots and roots [6].

There are many reports about the influence of P on nodulation; ultimately the amount of N fixed by the plants e.g. nitrogen contents of the legume, *Crotalaria micans*, were increased about 4 folds due to increased nitrogen fixation with the application of P at 90 kg ha$^{-1}$ [43]. Similarly Israel [15], studied the effect of P on accumulation of nitrogen in soybean (*Glycine max* L.). It was found that the concentration of nitrogen was increased with increasing the supply of P that was suggested to be due to increase in the symbiotic nitrogen fixation as P serves as an energy source. Plant dry matter, nodule number, and mass was also increased with increasing P supply. Enzyme activity of nitrogenase was also enhanced with increasing P. Earlier legumes are well renowned as P exhaustive crop plants due to the formation of nodules for symbiotic nitrogen fixation. In another experiment on different legumes (soybean, clover), similar effect of P on growth and nodulation parameters was recorded [44].

Another soil and sand culture experiment to study the interaction of N and P and their effect on growth, nodulation, nitrogen fixation, activity of nitrate reductase, and on the accumulation of nitrogenous compounds (ureides, amino acids, nitrate) in the xylem sap of common bean was conducted [3]. Both the soil and sand culture experiment showed that with increasing levels of N, nodulation parameters such as nodule number and mass, nitrogenase activity, and xylem ureides were decreased, while the concentration of asparagine in the xylem sap increases. Symbiotic nitrogen fixation was only increased at low N concentration with increasing P application. Similarly it was also found that the effect of N on the inhibition of nodulation including nodule number and biomass was systemic, while high dose of P had a systemic stimulatory effect on nodulation parameters as mentioned above. The systemic effect was confirmed by its direct effect on nodulation and not on the plant growth overall. There is still lack of information on whether there is effect of N and P on both the nodulation and nitrogen fixation or not and needs to be explored in future studies [32].

Similarly, another Leonard jar experiment was conducted to study the impact of P (0–2 mM P) on growth, symbiotic nitrogen fixation, N and C metabolism, as well as on the concentration of ATP, N and P contents of common bean (*Phaseolus vulgaris*). With the application of medium to high-P, not only the nodulation (nodule biomass: 4-fold) and growth parameters but also the P contents of the harvested plants were increased at the onset of the flowering. In the case of total soluble sugar and amino acid contents of leaf, root, and nodules of the plant, these were decreased with increasing the level of P application. Moreover, an increase of 20-fold in nodule-ARA and 70-fold in ARA per plant was observed with the application of 1.5 mM P [45]. Another split root experiment found that P has a specific effect on the nodulation not generally on the overall growth of the plant. More P was required in the earlier stage of nodule initiation and growth of the plant. It was also found that P could suppress the effect of N on the inhibition of symbiotic nitrogen fixation [32]. Many other researchers have reported the effect of P on nodulation, growth, and nitrogen fixation in many crops like clover, soybean, red clover, etc. [11, 15, 44, 46].

## 3. Phosphorus problems in the soils

Plants depend almost exclusively on the availability of nutrients from the soil as these are fixed in the soil. As far as P is concerned, its availability in the form of $PO_4^{3-}$, $HPO_4^{2-}$, and $H_2PO_4^-$ depends on the soil pH and is a significant determinant of plant growth [41]. There are two forms of P present in the soil: the organic (20–85%), which is the large proportion, and the inorganic. Phosphates ($PO_4^{3-}$, $HPO_4^{2-}$, and $H_2PO_4^-$) are the most available forms of inorganic P in the soil. While the organic portion of P, phytin and its derivatives constitute about 50%, lecithin and glycerol-phosphate are present in minor fraction, and these organic compounds could serve as a good source of P if mineralized [47].

Normally, the amount of total P present in the soil is about 0.05% (w/w) but the available fraction of P to the plants is very small (Reference). To overcome this problem and to maximize the production of crops per unit area, farmers have to apply costly inorganic synthetic phosphatic fertilizers, but their availability to the crop plants hardly reaches 20% [48]. This has led to the addition of 2–4 times more than the required amount of phosphatic fertilizers and has led to the maximization of cost to benefit ratio of growing crop plants per unit area [49]. Another issue with the phosphatic fertilizers, most available form of P, is that their cost has gone sky high and unaffordable for the poor farmers of the world especially of the developing countries like Pakistan. Moreover, it becomes an environmentally ill practice if we consider the production of chemical phosphatic fertilizers, which include the use of sulfuric acid [50]. Moreover, environmental problems such as eutrophication has also emerged which has led to the destruction of the habitat of the aquatic life and has caused environmental degradation. In conclusion, these problems have pessimistically posed a problem not only on the environment but also on the economics of growing crops. There are two reasons, which are mostly quoted for acidic and basic/alkaline soils depending on the soil pH. In acidic soils such as oxisols, inceptisols, and utisols, the added P quickly reacts with Fe and Al oxides as these are the dominant cation oxides in these types of soils and make it unavailable to the plants. In case of alkaline soils, Ca and Mg salts are abundantly present, so these cations react with phosphatic compounds thus making them unavailable to the plants [41].

## 4. Expected depletion of RP

The high pH of the soils from the Savannah zones of Nigeria resulted in increased adsorption of P when varying quantities of phosphate were added in these soils. The suggested responsible factor causing this fixation was the high activity of hydroxy-aluminum at high pH which has strong attraction with phosphate compared to hydroxyl. This attraction was found enough to displace hydroxyl from the hydroxy-aluminum-phosphate attraction. Due to this, increase in phosphate buffer capacity and the amount of phosphate required to attain the desired level of P in the equilibrium solution was noted [51]. In order to study the mechanisms behind the fixation of P in the soils, another study was conducted which showed that P adsorption by the amphoteric soil surface decrease with increasing pH from 4.0–7.0. But in soils high in exchangeable Al, increasing pH results in the formation of highly reactive adsorbing surfaces for P as Al-ions precipitate and insoluble polyhydroxy-Al cation species. So if acidic soils are reacted with lime without intervening air drying, this will result in the adsorption of more P in the soils. Alternatively, it was found that the application of lime to the acidic soils after intervening air-drying results in decreasing the P adsorption in the soil as clear through isotherm studies [52].

As mentioned earlier, precipitation, and adsorption of P with the soil colloids are the main responsible mechanisms/reactions for the removal of P from the soil solution. The former is induced by the presence of $Ca^{2+}$ ion in the soil solution while latter depends on the chemical properties of the soil colloids. There is high probability of having the precipitates of calcium phosphate in the soils rich in exchangeable cations. It has also been found that calcareous soils were poor in plant available P compared to the limed acid soils [53].

If we look at the manufacturing process of phosphatic fertilizers, we would found that the major factor responsible for their high prices is the use of very high energy in their manufacturing process. Coming to the initial step of the process is the raw material that is used for the manufacture of most of the phosphatic fertilizers, the phosphate rock or RP that is a naturally occurring mineral source of P. One strategy could be the use of this raw material, the cheaper source of P, directly in the field and getting the benefit of phosphatic fertilizers. To cope with this alarming situation, there is a need to find alternative cheaper sources of P, so that we could have a sustainable agriculture to feed out the ever growing population of the world.

## 5. Rock phosphate (an indigenous and economical source of P)

Phosphate rock or the RP is a non-renewable alternative natural source of P. It serves as raw material for the so-called chemical phosphatic fertilizers. It has been found on every continent of the world, however, quality may differ depending on the percentage of P present in it. Distribution of RP on the earth is given in Figure 1 [54].

In literature, there have been many reports showing effectiveness of directly applied RP being less expensive compared to the chemical phosphatic fertilizers like SSP or TSP. It has also been found to be effective for the perennial crops [55]. It has been directly applied in the soil of Nigeria in place of very expensive phosphatic fertilizers [50, 56]. The demand for the direct application of RP is increasing day by day as it would help reduce pollution and the burden on manufacturing industry for the high demand of chemical phosphatic fertilizers, and this would ultimately serve as a cheaper source of P. It has been found that RP results in increasing

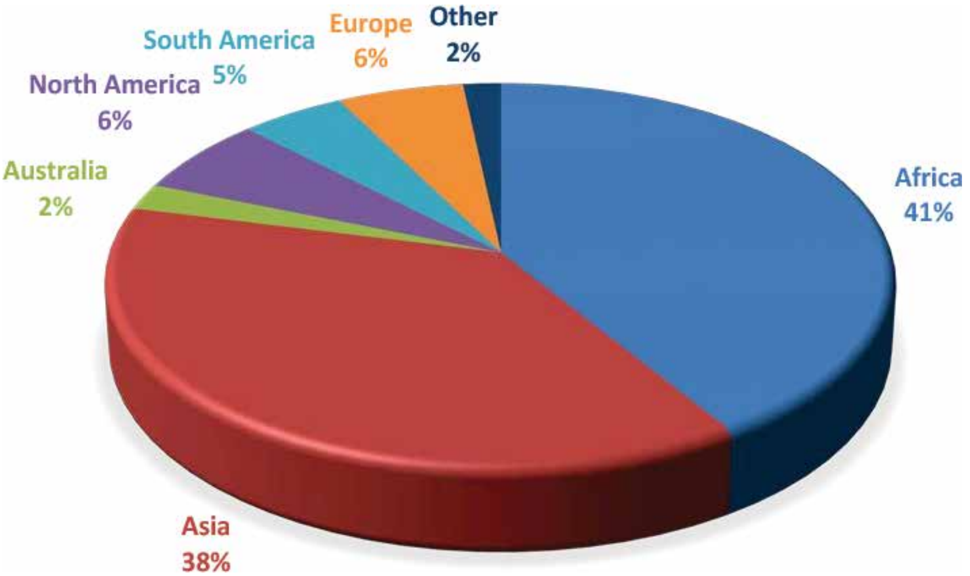

**Figure 1.** Rock phosphate (RP) reserves around the world.

the relative agronomic efficiencies of the crops grown on P deficient soils. It has sedimentary origin and its direct application might be feasible due to the presence of somewhat open, roughly consolidated aggregates of microcrystals with large surface area [57].

In a study, the direct application ortho rock phosphate (ORP) as a P source increased the crop yield and was comparatively found to be more effective than chemical phosphatic fertilizers [58]. Another greenhouse experiment was conducted to assess the relative effectiveness of directly applying Togo and Egypt RP sources and these sources were compared with TSP, using Lucerne as a test crop. From the results, it was found that the application levels of 60 and 43 kg P ha$^{-1}$ were the effective levels of Togo and Egypt RP, respectively. At these levels, the Egyptian and Togo RP were 92 and 64% as effective as that of TSP, respectively [59]. The direct application of RP to vertisols, oxisols, and ultisols, having pH less than 7, has reported to yield similar results as that caused by TSP. Moreover, the initial and residual effects of RP on volcanic ash soils like that of vertisols, oxisols, and ultisols are less compared to the TSP. It has also been found that soils with pH >7, like in andepts, should be taken with care when applying RP directly to the soils as these soils have greater tendency of P sorption [59–61].

It has been found that most of the phosphate rocks or RPs yield better results in the acidic soils like that of oxisols and ultisols compared to alkaline and neutral soils like andepts with high pH, high P sorption capacity, low cation exchange capacity (CEC), low rainfall, low organic matter, low microbial activity, etc. Moreover, RP has significant proportion of isomorphic substitution in the crystal lattice and variable proportion of impurities and accessory minerals. Thus, it has been found to be more beneficial if applied in acidic soil conditions as compared to the neutral and alkaline conditions [57].

One strategy for the direct application of RP on andepts soils might be the application of partially acidulated RP by using 20% $H_2SO_4$. It has been reported to increase the plant response from applied RP from 0–16% to 59–77% relative agronomic efficiency (RAE) on Andepts [61]. However, the application of inorganic acid like 20% $H_2SO_4$ could have detrimental effects on the soil microbiota. Moreover, some other procedures like mixing with elemental sulfur, partial acidulation with an acid, thermal alteration, combination with chemical phosphatic fertilizers, preparation of RP enriched compost, and dry compaction with water-soluble chemical phosphatic fertilizers [62–64] have been reported to increase the efficiency of RP in increasing the availability of P for the crops. However, these procedures are labor intensive, costly, and inappropriate to be practiced at large scale. Due to these problems, there is a growing interest in manipulation certain biological procedure like the application of PSMs. Composting have been proposed, which are discussed in the next sections separately.

## 6. Integrated application of RP and PSMs

It is well renowned that RP has no substitute as a source of P however; minimum processing is required for its direct application to the soils especially the non-acidic soils. It has been found that RP could be an effective source of P after four years of its application in soils with pH 5.5–6.0 [65]. Moreover, RP is not economically feasible for the soils with high adsorption capacities, low CEC, high pH, low rainfall, low organic matter content, and low microbial activity [66]. For these reasons, various strategies like that of the application of PSMs have been proposed in order to increase the solubilization of P from RP [67].

PGPR are the bacteria present in the rhizosphere (the area near plant roots up to where the effect of roots and its exudates is found), which helps directly or indirectly to the growth and yield attributes of the plants. Many species of PGPR have been identified like PSMs, 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) producing bacteria depending on the mechanism of action employed [68]. As mentioned in the above section, phosphate solubilizing bacteria (PSBs) and/or plant growth-promoting rhizobacteria not only improves the physicochemical properties of the soils, but also help in the solubilization of RP, which leads to increased availability of P to the crop plants. Certain mechanisms employed by these PGPR and PSMs have been identified regarding the solubilization of RP. This includes: 1) production of certain organic acids formic, acetic, propionic, lactic, gluconic, fumaric, and succinic which results in lowering the pH of microclimate in the rhizosphere, 2) synthesis of chelating compounds which help in easy provision of nutrients to the crop plants and many more [19, 21]. Other mechanisms reported in literature include the production of phytohormones, siderophores, nutrient assimilation, protection of seeds from pathogens through antagonistic action, competition for the nutrients and space, induction of systemic resistance and emission of volatile organic compounds [69–76]. Moreover, the presence of microbiota in the soil is an indicator of good soil health. In this way, the application of these bio-inoculants not only helps in the solubilization of fixed P in RP but also reduce the amount of costly prepared chemical phosphatic fertilizers being applied to the soil, thereby providing a cheaper and sustainable source of P for the plants [77–78].

Previously, it has been found that under biotic and abiotic stress like that of nutrients; there is increased production of ethylene that is a well renowned stress hormone and causes senescence, abscission, and chlorosis in the environment where it is produced. So as we have phosphorus stress due to the low recovery efficiency of phosphatic fertilizers as mentioned earlier, we have to adopt certain strategy that can reduce the amount of ethylene in the rhizosphere of the plants. Studies aimed on finding the biosynthetic pathway of ethylene have found that ACC is the precursor of ethylene.

One strategy could be to use the microorganisms that can use its precursor the ACC as a nutrient source and reduce the amount of ethylene produced. Scientists have isolated the so-called PGPR with ACC-deaminase activity. These rhizobacteria contain an enzyme ACC-deaminase that can convert ACC, the precursor of ethylene, into ammonia and $\alpha$-ketobutyrate, thereby reducing the ethylene stress and ultimately increase the plant growth especially through the proliferation of root growth with increased surface area to explore more soil volume [80]. Many studies have confirmed the efficacy of these PGPR in increasing the root growth of the crop plants, and hence improved yield through increased absorption of nutrients [71–72, 74–76]. In literature, there are many reports about the use of RP along with PSBs as an alternative cheap source of P for costly chemical fertilizers [22–23]. Zaidi and Khan [80] have suggested a synergistic interaction between PSBs and nitrogen fixers like *Azotobacter chroococcum* that helped in the better utilization of poorly soluble RP. Organic acids produced by different PSBs, are mainly responsible for this solubilization as reported earlier [20, 82]. The degree of P-solubilization of RP by the application of PSMs and their impact on nodulation, growth, and yield of mung bean was studied on an acidic and alkaline soil. The results clearly showed an increased nodulation, growth, and yield attributes of mung bean over control. Moreover, it was suggested that optimum results regarding nodulation, growth, and yield of mung bean could be because of PSMs applied along with an initial dose of chemical phosphatic fertilizer [83].

From the above discussion, it could be imperative to use both types of microorganisms with the ability to solubilize P from the RP, and decrease the level of ethylene through the enzyme ACC-deaminase. In soil microbiology, we usually use a term co-inoculation which involves the application of microbes with more than two traits. In plant sciences, it would be the application of microbes with phosphorus solubilizing activity and ACC-deaminase activity i.e. the application of PSBs and PGPR with P-solubilizing and ACC-deaminase activity.

This strategy has been opted under pot and field conditions and many success stories have shown its effectiveness. However, this strategy has been studied for increasing the availability of P from the so-called chemical phosphatic fertilizers, which are becoming a burden for the farmers to use them due to high cost as already mentioned in the previous section, and not from the RP. Similarly, there are certain problems associated with biofertilizers like shelf life and lack/limited knowledge about their mechanism of action. Poor handling and lack of availability in remote areas are also one of the problems due to poor extension work to disseminate their beneficial effects to the crops in remote areas. These issues could be solved by employing biotechnological approaches to produce certain genetically modified organisms

(GMOs) which could sustain the harsh environmental conditions and thereby improved shelf life.

## 7. Integrated application of RP and compost

As mentioned earlier, the direct application of RP has been useful only in soils with acidic pH due to poor solubility in alkaline and neutral soils [85]. However, a limited number of climatic and soil situation are available in which direct application of RP would sufficiently provide nutrients for the fast growing crops to feed the fast growing population of the world. So the scientists are on the way to find out alternate strategies to increase the solubility of directly applied RP. One such strategy is the use of bio-inoculants, i.e. the use of PSMs and PGPRs and as explained in the earlier section. The other strategy is the mixing of RP with a well rotten product of crop residues and daily waste materials through a process known as composting. Many researchers have reported increased availability of P to the plants by increasing the solubility of RP in the soil through composting [86–89]. The mechanisms behind this solubilization includes the release of organic acids during the decomposition of organic residues which helps solubilize RP by lowering the pH, and by the conversion of inorganic P in RP into organic P which might become available to the plants after mineralization in the soil [89–91].

The processing of RP through the processes like that of composting is essential before applying to the alkaline soils found in Pakistan. In Pakistan, most of the phosphatic fertilizers are imported, as most of the reserves of available RP are of poor quality, which hinders its use for the preparation of phosphatic fertilizers. So as mentioned earlier, the possible strategy to cope with this situation would be to utilize the capacity of composting in increasing the solubility of raw RP. It is one of the most efficacious strategies for the recycling of organic waste materials which helps in boosting the level of organic matter, thereby playing an important role in productivity and sustainability of the soil [92]. Many researchers have authenticated the role of composting in ameliorating the soil fertility, structure, and plant growth [84, 89, 93–95]. They have found that organic acids (humic, fulvic acids, etc.) are released during this process which helps solubilize the fixed or unavailable P present in RP, thereby increasing the availability of P to the plants.

For composting process, a variety of waste material like cow dung, rice husk, poultry waste, fruit peels, mango stones, and many others could be used with RP, which does not only improve the physicochemical properties of the soil and a good source of nutrients [26], but also helps in cleaning our polluted environment. During this process, a variety of organic acids are produced, which helps reduce the pH, and ultimately results in better solubilization of P from the RP [89]. Composting process is very old and has been used for many centuries but up until now, very few or no studies have considered this process to be utilized for increasing the availability of P from the raw source of P, the RP. In a field experiment at the Agricultural Research Farm of NWFP Agricultural University, Peshawar, Pakistan, the effect of different levels of the combined application of chemical phosphatic fertilizer (0, 30, 60, 90 kg $P_2O_5$ ha$^{-1}$) and that of the compost (0, 5, 10, 15 Mg ha$^{-1}$) was investigated for improving nodulation, growth, and yield of chickpea. There was no significant interaction effect of compost and phosphatic fertilizer that was suggested to be due to the P-deficiency of the experimental site. However, there was a significant effect of P on the nodulation and yield parameters studied, and 60 kg $ha^{-1}$ $P_2O_5$ from the phosphatic fertilizers was found to be optimum [96].

The quantity of available P varies with the nature of organic residues being used in the composting process and its rate of decomposition [84]. They found an increased citric acid solubility of RP by the composting of unreactive Mussoorie RP, chopped grasses, and tree leaves. Moreover, when this product was applied to the crop on equivalent total P basis, the grain and straw yield of the test crop, Guar, were similar to that of the application of SSP. Similar results were obtained using pigeon pea as test crop. The reason behind increased P availability from the phosphocompost might be due to the conversion of unavailable P in RP to water soluble P, thus increased efficiency of the dissolved P for the plant [97].

To investigate the impact of two RPs (nutriphos guano powder and Indian potash limited) with and without the composts (compost mulch and compost residues) on soil P pools and P uptake, a glasshouse experiment was conducted using wheat as test crop for 75 days in a loamy sand soil [98]. The composts were applied as a thick layer of 2.5 cm on the soil surface and nutriphos guano powder and Indian potash limited were applied at the rate of 35 and 26 mg P $kg^{-1}$, respectively. There was a control with an un-amended soil and amended soil with a soluble source of P ($KH_2PO_4$) at the rate of 50 mg P $kg^{-1}$ soil (inorganic P fertilizer applied soil). The results showed that there was no significant effect of the treatments on the total organic carbon concentrations and pH of the soil. However, the soil respiration was significantly higher in case of compost applied soil with and without RP compared to un-amended and inorganic P fertilizer applied soil. The plant growth was increased by 30–50% in the case of soils applied with compost alone or with RP sources. In case of the concentration of $NaHCO_3$-P and microbial P after 75 days of the applied treatments, an increase of 30% was noted in the case of the combination of compost with either RP compared to compost or RP applied alone, which suggested that compost helps in the mobilization of P present in RP. A significant change in labile pools of P was observed in case of the combined application of compost and RP compared to RP applied alone. Moreover, there was no significant effect on the plant growth and P uptake of wheat in case of combined application of compost and RP compared to compost applied alone, which shows that compost contains sufficient amounts of nutrients for the plants.

The organic matter in the soils or soil organic matter (SOM) is a good indicator of the productivity and quality of the soil as it mediates the physicochemical and biological properties of the soil. Many studies have authenticated the beneficial effects on the physicochemical, e.g. improves structure and water holding capacity of soils, decreases P fixation, increases CEC and buffer capacity of soils to resist pH change, and biological properties of the soil, e.g. provides energy to a variety of soil fauna and flora. For example, P sorption capacity of the soil was reduced through the addition of the SOM, which resulted in the alteration of chemical properties of the soil, e.g. complex formation of P compounds on the reaction sites [85]. The organic matter in the soil also serves as a reservoir of micro- and macronutrients.

The SOM exists as partially decomposed residual layer of plants and animals, microorganisms, and humus. The humus, a stable compound of carbon, comprises about 50–75% of the total soil carbon. It has been found that the humus content of the soil are increased by the addition of organic fertilizers, and it also enhances the microbial activities in the soil [100]. It has also been speculated that farming methods aiming at increasing the organic matter in the soil would ultimately reduce the requirement of P application [100].

As mentioned earlier, SOM also serves as a basic source of mineral nutrients in the soil, e.g. nitrogen, sulfur, and phosphorus. About 95% of the total N and S, and up to 75% of P in the surface soil is in organic forms [101, 102]. The organic P in the soil exists in various forms of which phytic acid is the most important one. It is stored in the plant seeds to accomplish early establishment of the seedlings from the germinating seeds. Other organic compounds include mono and di-esters, phospholipids, nucleotides, sugar phosphate, phosphoproteins, and phosphonates [103]. The release of P from its organic compounds is not a very simple process and depends on many factors, like the relative stability of the organic substances and their chemical composition, climatic conditions, physicochemical properties of the soil, cropping scheme, and their interaction with mineral fertilizers [104–106]. Organic fertilization through compost application is a common practice for sustainable agricultural production and P cycling [107]. However, the amount or concentration and type of P compounds depend on the source of the material being used for composting [108]. Long term application of organic fertilizers in the form of composts results in boosting the organic P in the soil [109–111]. The conversion of this organic P into inorganic P or the mineralization depends on the type of compound being mineralized, e.g. orthophosphate di-esters are quickly mineralized compared to orthophosphate monoesters [112, 113]. Mineralization helps in increasing the total available P, which has been suggested to be due to reduction in the P adsorption and increased rates of microbial enzyme activities, which boost up the biologically mediated turnover of organic P into inorganic P [114].

It has been well established that the addition of organic fertilizers not only increases the organic matter and nutrient status of the soil, but also reduces the amount of costly chemical phosphatic fertilizers to be added into the soil, thereby, promoting healthier and sustainable environment of the soil [115]. It has also been documented that sustainable production from the continuous cropping system through the application of recommended levels of chemical fertilizers may not be possible in future [116]. The integrated use of chemical and organic fertilizers would be a sustainable strategy and has achieved substantial attention throughout the world. Chemical and organic fertilizers both supplement each other's efficiency for nutritional deficiencies and would ultimately decrease the exclusive dependence on chemical fertilizers [117].

Another field experiment was conducted to study the effect of different coir dust based composts on dry matter production and nutrient uptake of maize. It was found that the C:N ratio of the soil samples taken after 120 days of crop growth was decreased, and more biomass was produced with the application of organic fertilizer [118]. Another pot experiment using four different combinations of organic wastes including horse manure and bedding, sewage sludge along with clarifier solids from pulp mill, mink farm wastes, and municipal solid waste (MSW) was conducted to compare the capacity of different organic wastes to improve the growth and yield of tomato, and to assess the phytotoxicity of these organic wastes in radish and cress (*Lepidium sativum*) seedlings. It was concluded that paper mill waste, applied alone without the application of organic fertilizer, causes toxic effect in vegetables, i.e. radish and cress [119].

During composting, organic acids like humic and fulvic acids are released, which decreases the pH of the material being composted [120], and increases the solubility of fixed P in the soils with high pH. These composts act like plant growth regulators when applied to the soil. The effect of these humic acids isolated from the cattle vermicompost was tested on the earliest stages of lateral root and on the plasma membrane $H^+$-ATPase activity in an experiment using maize as a test crop. From the results, it was clearly found that the humic acids significantly increased the overall root growth of maize seedling in conjunction with lateral root emergence. Also a significant stimulatory effect on the activity of $H^+$-ATPase was observed, which implies that the humic acids enhance the expression of this enzyme. Moreover, exchangeable auxin groups in the macrostructures of humic acid-containing compost, as revealed through structural analysis, shows that the hormonal activity is enhanced by the application of organic wastes containing humic acids [121]. In a pot experiment, to confirm the production and impact of organic acids on the growth and yield of crop plants during the composting process, the comparison of the impact of natural and synthetic humates like that of humic acids found in composts and exogenously applied synthetic humates, e.g. potassium humate on the growth of chicory plants and behavior of soil microbial population was studied. The results confirmed that during composting, there is production of organic acids, and these acids have a stimulatory effect on the growth and yield of crop plants and also increases the microbial population of beneficial microorganisms as observed in this study [122].

The impact of commercially produced vermicomposts produced from the mixture of cattle manures and food and paper wastes was tested in a two year filed study using pepper (*Capsicum annuum* L.) as a test crop. It was suggested that increased growth and yield of pepper resulted due to the production of humic material and plant growth hormones by the increased microbial biomass present in the compost [123]. Similarly, they also found that the composted material significantly increase the soil microbial biomass and their dehydrogenase activities. Similar results were observed by Manna *et al.*, [124] who tested the effect of the application of compost and chemical fertilizers on the growth and yield of chickpea and wheat. They found that the compost application not only significantly enhance water soluble, citrate soluble, and total P in the soil, which resulted in an increased growth and yield parameters of chickpea and wheat, but also increase the microbial biomass and their enzyme activities.

The impact of different rates of application of vermicompost on the physicochemical properties of the soil was investigated in a field experiment using tomato (*Lycopersicum esculentum* L.) as test crop. The compost was applied into the upper 15 cm layer of the soil at the rates of 0, 5, 10, 15 Mg $ha^{-1}$. From the results, it was found that the application rate of 15 Mg $ha^{-1}$ significantly enhanced the electrical conductivity, total organic carbon, total N, P, K, Ca, Zn and Mn, as compared to that of the control. Overall, it was concluded that vermicompost can significantly improve the physicochemical properties of the soil [125].

As mentioned earlier, the effect of every compost/vermicompost depends on the chemical nature of that compost. It has been found that with the application of vermicomposts, there was an improvement in the biological properties, which resulted in an increased yield of the rice, while the impact of cow dung in improving the biological properties of the soil was more pronounced as compared to that of the green forage [126]. Similar results were observed regarding the biological properties and fertility status of the soil [127, 128].

From the above discussion, it has been clear that different composts have a well renowned effect on improving the fertility status, physicochemical, and biological properties of the soil which ultimately results in improving the growth and yield of crop plants. Intensive agriculture has become essential to feed an ever-increasing population of the world. Similarly, burning of farm waste not only increases the environmental pollution due to the emission of $CO_2$ but also causes the wastage of nutrients and very precious organic matter. So depending solely on organic fertilizers would not be a wise strategy, instead the combined use of organic and an economical source of P like that of RP would serve better compared to the use of organic or chemical fertilizers alone [50]. Moreover, the composting process helps in the recycling and stabilization of organic wastes, which reduces their contribution to the environmental pollution, and this stable product can increase the plant production [129]. In this area of research, the interest has been increased and new strategies are underway to make a valuable product. These include partially acidulating RP with natural or synthetic organic acids as through composting, decreasing the particle size [130] and through the addition of bio-inoculants [131].

The properly employed process of composting converts the organic wastes into a stable and mature product of carbon, i.e. humus [132], while improperly composted organic wastes lead to the immobilization of plant nutrients and cause phytotoxicity [127, 133, 134]. During composting, heat is produced which helps in the destruction of pathogens as well [135]. The properties of composts depend not only on the chemical nature of organic wastes being composted but also the make-up of the organic material during composting [136]. In other words, stability and maturity indices are good indicators of the worth of composted material. However, it is very difficult to measure these indices and still no standards have been devised yet [137–140]. In general, the following parameters including C:N ratio, carbon contents (water soluble), CEC, humus contents, and the evolution of carbon dioxide from the finished composted material have been used to evaluate the stability and maturity of the composted material [141, 142]. For the measurement of the phytotoxicity of the matured composted material, germination index is used [143].

Many studies have proved that if we could find a natural and non-polluting way of increasing the solubilization of RP, its use could serve as a valuable substitutional source of chemical phosphatic fertilizers [144]. There have been many reports about the preparation of RP-enriched compost and their ability to increase the total P in the soil compared to the straw compost alone without RP, but the quantity of water soluble P is decreased in the RP-enriched compost compared to the straw compost or RP alone due to the dilution effect of RP being mixed with a large amount of composted material reaction of soluble P with $CaCO_3$ present in RP [64, 83]. On the other hand the RP-enriched compost has considerably more citrate soluble P compared to the straw compost, which is due to the production of organic acids like citric, oxalic, tartaric, 2-ketogluconic, acetic, malic, and succinic acids, etc., which enhance the dissolution of RP-P [64, 145]. These organic acids are in anionic form and produced during the degradation of complex organic compounds [146]. A lot of $CO_2$ is produced during composting which results in the formation of carbonic acid, ultimately increasing the solubility of P in RP decreasing the pH [64, 147]. The RP-enriched compost has low microbial biomass carbon compared to the straw compost due to the dilution of carbon over a large biomass of compost material. The levels of organic P are also higher in case of RP-enriched compost compared to the straw compost as clear from the higher amount of alkaline phosphatases activities and acids [64]. The level of citrate soluble and organic P increases with increasing the addition of RP but up to a certain level and then decrease. So overall, during this organic matter decomposition, the available P and calcium contents are increased to the plants [147].

An experiment was conducted to compare the impact of pill millipede (*Arthrosphaera magna*) compost and farm yard manure, using black gram (*Phaseolus mungo*) and finger millet (*Eleusine coracana*) as test crop. From the results, it was found that the former compost resulted in better growth and yield of both the crops as compared to the farm yard manure, which was suggested due to the provision of plant nutrients present in both the types of composts [25]. Similarly the combined effect of mimosa compost and phosphatic fertilizers was more pronounced compared to the application of mimosa compost and phosphatic fertilizer alone [28]. Another field experiment found that RP-enriched compost performed better in case of yield and nutrients concentration in cow pea [24].

However, the extent of P solubilization depends on many factors like that of the ratio between the RP and compost, time and rate of application to the soils, which has not been done yet. It has been reported that P from this RP-enriched compost is available even at high pH of 8.5 or more [81], so it would ultimately serve as an economical and environment friendly way to reduce the use of chemical phosphatic fertilizers.

## 8. Integrated application of RP, PSMs, and compost (bio-organo-phos)

The PGPR include all the bacteria found in the rhizosphere, which directly or indirectly enhance plant growth. These may be involved in enhancing the availability of nutrient directly or through other indirect mechanisms like lowering the pH of rhizosphere. PSMs are the PGPR and can be utilized for enhancing the availability of P, which has a direct effect on nodulation. The combined application of RP-EC with PSMs could be helpful in improving the nodulation and the plant growth. There are few reports about their combined application. Shahzad and his co-workers [30] studied the effect of integrated use of plant growth promoting bacteria, and compost enriched with single super phosphate (SSP) for improving growth, yield, and nodulation of chickpea. Their results revealed that combined application of rhizobacteria and P-enriched compost resulted in an increase of 84, 97, and 79% in fresh biomass, number of pods plant$^{-1}$ and grain yield, respectively compared to uninoculated control without compost. So PGPB along with SSP-enriched compost was found highly effective in improving growth, yield, and nodulation of chickpea as compared to their application alone.

A field experiment was conducted to study the impact of four different agro-industrial wastes inoculated with PSMs (*Aspergillus niger* and *Phanerochaete chrysosporium*) in increasing the availability of P from RP. From the results it was found that with the application of *Aspergillus niger*, 59.7, 42.6, and 36.4% of the total P present in the RP was released in the case of the application of sugar beet wastes, olive cake, and olive mill wastewaters, respectively which was suggested to be due to the secretion of organic acids. Overall, the growth and yield of the plants was increased with the combined application of RP, *Aspergillus niger* and different agro-industrial wastes, i.e. sugar beet wastes, olive cake, olive mill wastewaters, and dry olive cake [27]. Series of field and pot experiments were conducted in our lab to check the combined effect of RP, compost and PSMs in increasing the growth and yield of wheat. From the results, it was found that the combined application of RP-enriched compost with PSMs could serve as an alternate source of P for increasing the growth and yield of many crops, thereby leading to sustainable agriculture and cleaner environment [29]. Recently, we have conducted a series of pot and field experiments and their results have clearly shown better improvement in growth, nodulation, and yield of lentil compared to conventional use of chemical phosphatic fertilizers [31].

## 9. Conclusions and perspectives

For sustainable agriculture, there must be a positive link between the nutrients applied to the soil and crop uptake. This link could be more firm and sustainable if we employ an integrated approach, i.e. the use of bio-augmented RP-enriched organic fertilizer. In this way, we would be employing a sustainable approach to meet the needs of crops for P in an environment friendly way. The use of this approach would not only be helpful in the restoration of degraded soils, but would also be helpful in minimizing organic wastes that could be composted to make organic fertilizer. The use of indigenous sources of RP would help minimize the energy use during its conversion into chemical phosphatic fertilizers. The product would help the farmers in reducing their expenditures to purchase chemical fertilizers, and would also reduce the import budget on national level. However, a few studies have reported its efficacy under pot and field conditions. More studies under controlled and field condition are needed to confirm these reports. Also there is a need to find the optimum ratio to mix organic fertilizer and RP, its time, and rate of application. Relative efficacy of different sources of organic fertilizers to make bio-organo-phos could also searched out. Their post-harvest effects on soil physico-chemical properties and on microbial community structure could also be found out in future.

A variety of organisms are involved in P cycling in soils, and microorganisms are probably the most important ones. However, most of the soil microbes have not been cultured successfully [148]. In the future, new culture-independent methods like LMW RNA profiling and PCR based on nucleic acid composition are required to study the function and ecology of microbes involved in P cycling in soils [149]. The techniques mentioned have been found not only independent of culture media composition or growth phase of microorganisms but also are precise and reproducible. These techniques also made it possible to utilize different biotechnological tools like amplification of targeted genes or to quantify their expression. Overall, these techniques have opened new horizons in order to solve out the puzzle related to microbial community ecology and to assess the survival and persistence of specific inoculants under different environmental conditions.

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# Integrated Use of Phosphorus, Animal Manures and Biofertilizers Improve Maize Productivity under Semiarid Condition

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Additional information is available at the end of the chapter

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## Abstract

Phosphorus unavailability and lack of organic matter in the soils under semiarid condition are the major reasons for low crop productivity. Field trial was conducted to investigate the impact of different animal manures (poultry, cattle, and sheep manures) and phosphorus levels (40, 80, 120, and 160 kg $P_2O_5$ $ha^{-1}$) on yield and yield components of hybrid maize (CS-200) with (+) and without (−) phosphate-solubilizing bacteria (PSB) seed treatment at the Agronomy Research Farm of The University of Agriculture Peshawar, during summer 2014. Our results confirmed that the application of poultry manure significantly ($P \leq 0.05$) increased yield and yield components of maize. Phosphorus applied at the rate of 120 kg $P_2O_5$ $ha^{-1}$ increased ear length, grains $ear^{-1}$, and shelling percentage, while the highest rate of 160 kg P $ha^{-1}$ increased grains weight, grain yield, and harvest index. Maize seeds treated with PSB (+) before sowing had produced higher yield and yield components than untreated seeds (−). We concluded from this study that combined application of 160 kg $P_2O_5$ $ha^{-1}$ + poultry manure and seed treatment with PSB (+) could improve crop productivity and profitability under semiarid condition.

**Keywords:** *Zea mays* L, hybrid, yield components, phosphate-solubilizing bacteria, animal manures, phosphorus levels, semiarid climate

## 1. Introduction

Maize (*Zea mays* L.) is the third most important cereal crop in Pakistan after wheat and rice [1]. In Northwest Pakistan (Khyber Pakhtunkhwa province), maize ranked second after wheat in its importance [2]. Maize average yield in Northwest Pakistan is too low as compared with the average yield of the country [3, 4]. Maize is a $C_4$ mode of carbon fixation plant efficiently utilizes inputs, shows rapid growth, producing large quantity of organic matter per unit area [5]. In Pakistan, maize was cultivated on an area of 1059.5 (000 ha) with a total production of 4220.1 (000 tones), while during the same season its area of cultivation and production in the Khyber Pakhtunkhwa Province (semiarid condition) was 463.4 (000 ha) and 858.3 (000 tones), respectively. Its average yield in Pakistan is 3983 kg $ha^{-1}$, which is much lower than the other corn growing countries of the world (USA, China, Brazil, Argentina, Canada, Italy, Egypt) [6].

The major problems in the way of increasing yield at farmer's fields are the inappropriate nutrients supply [7–9]. Phosphorus unavailability and lack of organic matter under calcareous soils in semiarid climates are some of the major reasons for low crop productivity [10, 11]. Phosphorus is very important for improving crop growth and yield [12–14]. Phosphorus is a macronutrient that plays a number of important roles in plants. Adequate phosphorus results in higher grain production, improved crop quality, greater stalk strength, increased root growth, and earlier crop maturity [3, 4]. Crop phosphorus nutrition depends on the ability of the soil to replenish the soil solution with phosphorus as the crop removes it and on the ability of the plant to produce a healthy and extensive root system that has access to the maximum amount of soil phosphorus. Application of P fertilizers must be done in a way to maximize the P availability to crops and to minimize the risk that P might be lost to the environment by runoff or erosion. According to [15], phosphorus deficiency symptoms appear in the lower part of the plants and results in (1) decreased leaf number, (2) decreased leaf blade length, (3) reduced panicles/ears/spikes per plant, (4) reduced seeds per panicle/ear/spike, and (5) reduced filled seeds per panicle/ear/spike.

Phosphorus is the second most important crop nutrient after nitrogen that increases crop productivity and profitability on P-deficient soils in Khyber Pakhtunkhwa [4, 10, 11]. Phosphorus has been reported to increase the strength of cereal straw, stimulate root development, promote flowering, fruit production, and formation of seeds, and it hastens maturity of the crops [16]. In most of the cropping system especially under semiarid condition, phosphorus is one of the least available mineral nutrient [17] especially in soils high in $CaCO_3$ [18] and high soil pH [19], which reduces P availability to crops [20, 21]. Because large applied phosphorus application as fertilizer moves in to immobile pools through precipitation reaction with highly reactive $Al^{3+}$ and $Fe^{3+}$ in acidic, and $Ca^{2+}$ in calcareous or normal soil [22]. At least 60% water soluble P fertilizer was more effective in calcareous soils because P uptake by corn plants [23]. Percent utilization fertilizer P and available P content of soil generally decreased with increasing $CaCO_3$ concentration [24]. The use of microorganisms such as phosphate-solubilizing bacteria (PSB) as inoculants with the seed increases P availability and uptake by the plant [25] by production of organic acid, which reduces the pH of the surroundings rhizosphere [26]. Which either dissolve phosphates as a result of anion exchange or can chelate Ca, Fe, or Al ions associated with the phosphates [22].

Application of organic matter to field crops provides nutrients to the plants, also improves water holding capacity, and helps the soil to maintain better aeration for the seed germination and plant root development [27]. Therefore, the combine use of organic fertilizers along with chemical fertilizers may be utilized as an effective tool to improve growth and increase yields

[28]. Applications of organic manures, such as crop residues, animal manures (AM), chicken manures, green manures, composts, farm yard manure, biochar, and ash, increase the beneficial microbes in the soil and improve soil health and sustainability. Organic fertilizers consisted of farmyard manure, poultry manure, sheep manure, and biofertilizer may be used for crop production as an alternate of inorganic fertilizers [29]. However, most of Pakistani soils comprise <1% organic matter [30], because of lower organic matter added to soils [31, 32]. Pakistan is rich in farm manures with immense livestock population. In Pakistan, about 50% AM is used as fuel and more than 50% is not consumed [33]. Poultry manure mineralizes greater than other manure such as cattle or pig dung: plant absorbed nutrient and utilized rapidly [34]. Basic nutrients required for higher growth and yield of crops contains in poultry manure and increases carbon content, water holding capacity, soil structure, and decreases bulk density [35, 36]. Subsistence farmers should apply organic manure directly to the soil as a natural means of recycling nutrients in order to improve soil fertility and yield of crops [37]. Application of cattle dung increases plant height, leaf area, pod number, pod weight in cowpeas [38] and in maize [39].

Biofertilizers are known to play a number of vital roles in soil fertility; crop productivity and profitability [40]. Biofertilizers are the products containing living cells of different types of beneficial microbes (bacteria, fungi, protozoa, algae, and viruses). Some of the commonly used beneficial microbes in agriculture include *Rhizobia, Mycorrhizae, Azospirillum, Bacillus, Pseudomonas, Trichoderma, Streptomyces* species. According to [40], beneficial microbes are essential for decomposing organic matter in the soil and increase essential macronutrients (nitrogen, phosphorus, potassium, sulfur, calcium, and magnesium) and micronutrients (boron, copper, chlorine, iron, manganese, molybdenum, and zinc) availability to crop plants. Beneficial microbes also play significant role in solid wastes and sewage management. Beneficial microbes increase plants tolerance to different environmental stresses (drought, heat, cold, salinity etc.) and increase plant resistance to insects and diseases attacks. Beneficial microbes not only improve crop growth and productivity by increasing photosynthesis and producing hormones and enzymes but also improve crop quality by controlling different insects and various plant diseases. Beneficial microbes reduce the use of chemical fertilizers and thereby reduce environmental pollution caused by chemical fertilizers. Beneficial microbes reduce cost of production and so increase grower's income and profitability. Beneficial microbes are therefore very important for increasing crop productivity, profitability, and sustainability. PSB increase the growth and yield of different crops as reported in maize [10, 11, 41] and wheat [42]. The use of beneficial microorganisms (biofertilizers) such as PSB as inoculants with the seed increases P availability and uptake by the plants [25], which are important not only for the reduction of the quantity of chemical fertilizers and environment friendly [43] but also increased crop productivity [41].

As phosphorus and organic matter are some of the major limiting factors for crop production under semiarid condition. Therefore, the application of biofertilizer especially PSB and AM could increase phosphorus availability and crop productivity under semiarid condition. However, there is no research to investigate the interactive effects of AM × PSB × P under semiarid condition. This research work was therefore designed with the objectives (1) to find out suitable AM source, (2) to find out proper P level, (3) to find out proper combination of AM × P, (4) to find out proper combination of AM × PSB, (5) to find out proper combination of P × PSB, and (6) to find out proper combination of AM × PSB × P for improving yield and yield components of maize hybrid (CS200) under the semiarid condition at Peshawar (Pakistan).

## 2. Materials and methods

### 2.1. Site description

Field trial was conducted to investigate effects of AM (poultry, cattle, and sheep manures) and phosphorus levels (40, 80, 120, and 160 kg $P_2O_5$ $ha^{-1}$) on growth and yield of maize with (+) and without (−) PSB at the Agronomy Research Farm of The University of Agriculture Peshawar, during summer 2014. The experimental farm is located at 34.01°N latitude, 71.35°E longitude at an altitude of 350 m above sea level. The farm soil is silt clay loam, low in organic matter (0.87%), extractable P (5.6 mg P $kg^{-1}$), exchangeable, alkaline (pH 8.2), and calcareous in nature [9].

### 2.2. Experimentation

The experiment was laid out in randomized complete block design with split plot arrangement using three replications. Combinations of three AM and to PSB levels (with and without PSB) were applied to the main plots, whereas four phosphorus (P) levels were applied to subplots. All the AM sources (poultry manure, sheep manure, and cattle manure) were applied at the rate of 5 t $ha^{-1}$ 2 weeks before seed bed preparation, while P was applied at sowing time. The PSB obtained from NARC, Islamabad, was mixed with the seed just before sowing time. A subplot size of 4 m × 3.5 m, having five rows, 4 m long, and 70 cm apart was used. A uniform dose of 120 kg N $ha^{-1}$ as urea in two equal splits, that is, half at sowing, and half at knee height was applied. Maize hybrid "CS-200" was used as a test crop. All other agronomic practices were kept uniform and normal for all the treatments.

### 2.3. Data recording

Data on ear length (cm) were recorded with the help of meter rod by selecting ten plants randomly from each subplot, and then, the average was worked out. Number of grains $ear^{-1}$ was calculated on 10 randomly selected ears from each subplot, and then, average was worked out. Thousand grain weights (g) of randomly 1000 grains were taken from seed lot of each subplot and were weighted with the help of electronic balance. For grain yield data, the three central rows of each treatment were harvested, dried, threshed, weighted, and then were converted into grain yield (kg $ha^{-1}$) using following formula:

$$\text{Grain yield}\left(\text{kg ha}^{-1}\right)=\frac{\text{Grains weight in three rows}\left(\text{kg}\right)}{\text{No. of rows}\times\text{row length}\times\text{R}-\text{R distance}}\times 10{,}000\ \text{m}^2$$

Harvest index and shelling percentage for each treatment were calculated using the following formulae.

$$\text{Harvest index}=\frac{\text{Economic yield}}{\text{Biological yield}}\times 100$$

$$\text{Shelling \%}=\frac{\text{Grains weight of 10 ears}}{\text{Total weight of 10 ears}}\times 100$$

### 2.4. Statistical analysis

Data were statistically analyzed according to [44] for randomized complete block design with split plot arrangement, and means among different treatments were compared using least significant differences (LSD) test ($p \leq 0.05$).

## 3. Results and discussion

### 3.1. Effect of phosphorus

Phosphorus (P) levels significantly affected ear length, number of grains ear$^{-1}$, thousand grains weight, grain yield, harvest index, and shelling percentage of maize (**Table 1**). Higher ear length (25 cm) was obtained with the application of P at the rate of 120 kg ha$^{-1}$, followed by 160 kg P ha$^{-1}$ (23 cm), whereas lower ear length (20 cm) was produced in plots receiving 40 kg P ha$^{-1}$. The higher number of grains ear$^{-1}$ (417) was obtained at 120 kg P ha$^{-1}$, followed by 160 kg P ha$^{-1}$ (406), whereas lower number of grains ear$^{-1}$ (385) was recorded with 40 kg P ha$^{-1}$. Phosphorus application at the rate of 120 kg P ha$^{-1}$ produced heavier thousand grains weight (346.5 g) being statistically at par with 160 kg P ha$^{-1}$ (347.8 g), while the lower thousand grains weight (331.2 g) was recorded with 40 kg P ha$^{-1}$. The highest grain yield (5245 kg ha$^{-1}$) was obtained with the highest P level of 160 kg P ha$^{-1}$ being at par with 120 kg P ha$^{-1}$ (5164 kg ha$^{-1}$). The lowest grain yield (4284 kg ha$^{-1}$) was noted in the plot received the lowest rate of 40 kg P ha$^{-1}$. Application of 160 kg P ha$^{-1}$ resulted in the highest harvest index (42.13%) being statistically at par with 120 and 80 kg P ha$^{-1}$, while plots with 40 kg P ha$^{-1}$ resulted in the lower harvest index (38.49%). The higher shelling percentage (83.6%) was calculated with 120 kg P ha$^{-1}$; however, it was statistically at par with 160 kg P ha$^{-1}$ (82.9%), whereas lowest shelling (80.3%) was recorded with 40 kg P ha$^{-1}$.

| **Phosphorus (kg ha$^{-1}$)** | **Ear length (cm)** | **Grains ear$^{-1}$** | **Thousand grains weight (g)** | **Grain yield (kg ha$^{-1}$)** | **Harvest index (%)** | **Shelling percentage (%)** |
|---|---|---|---|---|---|---|
| 40 | 20 d | 385 d | 331.2 c | 4284 c | 38.49 c | 80.3 c |
| 80 | 21 c | 400 c | 338.9 b | 4754 b | 40.28 b | 81.5 b |
| 120 | 25 a | 417 a | 346.5 a | 5164 a | 40.55 ab | 83.6 a |
| 160 | 23 b | 406 b | 347.8 a | 5245 a | 42.13 a | 82.9 a |
| **LSD$_{0.05}$** | **0.94** | **4.43** | **1.77** | **159.01** | **1.63** | **0.99** |

Means followed by different letters within each category are significantly different using LSD test ($P \leq 0.05$).

**Table 1.** Ear length, number of grain ear$^{-1}$, thousand grains weight, grain yield, harvest index, and shelling percentage of maize hybrid as affected by phosphorus levels.

The increase in the yield components (length, number of grains ear$^{-1}$, and thousand grains weight), grain yield, harvest index, and shelling percentage with the application of higher P rates (120 and 160 kg P ha$^{-1}$) over lower rates of P (40 and 80 kg P ha$^{-1}$) as shown in **Table 1** could be due to the higher requirement of P by maize hybrid. These results are in accordance with those of [32, 33] that number of cobs increased with the increase in the level of organic and inorganic fertilizers. According to [34], ear lengths in maize increased while increasing P level, while [35] reported that the application of P significantly increased the number of grains ear$^{-1}$ in maize. These results are in agreement with those of [6, 35] that increase in P level increased maize grain yield. According to [41], grain yield in maize increased to maximum level with the application of 90 kg P ha$^{-1}$. The increase in harvest index with higher P levels might be due to the increase in yield and yield components of maize with higher P rates [6]. The increase shelling percentage with increase in the P level probably may be due to the increase in ear length, number of rows, and number of grains per ear as well as heaviest grain weight [4]. The results published from the same study [11] indicated that maize phenology (tasseling, silking, and physiological maturity) was delayed with lower P levels (40 and 80 kg ha$^{-1}$) levels. Phenological development enhanced (early development) was observed with the application of higher P levels (120 and 160 kg ha$^{-1}$). The reason for early phenology with the application of higher P levels might be due to better root development and thus facilitated the plants obtained more P and other nutrient from poultry manure for rapid plant growth and development. These findings are in line with those of [31] who reported that early phenological development with higher P levels. Growth parameters (plant height, mean single leaf area, and leaf area index) were significantly improved with the application of two higher P levels [11]. The biomass yield was significantly increased with the application of 120 or kg P ha$^{-1}$ and poultry manure. Reduction in biomass yield was observed with the application of 40 kg P ha $^{-1}$ and cattle manure. The increase in biomass yield reflects the better growth and development of the plants due to balanced and more availability of nutrients, which was associated with increased root growth due to which the plants explore more soil nutrients and moisture throughout the growing period. The increase in biomass yield with integrated use of 120 and 160 kg P ha$^{-1}$ + poultry manure in our experiment was attributed to the improvement in growth parameters that increased yield and yield components in maize. These results are in line agreement with [45] who stated that the application of P fertilizer significantly increased the biomass and grain yield of maize.

The results from another research on wheat crop [46] revealed that the increase in P level accumulated more total dry matter (DM) and portioned more DM into leaf, stem, and spike at both anthesis and physiological maturity. A large amount of DM was accumulated in response to the application of the highest rate of (90 kg P $ha^{-1}$). The increase in total DM accumulation with increase in P probably may be due P being the components of ATP might have contributed to a higher photosynthetic rate, abundant vegetative growth and assimilates formation and partitioning [47]. The results are also in accordance with those of [48] who reported increase in DM partitioning and accumulation while increasing rate of P. The increase in number of spikes $m^{-2}$ and grains per spike with increase in P level probably may be the major cause for increasing total DM accumulation and greater amount of partitioning into various plant parts especially the reproductive parts which increased grain yield. Memon et al. [49] and Rahim et al. [50] reported that grains per spike in wheat increased with increase in P level. Recently [10], we found that phosphorus levels had significantly ($P \leq 0.05$) affected number of grains $ear^{-1}$ and grain $srow^{-1}$, 1000 grains weight, grain yield, harvest index, and shelling percentage in maize. Phosphorus applied at the two higher rates (75 and 100 kg P ha $^{-1}$) had increased number of grains $ear^{-1}$ and grains $row^{-1}$, 1000 grains weight, grain yield, harvest index, and shelling percentage in local variety "Azam" [10]. Decrease in P level not only decreased yield and yield components of maize "Azam" but also declined the income of maize growers under semiarid climates [4, 10].

### 3.2. Effect of AM

AM significantly affected ear length, number of grains $ear^{-1}$, thousand grains weight, grain yield, and harvest index (**Table 2**). Plots applied with poultry manure resulted in higher ear length (24 cm), followed by sheep manure (22 cm), which is statistical at par with cattle manure (21 cm). In case of AM, application poultry manure produced higher number of grains $ear^{-1}$ (414) lower (391) was observed under cattle manure being at par with the sheep manure (401). In the three AM used, application of poultry manure produced heavier thousand grains weight (348.2 g), followed by sheep manure (341.0 g), while cattle manure resulted in lower thousand grains weight (334 g). The highest grain yield (5216 kg $ha^{-1}$) was recorded with application of poultry manure, followed by sheep manure (4786 kg $ha^{-1}$), whereas the lowest grain yield (4583 kg $ha^{-1}$) was obtained with cattle manure. Maximum harvest index (42.09%) was observed with poultry manure, while minimum harvest index (39.14%) was recorded with cattle manure being at par with sheep manure (39.85%). In case of AM, poultry manure application increased shelling percentage (84%); however, lower shelling percentage (81.1%) was calculated with cattle manure being at par with the sheep manure (82.2%). The increase in the yield components (length, number of grains $ear^{-1}$, and thousand grains weight) with the application of poultry manure (**Table 2**) over cattle and sheep manures probably may be due to the higher availability crop nutrients specially P and other macronutrients and micronutrients. These results are in accordance with those of [51, 52] that number of cobs increased with the increase in the level of organic and inorganic fertilizers. According to many studies, application manure increased tassel length [53], 1000 grains weight [54], and number of grains ear$^{-1}$ in maize [55]. In our recent study, we found that the residual effect of poultry manure was also found better on the yield components of wheat under rice–wheat cropping system as compared with sheep manure and cattle manure [32]. Many researchers [56, 57] reported that poultry manure significantly increased the grain yield in maize. According to [53], increase in the poultry manure doses had significantly increased harvest index in maize, while [58] reported that poultry manure increased the shelling percentage in maize. The results published from the same study [11] indicated that phenological development delayed with the application of poultry manure as compared with other two manures (sheep and cattle manures) reported delayed in phenology with the application of poultry manure. The growth parameters were also significantly improved with application of poultry manure and there by increased grain yield and yield components in maize over sheep and cattle manures [11] suggested that poultry manure enhanced the LAI in maize. The improvement in growth and yield with the application of poultry manure probably may be due to the enhanced lead area, total chlorophyll content, carbon content, water holding capacity, and decrease bulk density of soil [59]. Earlier the results published from the same study [11] indicated that maize biomass yield was significantly increased with the application of poultry manure as compared with cattle and sheep manure (poultry manure > sheep manure > cattle manure). The increase in biomass yield was attributed to the better growth and development of the plants due to balanced and more availability of nutrients which was associated with increased root growth due to which the plants explore more soil nutrients and moisture throughout the growing period [11, 60]. The increase in biomass yield showed positive relationship with grain yield.

| **Animal manure (5 t ha$^{-1}$)** | **Ear length (cm)** | **Grains ear$^{-1}$** | **Thousand grains weight (g)** | **Grain yield (kg ha$^{-1}$)** | **Harvest index (%)** | **Shelling percentage (%)** |
|---|---|---|---|---|---|---|
| Sheep manure | 22 b | 401 b | 341.0 b | 4786 b | 39.85 b | 81.2 b |
| Poultry manure | 24 a | 414 a | 348.2 a | 5216 a | 42.09 a | 84.0 a |
| Cattle manure | 21 b | 391 b | 334.0 c | 4583 c | 39.14 c | 81.1 b |
| **LSD$_{0.05}$** | **1.38** | **10.93** | **2.16** | **194.93** | **2.12** | **1.14** |

Means followed by different letters within each category are significantly different using LSD test ($P \leq 0.05$).

**Table 2.** Ear length, number of grain ear$^{-1}$, thousand grains weight, grain yield, harvest index, and shelling percentage of maize hybrid as affected by animal manures.

### 3.3. Effect of PSB (biofertilizer)

The PSB had no nonsignificant effect on ear length and harvest index, while number of grains ear$^{-1}$, thousand grains weigh, grain yield, and shelling percentage was significantly affected by PSB (**Table 3**). Higher number of grains ear$^{-1}$ (409) was obtained in the plots applied with PSB than without PSB (395). Plots with PSB had produced heavier thousand grains weight (342.0 g) than plots without PSB. The +PSB vs. −PSB comparison indicated that the plots with

PSB (+PSB) produced higher grain yield (4993 kg ha$^{-1}$) than plots without PSB (4730 kg ha$^{-1}$). Higher shelling percentage (82.9%) was obtained with PSB and lower (81.2%) at without PSB. The increase in the yield components (number of grains ear$^{-1}$ and thousand grains weight), grain yield, and shelling percentage of maize seed treatment with PSB over not treated maize seeds (**Table 3**) probably may be due to the higher availability of crop nutrients and increase in beneficial soil microbes. According to [10, 45, 61, 62], PSB application resulted in higher yield components and grain yield. The results published from the same study [11], however, indicated that plots with (+) and without (−) PSB had showed no significant differences in the phenological development of maize. However, other growth parameters were improved in maize crop grown under plots with (+) PSB than without (−) PSB. Application of phosphate-solubilizing microorganism improving soil fertility by releasing bound P therefore improves crop growth and increases crop productivity [29]. In contrast to our results, [45, 61] reported that the inoculation of maize with PSB under greenhouse and field conditions increased biomass yield of maize. According to [62], biofertilizer (*Pseudomonas*) significantly increased the biomass yield of maize over control. Our recent results on wheat [46] also revealed that the application of beneficial microorganisms (BMO) at the two higher levels (20 and 30 L ha$^{-1}$) accumulated more total DM and partitioned more DM into leaf, stem, and spike at both anthesis and PM. Because BMO applications increase the availability of plant nutrients, especially P availability to the plants that resulted in better plant growth and higher production [63–65]. Dobblaere et al. [66] assessed the inoculation effect of BMO on growth of spring wheat and observed that inoculated wheat plants had better growth, more number of grains spike$^{-1}$, and grain yield. Significant differences were found in number grains ear$^{-1}$ and grains row$^{-1}$, 1000 grains weight, grain yield, and shelling percentage between the plots treated with PSB (+) and without PSB (−) [10]. Plots applied with PSB (+) had produced more numbers of grains ear$^{-1}$ and grains row$^{-1}$, heavy 1000 grains weight, higher grain yield, and shelling percentage than plots without PSB (−). However, no significant differences were observed for harvest index between the plots with PSB (+) and without PSB (−).

| PSB | Ear length (cm) | Grains ear$^{-1}$ | Thousand grains weight (g) | Grain yield (kg ha$^{-1}$) | Harvest index (%) | Shelling percentage (%) |
|---|---|---|---|---|---|---|
| With PSB (+) | 22 | 395 b | 340.2 b | 4730 b | 40.00 | 81.2 b |
| Without PSB (−) | 22 | 409 a | 342.0 a | 4993 a | 40.73 | 82.9 a |
| **Level of significance** | **ns** | * | * | * | **ns** | * |

Means followed by different letters within each category are significantly different using LSD test ($P \leq 0.05$). ns, nonsignificant at 5% level of probability.
*Significant at 5% level of probability.

**Table 3.** Ear length, number of grain ear$^{-1}$, thousand grains weight, grain yield, harvest index, and shelling percentage of maize hybrid as affected by with (+) and without (−) PSB inoculation.

| Interactions | Ear length (cm) | Grains ear$^{-1}$ | Thousand grains weight (g) | Grain yield (kg ha$^{-1}$) | Harvest index (%) | Shelling percentage (%) |
|---|---|---|---|---|---|---|
| AM × PSB | ns | ns | (**Figure 4**)* | ns | ns | (**Figure 7**)* |
| AM × P | ns | (**Figure 1**)* | (**Figure 5**)* | ns | ns | (**Figure 8**)* |
| PSB × P | ns | (**Figure 2**)* | ns | ns | ns | ns |
| AM × PSB × P | ns | (**Figure 3**)* | (**Figure 6**)* | ns | ns | ns |

ns, nonsignificant at 5% level of probability.
*Significant at 5% level of probability.

**Table 4.** Ear length, number of grain ear$^{-1}$, thousand grains weight, grain yield, harvest index, and shelling percentage of maize hybrid as affected by interactions.

### 3.4. Interactive effect

All interactions (AM × PSB, AM × P, PSB × P, and AM × PSB × P) had no significant effect on ear length, grain yield, and harvest index of maize **(Table 4)**. Number of grains ear$^{-1}$ was significantly affected by AM × P, PSB × P, and AM × PSB × P interactions **(Table 4)**. Interaction between AM × P indicated that the increase in number of grains ear$^{-1}$ with the combination of poultry manure + 120 kg P ha$^{-1}$, and the plots that received cattle manure along with 40 kg P ha$^{-1}$ produced minimum number of grains ear$^{-1}$ (**Figure 1**). Significant increase in number of grains ear$^{-1}$ was observed with PSB + 120 kg P ha$^{-1}$, and plots that received 40 kg P ha$^{-1}$ without PSB application resulted in the lowest number of grains ear$^{-1}$ (**Figure 2**). The three-way interaction among AM × PSB × P indicated that the highest number of grains ear$^{-1}$ was recorded in plots under poultry manure + 160 kg P ha$^{-1}$ + PSB. Plots having cattle manure + 40 kg P ha $^{-1}$ without PSB produced the lowest number of grains ear$^{-1}$ (**Figure 3**). The results published from the same study [11] indicated that higher mean single leaf area and maximum leaf area index (4.28) were recorded with the combined application of higher P levels, viz 120 or 160 kg P ha$^{-1}$ + poultry manure along with PSB inoculation. The lower mean single leaf area and minimum leaf area index (3.65) were recorded with the combined application of sheep manure + 40 kg P ha$^{-1}$ without seed inoculation with PSB (−) and therefore resulted in significant AM × PSB × P interactions [11].

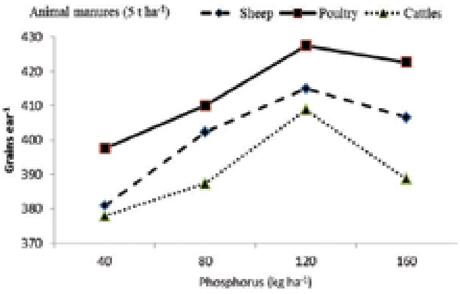

**Figure 1.** Response of number of grains ear$^{-1}$ in hybrid maize to animal manures and phosphorus interaction (AM × P).

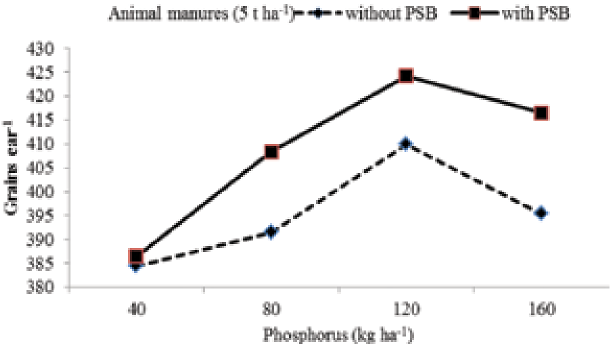

**Figure 2.** Response of number of grains ear$^{-1}$ in hybrid maize to phosphate-solubilizing bacteria and phosphorus interaction (PSB × P).

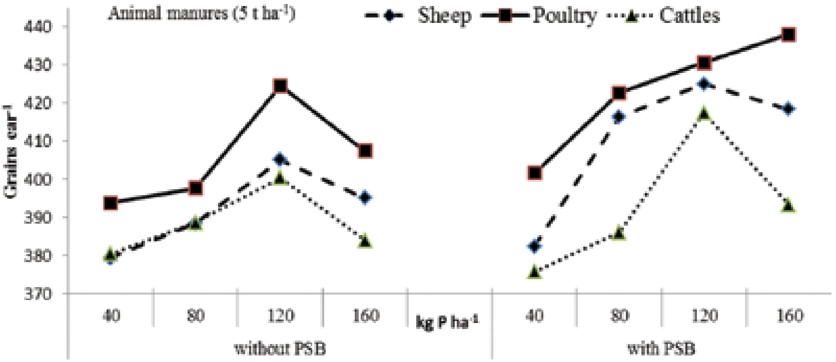

**Figure 3.** Response of number of grains ear$^{-1}$ in hybrid maize to animal manures, phosphate-solubilizing bacteria and phosphorus interaction (AM × PSB × P).

Thousand grains weight was significantly affected by AM × PSB, AM × P, and AM × PSB × P interactions (**Table 4**). Interaction between AM × PSB indicated that heavier thousand grains weight was recorded in plots applied with poultry manure under PSB, and plots that received cattle manure with no PSB applied reduced thousand grains weight (**Figure 4**). The results from the same study [11] indicated that delayed physiological maturity (104 days) was recorded with the application of poultry manure along with PSB inoculation, while early days to physiological maturity (101 days) was observed with the application of cattle manure along with PSB inoculation. The P × PSB interaction indicated that the highest thousand grains weight was observed in plots under poultry manure along with 160 kg P ha$^{-1}$, and plots received cattle manure with 40 kg P ha$^{-1}$ resulted in lowest thousand grains weight (**Figure 5**). Interaction among AM × PSB × P showed that the highest thousand grains weight was recorded in plots under poultry manure + 160 kg P ha$^{-1}$ + PSB (**Figure 6**). Plots having cattle manure + 40 kg P ha$^{-1}$ without PSB application reduced thousand grains weight to minimum (**Figure 6**).

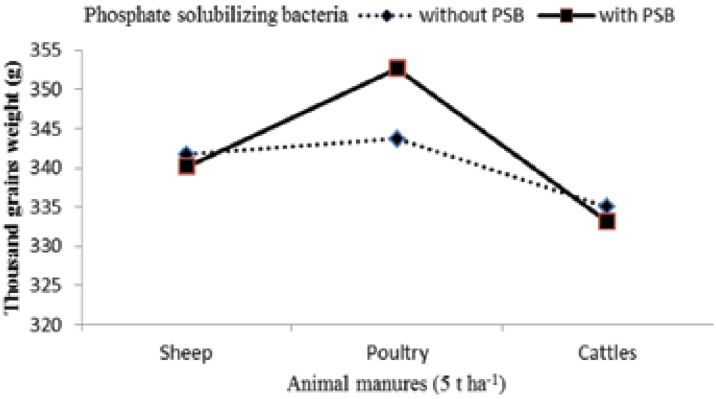

**Figure 4.** Response of 1000 grains weight (g) in hybrid maize to animal manures and phosphate-solubilizing bacteria interaction (AM × PSB).

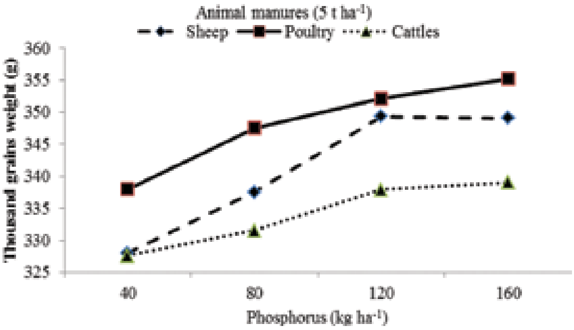

**Figure 5.** Response of 1000 grains weight (g) in hybrid maize to animal manures and phosphorus interaction (AM × P).

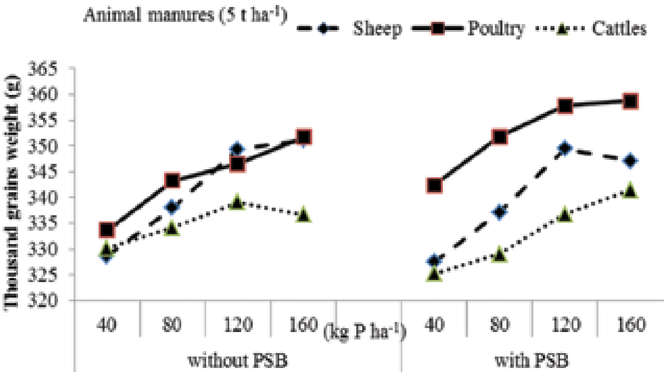

**Figure 6.** Response of 1000 grains weight (g) in hybrid maize to animal manures, phosphate-solubilizing bacteria and phosphorus interaction (AM × PSB × P).

Amanullah and Khan [10] reported that the interaction between P levels and PSB (P × PSB) had significant effect on number of grains per row and grain yield. They found that the two higher P levels (75 and 100 kg P ha$^{-1}$) had produced significantly more number of grains per row in maize grown under both with (+) and without PSB (−) treated plots [10]. The two higher P levels (75 and 100 kg P ha$^{-1}$) had produced significantly higher grain yield grain yield than the two lower levels of P (25 and 50 Kg P ha$^{-1}$) in the plots treated with (+) and plots, where PSB was not applied [10]. Many researchers [26, 67, 68] also suggested that the seed inoculation with PSB along with the application of soluble phosphatic fertilizer decreases P fixation on calcareous, thereby increasing P use efficiency and grain yield.

Shelling percentage was also significantly affected by the interaction between AM × PSB and AM × P **(Table 4)**. Interaction between AM × PSB indicated that the application of poultry manure with PSB increased while the application of sheep manure without PSB decreased shelling percentage in maize (**Figure 7**). Interaction between AM × P indicated that the highest shelling percentage was calculated with poultry manure + 120 kg P ha$^{-1}$, and the lowest shelling percentage was calculated for the combination of sheep manure + 40 kg P ha$^{-1}$ (**Figure 8**). Increase in yield and yield components of maize was reported earlier by Cheema et al. [69], Zafar et al. [70], Khan et al. [71], and Iqbal et al. [39] with integrated application of organic and inorganic fertilizers under semiarid climates. Amanullah and Stewart [72] found that wheat and rye responded differently in growth under different soil types. Both crops had better performance in terms of higher leaf area per plant, leaf area expansion rate, specific leaf area, leaf area ratio, plant height, stem elongation rate, root length, number of roots per plant, number of tillers per plant, absolute growth rate, and crop growth rate under organic soils as compared with inorganic soils at different growth stages [72]. Recently Amanullah and Khan [10] reported that integrated use of inorganic P fertilizer at higher rates + organic matter (in the form of compost) along with the seed inoculation with PSB significantly increases grain yield and yield components of maize under the semiarid climate of Peshawar Valley. Organic agriculture is important for the improvement of the environmental conditions and human health [73, 74].

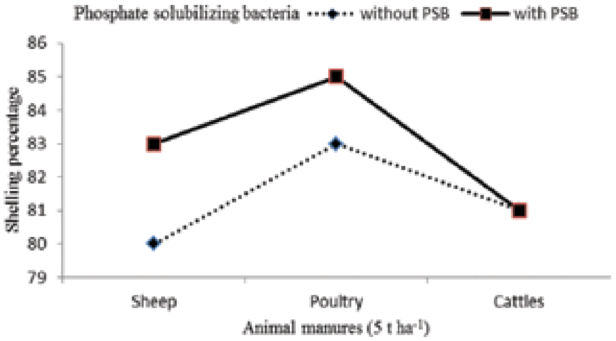

**Figure 7.** Response of shelling (%) in hybrid maize to animal manures and phosphate-solubilizing bacteria interaction (AM × PSB).

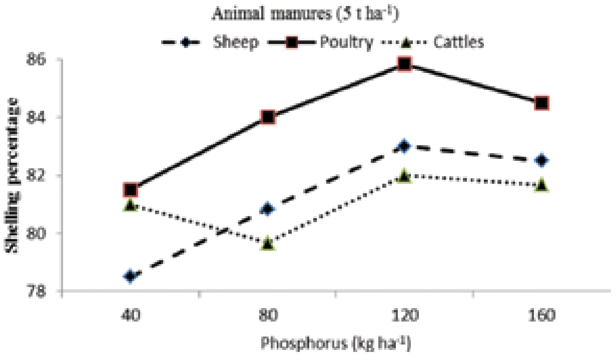

**Figure 8.** Response of shelling (%) in hybrid maize to animal manures and phosphorus interaction (AM × P).

## 4. Conclusions

Phosphorus unavailability and lack of organic matter in the soils in semiarid condition are the major reasons for low crop productivity. The results obtained from this field research work indicated that among the three AM used in the experiment (cattle manure, sheep manure, and poultry manure), poultry manure was found more beneficial in terms of better yield components (longer ear lengths, higher number of grains ear$^{-1}$, heavier grains) that resulted in higher grain yield, harvest index, and shelling. Among the four P levels (40, 80, 120, and 160 kg $P_2O_5$ ha$^{-1}$), application of P at the rate of 120 kg ha$^{-1}$ had increased the ear length, number of grains ear$^{-1}$, and shelling percentage. Application of P at the highest rate of 160 kg P ha$^{-1}$ increased grains weight, grain yield and harvest index. The plots treated with PSB (seed inoculation) had produced significantly higher yield and yield components of maize as compared with non-inoculated seeds. We concluded from these results that the integrated use of phosphorus (160 kg ha$^{-1}$) + poultry manure along with the seed inoculation with PSB could improve maize productivity under semiarid condition. Organic agriculture is also important for the improvement of the environmental conditions and human health.

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Chapter 7

# Soil Amendments for Agricultural Production

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Additional information is available at the end of the chapter

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## Abstract

The word organic, applied to fertilizers, indicates that the nutrients are derived from the remains or by-products of a once-living organism. Farmers are continually searching for alternatives to synthetic inorganic fertilizers to alleviate the escalating production costs associated with the increasing costs of energy and fertilizers and the problems of soil and surface water deterioration associated with intensive use and release of inorganic fertilizers such as N and P fertilizers. One of the advantages of organic fertilizers is that they provide their nutrients especially the principal nutrients (NPK) to growing plants over a long period of time in a slow release process. The soil has to be moist and warm enough to allow soil microorganisms to decompose and breakdown the complex forms of organic fertilizers. Generally, the application of organic amendments to agricultural soils makes good use of natural resources and reduces the need of synthetic inorganic fertilizers. Soil structure, nutrient composition, and microbiological activity of soil are usually increased following the application of organic amendments. This is because of the presence of sugars and amino acids as simple molecules in organic amendments that contribute to microbiological activity and fertility and elevated levels of enzymes secreted by soil microbes. To investigate the soil microbiological activity after the addition of soil amendments, three enzymes that control the C, N, and P cycles should be monitored in the plant rhizosphere zone, which is defined as the zone of increased microbial and enzyme activity where soil and root make contact. An increase of organic waste originated from different humans and productive activities is a continuous concern. Waste application (i.e., municipal sewage sludge, chicken manure, horse manure, and cow manure) to soil is proposed as a solution to disposal problem. This practice is popular in the agricultural fields because of the value of this waste as organic fertilizer. At KSU, numerous studies have been conducted on organic soil amendments and their impact on crop yield and quality, soil erosion and nutrient availability, soil enzymes activity, and bioremediation of heavy metals in organic amendments.

**Keywords:** sewage sludge, chicken manure, horse manure, yard waste, soil enzymes, antibiotics, heavy metals

## 1. Introduction

Organic fertilizers are derived from municipal sewage sludge (SS), or chicken manure (CM), horse manure (HM), blood and bone meal, and all manures are examples of organic fertilizers. Organic fertilizers also include vegetable matter (i.e., cottonseed meal, vegetable remains, and yard waste compost). There is often low available concentrations of nutrients in organic fertilizers used in agricultural production. However, organic fertilizers have important functions that cannot be gained from synthetic inorganic fertilizers, they increase soil organic matter, improve soil physical structure, enhance soil fungal and bacterial activity, and reduce eutrophication (excess N and P in natural water resources), provide low-cost adsorbents that binds with agricultural contaminants and prevent natural water contamination by pesticides and inorganic fertilizers [1], and hence, reducing the impact of xenobiotics on surface and groundwater quality. In addition, over the last 50 years, the amount of N and P pollution entering our nation's waters has escalated dramatically. Thirty percent of US streams have high levels of N and P contamination and drinking water violations due to nitrates and phosphates that have been doubled in the last 8 years [2] due to over application of inorganic fertilizers. Accordingly, environmentally and economically viable agriculture requires the use of cultivation practices and innovative technologies that maximize agrochemical efficacy while minimizing their side-effects.

Organic farming “farming without chemicals” requires organic fertilizers. While such a definition is concise and clear, it is unfortunately untrue and misses out on several characteristics which are of fundamental importance. All materials, living or dead, contain chemical compounds; therefore, organic farming utilizes chemicals. The United States Department of Agriculture (USDA) has framed a handy definition of organic farming: “Organic farming is a production system, which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators, and livestock feed additives.” The potential health hazards of pesticide residues, nitrates, and phosphates resulting from conventional agriculture are now receiving attention. There is growing scientific evidence about the positive quality aspects of organically produced food like higher dry matter and vitamin content and improved storage quality. Unlike conventional agriculture, organic farming has not been blessed with extensive research and development, nor have organic farmers had the back-up of advisory services. Organic farming needs a continued research efforts, and it is agriculture for our future.

## 2. Justification for using organic amendments in agricultural production

As more municipal sewage sludge (SS) treatment districts turn to composting as a means of sludge stabilization and because of the rapid growth in the poultry industry, significant chicken manure (CM), and municipal SS generation will become available in increasing quantities. Recycling wastes such as SS and CM for use as a low-cost organic fertilizer resulted in a positive effect on the growth and yield of a wide variety of crops and promoted the restoration of ecologic and economic functions of soil. The organic matter content of composted soil amendments is high, and its addition to agricultural soils often improves soil physical and chemical properties and enhances soil biological activities. Composts provide a stabilized form of organic matter that improves the physical properties of soils by increasing nutrient and water holding capacity, total pore space, aggregate stability, erosion resistance, temperature insulation, and decreasing apparent soil density [3, 4]. Antonious et al. [5–8] reported that SS and CM, that must be disposed, are excellent fertilizers.

Demand for food is ever increasing and much of future plant production systems will depend on fertilizers. In the United States, about 317 million tons of animal manure is produced annually from about 238,000 animal feeding operations [9], and nearly 90% of about 11.4 million tons of poultry litter produced annually is applied as fertilizer [10]. The current rapid growth in the poultry industry has resulted in significant manure generation [11]. Poultry litter contains all essential plant nutrients (N, P, K, S, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn) and has been documented as an excellent fertilizer [12]. SS is rich in organic matter, and it acts much like slow release organic fertilizer that maintains productive soil and stimulates plant growth [13, 14]. The use of CM and SS as soil amendments in land farming provides a constructive means of waste disposal and a viable method for improving soil fertility and physical properties [14, 15]. Agricultural uses of SS have shown promise for a variety of field crops (e.g., maize, sorghum, and forage grasses) and production of vegetables (e.g., lettuce, cabbage, beans, potatoes, and cucumbers) [3,4]. The literature review revealed that there is lack of information regarding the impact of organic amendments on plants nutritional and antioxidant properties. Investigators have focused on the plant yield and soil physical and chemical properties following the incorporation of soil amendments with very little information on the plant nutritional and antioxidant contents. Chemical analysis of soil amended with CM and SS revealed a significant increase in organic matter, N, P, and K content, the primary nutrients required to achieve target crop yields. Vitamin C concentration decreased in the leaves of collard and kale greens grown in no-mulch native soil compared to plants grown in CM and SS amended soil [16].

Addition of organic amendments, such as yard waste compost [17], straw [18], manure [19], tree leaf mulch [20], wood products [21], chipped wood from twigs [22], have been found to increase soil organic matter. The literature review indicated that leaves of collard plants grown in soil amended with SS contained the greatest concentrations of glucosinolates (bioactive compounds) which could play a significant role in sustainable agriculture as alternative organic tools for soil-borne disease management in conventional and organic agriculture [1, 23, 24]. High-quality kale plants (US No. 1) obtained from SS and CM amended soil were also greater compared to no-mulch native soil. In a similar study, total pepper fruit harvest was increased by 15 and 34% after the addition of CM and SS, respectively, to native soil. Whereas the number of cull fruits, the fruits that failed to meet the requirements of the USDA grades, was low in soil amended with yard waste (YW) compared to SS and CM amended soils [25].

## 3. Organic farming and the market for organically produced food

Consumer concern, over high levels of saturated fats, sugar and salt in food, as well as the risks from food additives and synthetic pesticide residues, has stimulated the demand for healthy food and led to significant changes in the food sector, including the active promotion of additive-free foods. These concerns have contributed to the development of the market for organically produced food that uses organic fertilizers. The development of the market for organically produced food has been largely consumer led. As a result, organic farming became one of the fastest growing segments of US agriculture since 1990s; producers, exports, and retailers are still struggling to meet consumer demand for a wide range of organic products. Organic farming, the use of organic fertilizers and organic pesticides, is increasingly being recognized as a potential solution to many of the policy problems facing agriculture in both developed and developing countries and has become an established part of the farming scene.

During the past 15 years, field studies were conducted at Kentucky State University (KSU) Research Farm (Franklin County, KY, USA) on a Lowell silty-loam soil (2.2% organic matter, pH 6.7) to study the impact of manure (SS and CM) and YW on chemical composition of treated crops, crop yield, and quality. Eighteen (18) standard plots 22 × 3.7 m each were established, and the field study area was a randomized complete block design with three replicates for each of the three tested soil management practices tested (SS, CM, and no-mulch treatments). The soil in six plots in this design was mixed with SS from the Metropolitan Sewer District, Louisville, KY (**Figure 1**) at 15 t acre$^{-1}$ on dry weight basis.

**Figure 1.** Sewage sludge granules obtained from the Metropolitan Sewer District, Louisville, KY, USA.

Six plots were mixed with CM obtained from the Department of Animal and Food Sciences (**Figure 2**), University of Kentucky, Lexington, Kentucky, at 15 t acre$^{-1}$ on dry weight basis, and six plots were used for comparison purposes. The plots were transplanted with collard seedlings (*Brassica oleracea* cv. Top Bunch) of 45 days old. Results revealed that soil incorporated with SS increased soil myrosinase activity compared to soil incorporated with CM and no-mulch native soil. Across all treatments, SS and CM increased soil organic matter content from 2.2% in native soil to 4.2 and 6.5%, respectively. The greater soil urease and invertase activities in soil amended with SS provided evidence of increased soil microbial population (data not shown).

**Figure 2.** Chicken manure obtained from the Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky, USA.

In summer 2015, a field trial was conducted at the University of Kentucky South Farm (Lexington, KY). Arugula (*Eruca sativa*) and mustard (*Brassica juncea*) were grown in 30' × 144' beds of freshly tilled soil. Each bed, measuring 12' × 30', was divided into three replicates in a randomized complete block design (RCBD) with four soil treatments. The entire study area contained 24 plots (2 crops × 3 replicates × 4 treatments). The treatments were (1) SS amended with soil, (2) CM amended with soil, (3) horse manure (HM, **Figure 3**) amended with soil, each at 15 t acre$^{-1}$, and (4) no-mulch bare soil used for comparison purposes. The results in **Table 1** revealed that soil amended with SS increased plant biomass production in arugula and mustard by 26 and 21%, respectively, compared to no-mulch (NM) native bare soil [24].

**Figure 3.** Horse manure obtained from Kentucky horse park, College of Agriculture, University of Kentucky, Lexington, Kentucky, USA.

| Soil amendment | Root weight, g | Shoot weight, g | Plant weight, g |
|---|---|---|---|
| **Arugula** | | | |
| SS | 74.24 a | 290.95 a | 365.20 a |
| HM | 54.00 b | 247.21 b | 301.21 b |
| CM | 41.64 c | 235.60 c | 277.23 c |
| NM | 30.08 c | 240.45 c | 270.54 c |
| **Mustard** | | | |
| SS | 61.43 b | 426.60 a | 488.00 a |

| Soil amendment | Root weight, g | Shoot weight, g | Plant weight, g |
|---|---|---|---|
| HM | 67.21 b | 393.10 b | 460.30 b |
| CM | 75.87 a | 307.40 d | 383.30 c |
| NM | 44.24 c | 341.90 c | 386.20 c |

Statistical comparisons were carried out among soil amendments for each parameter tested.
Each value is an average of three replicates.

**Table 1.** Mean weights of arugula and mustard plants grown under four soil management practices.

Knowledge about the environmental problems and adoption of appropriate solutions and practices to enhance and protect soil quality require timely delivery of research and educational technology. Attempts to improve the efficiency of biofumigation have focused on selection of biofumigant crops with high glucosinolates (GSLs) content [26]. The use of soil amendments might reduce the biomass needed to produce significant concentrations of isothiocyanate (ITCs) generating GSLs in Brassica plants for greater biofumigant potential. Soilborne organisms are becoming more difficult to control due to pathogen resistance and restricted use of some pesticides. Brassica species produce a significant amount of GSLs in their tissue. When GSLs are hydrolyzed by the enzyme myrosinase which is also present in the Brassica plant tissues, a range of products are produced which include the volatile biocidal ITCs that is similar to the active ingredient in the nematicide, metam sodium (Vapam). New soil management practices are needed to develop and expand our knowledge and technical means of agricultural production systems related to GSLs and plant protection. The problems of soil deterioration and erosion associated with intensive farming systems and the use of synthetic pesticides have generated considerable interest in less expensive and more environmentally compatible production alternatives such as recycling wastes from several processing operations for use as fertilizers in land farming to provide high-quality organic amendments. Approximately 41,511 water body impairments across the US are attributed to synthetic pesticides and of that total 1300 water body impairments are only from the state of Kentucky [27]. Brassica plants (such as mustard and arugula) have been shown to release biotoxic compounds (GSLs) or metabolic by-products against bacteria, fungi, insects, nematodes, and weeds. When plants containing GSLs are physically disrupted, the hydrolytic enzyme myrosinase is released from ruptured cells, hydrolyzing GSLs primarily to ITCs, glucose, and nitrile products. Incorporation of allelopathic Brassica tissues, such as mustard and arugula, into soil suppresses soil-borne pests due to the biofumigant properties of the highly toxic ITCs, and moderately toxic non-glucosinolate S-containing compounds [28]. ITCs, physiologically active compounds, are the major products of hydrolysis of GSLs that are released when myrosinase (thioglucosidase), a degradative enzyme, comes into contact with GSLs in plant damaged tissues. This technology could be explored in organic agriculture as alternative to synthetic fungicides.

## 4. Vermicomposting

In agricultural practices, vermicomposting is the product or process of composting worms, usually red wigglers, white worms, and other earthworms such as *Eudrilus eugeniae* to create a heterogeneous mixture of decomposing vegetable or food waste and animal bedding materials to promote biotransformation of organic waste into organic fertilizer and contribute to sustainable agricultural practices. Researchers, government agencies, and farmers are seeking new ways to manage and utilize agricultural wastes to beneficial use. This way of environmental management could be used as a green technology to bio-covert plant residues or animal manure residues into nutrient rich in organic fertilizers [29]. Many factors such as available moisture, particle size, and organic content contribute to the growth of earthworms [30]. Earthworms might lose weight and die if they are fed with materials such as rice residues that are rich in lignin, even if the substrate is fortified with nitrogen-rich amendments to decrease the initial C/N ratio [31]. According to Edwards [32], the basic principle in earthworm breeding systems is to regularly add small batches of wastes in the composting chamber to allow worms to process successive layers of waste.

Data revealed that earthworm growth was lower in treatments that contained high percentages of cow dung (CD). Results also showed that *E. eugeniae* growth was reduced when the proportion of CD in feed substances was increased. This could be due to the earthworm preference of feed substances that may favor rice straw (RS) than CD, and possibly due to inadequate control of ammonia volatilization, resulting from inadequate pre-treatment of cow manure to remove urea present in the initial material. Similarly, Chan and Griffiths [33] found that earthworms feed that contained untreated pig manure killed the worms within few hours. This is because of the high sensitivity of earthworms to ammonia and presence of high concentrations of cations in livestock manure. Accordingly, availability of pre-treated cow manure could be used to enhance the reproduction and growth of *E. eugeniae* for use in composting and organic agricultural production.

## 5. Soil organic carbon and nutritional composition of crops grown in organic vs. conventional fertilized soil

Soil carbon includes both inorganic carbon as carbonate minerals, and organic carbon as soil organic matter. Carbon sequestration is the long-term storage of carbon in oceans, soils, vegetation (especially forests), and geologic formations. Soil carbon sequestration is a process in which $CO_2$ is removed from the atmosphere and stored in the soil carbon pool. About 35% of $CO_2$ anthropogenic emissions are related to changes in land use [34]. Methane ($CH_4$) and nitrous oxide ($N_2O$) are usually found in the atmosphere at much lower concentrations than $CO_2$. However, these two gases are the big contributors to the greenhouse effect, which is 298 times greater for $N_2O$ and 25 times greater for $CH_4$ [35]. Carbon binds to minerals in soil that are just a few thousandths of a millimeter in size, and it accumulates there almost exclusively on rough and angular surfaces [36]. The rough mineral surfaces provide an attractive habitat for microbes that convert the carbon and play a part in binding it to minerals.

Organic wastes are usually rich in carbon and nitrogen, and their addition increases the soil content of labile carbon, accelerates the activity of soil microbes, and increases nitrification and denitrification rates. $CO_2$ emissions from the soil are the result of a combination of heterotrophic and autotrophic respiration, and both processes could have been stimulated by the addition of organic compost [37]. Increases in soil $CO_2$ fluxes in agricultural soils after the disposal of organic wastes are frequently observed [37–39]. De Urzedo et al. [40] reported that the application of organic wastes increased $CO_2$ emissions in the soil, as has been observed in other studies, possibly reflecting the supply of labile C to soil microorganisms, raising the microbial activity and consequently increasing the rate of soil respiration [39]. Cheng et al. [41] studied the impact of fertilizer type (organic or inorganic) on N, P, and K concentrations in runoff water from agricultural field. Although the N, P, and K loss in runoff were low, their concentrations were significantly higher from inorganic fertilizer (Scott's Turf Builder®) obtained from the Scotts Company LLC, Marysville, OH, USA, applied at the manufacturer's recommended rate of 57 kg $acre^{-1}$ than those treated with organic fertilizer (Nature's Touch® containing enzymes) obtained from Garden Way LLC, Cleveland, OH, USA, and applied at the manufacture's recommended rate of 79 kg $acre^{-1}$.

Organic systems eliminate synthetic agrochemicals and reduce external inputs to protect and improve environmental quality. The perception among organic food consumers is that organically produced crops possess higher nutritional composition. In fact, the comparisons between organically and conventionally grown crops are not experimentally valid due to the variation in crop varieties, timing in fertilization, and handling and storage after harvesting. Investigators are not in complete agreement about the nutritional composition of crops grown in organic vs. conventional fertilized soils. Some investigators found that fruits of organic crops contain more minerals and vitamins than conventional crops [42, 43]. Worthington [44] compared the nutritional quality of organic vs. conventional crops and concluded that organic crops have significantly greater levels of iron, magnesium, and phosphorus. Whereas several other authors have concluded that very few compositional differences exist, although there are reasonably consistent findings for higher nitrate contents in conventionally produced vegetables [42, 45, 46]. Another investigation found higher nitrate concentrations in organically grown compared to conventionally grown crops [47]. In organic grown carrot, lettuce, and potato, Hoefkens et al. [48] reported that they contained lower nitrate concentrations compared to the higher nitrate levels in organic spinach. The low concentration of nitrate in edible plants has many advantages for human health. This is because high concentrations of nitrate in edible plants may cause many health problems in humans, such as methemoglobinemia (a blood disorder in which an abnormal amount of methemoglobin, a form of hemoglobin is produced), and can cause some types of cancer [49]. Johansson et al. [50] assessed organically and conventionally grown tomatoes for a variety of different attributes. They found no differences in acidity, sweetness, and bitterness, but did find that organic tomatoes were less firm, less juicy, and redder. Hallmann and Rembiałkowska [51] found that bell pepper fruits of plants grown in organic agriculture have significant concentrations of vitamin c, $\alpha$-carotene, cis-β-carotene, total carotenoids, and total phenolic acids, such as chlorogenic acid and flavonoids, such as quercetin D-glucoside, quercetin, and kaempferol, compared to fruits of plants grown under conventional agricultural practices.

Nitrogen (N) is a nutrient element that plants require in amounts larger than that of any other soil supplied element. Most plants can utilize both $NH_4^+$ and $NO_3^-$; however, $NH_4^+$ at high concentration can be toxic to plants [52]. $NH_4^+$ as a sole source of N can result in a variety of negative effects to most plant species. The negative effects include a reduced plant photosynthetic rate, lower dry weights, reduced root growth, and a reduced rate of water uptake [52–54]. However, plants differ in their sensitivity to $NH_4^+$ toxicity. Onion, a member of the Amaryllidaceae Family, is known to be fairly tolerant to high concentrations of $NH_4^+$ [54, 55]. On the contrary, Hippeastrum, a member of the Amaryllidaceae, suffered reduced bulb size when fertilized with $NH_4^+$ as the sole source of N [56]. Shoot and roots of ornamental shrub, "*Doublefile viburnums*" (*Viburnum plicatum* var. *tomentosum*) contained lower $NH_4$ (ammonium) concentrations when $NH_4$ was supplied alone compared to $NH_4$ supplied with $NO_3$ (nitrate) or a combination of $NH_4$ and $NO_3$ [57]. Al-Ghamidi et al. [58] found that date palm seedlings grown in a soilless hydroponic media had the greatest leaf area and root weight when $NO_3$ was used as the sole nitrogen (N) source whereas date palm seedlings had a lower root: shoot ratio when $NH_4$- or urea-N were used as N source [58].

Addition of organic matter could affect the adsorption, movement, and biodegradation of pesticides. The addition of organic waste, specifically to agricultural soils, is a practice that has been carried out for centuries, due to its fertilizer properties and contribution to the physico-chemical and biological properties of the soil [59, 60], which is a common agricultural practice in diverse countries [61–63]. Addition of organic waste to soil contributes to the enhancement of active humified components, such as humic acid (HA) and fulvic acid (FA) [64], which exert an important role in geochemical processes as sources of nutrients for plants and microorganisms, in acid–base buffering capacity of soils, and promoting a good soil structure, thereby improving aeration and moisture retention [65, 66]. In agronomic aspects, addition of organic waste enhances biological activity and fertility, because through this addition, nutrients and diverse groups of microorganisms, such as bacteria, fungi, and actinomycetes [67–71] play an important role in the fate of xenobiotic compounds such as heavy metals, aromatic hydrocarbons, and pesticides [72–76].

Some organic wastes are associated with inorganic and organic toxic compounds, such as heavy metals and pesticides [60, 72, 73, 77], that when incorporated into soil, constituting a pollution problem in soils and therefore causing toxic effects on microorganisms and crops [77, 78]. Excessive concentrations of heavy metals in soil can be toxic to microorganisms [79]. On the other hand, soil microorganisms need heavy metals for their growth and activity. Elevated concentrations of heavy metals in soil have shown a reduction in the activity of enzymes secreted by beneficial soil microorganisms. SS contains heavy metals, and the release of these metals into the soil solution and uptake by plants growing in soil amended with SS could be phytotoxic.

The efficiency of some pesticides and their persistence and potential as environmental contaminants depends on their retention and degradation on soil constituents [80]. The OM in soil amendments is reported as a major controller component in the sorption, transformation, and transport of many organic pollutants in soil [81]. Soil amendments could be used to intercept pesticide-contaminated runoff from agricultural fields, and this practice might provide a potential solution to pesticide contamination of surface and seepage water from farmlands. Residues of two of the herbicides commonly used in agriculture, DCPA [1,4-

Benzenedicarboxylic acid, 2, 3, 5, 6-tetrachloro-, dimethyl] ester, and metribuzin [4-amino-6-tert-butyl-4, 5-dihydro-3-methylthio-1,2,4-triazin-5-one] were monitored in runoff and infiltration water from agriculture fields. Metribuzin was detected in Ohio Rivers and Iowa wells and groundwater [82, 83]. The major mechanism by which metribuzin is lost from soil is microbial degradation. Losses due to the volatilization or photodegradation are not significant under field conditions [84, 85]. Sharom and Stephenson [86] also found that metribuzin mobility was inversely related to soil organic matter content. Similarly, soil amended with CM or SS retained DCPA residues up to 99 days compared to NM treatments [87]. Accordingly, soil OM has a major role in the transformation, transport, and adsorption/desorption of most soil organic and inorganic pollutants [81]. Because organic amendments used in agricultural production contain significant amounts of OM, it has been found that this practice (recycling waste and use of organic amendments in land farming) has a significant impact on the fate and transport of pesticides under environmental conditions. On the other hand, the addition of organic amendment to soil normally results in an increase in the microbiological activity due to the availability of simple organic molecules such as sugar and amino acids [88]. The application rate of soil amendments for agricultural soil is proposed according to nitrogen, phosphorus, and potassium requirements. However, a specific rule for animal manure application to soil does not exist, but is proposed through good agricultural practices.

## 6. Heavy metals in organic soil amendments and potential bioaccumulation in edible plants

In consideration of the enormous worldwide consumption of fruit and vegetables, that is, various *Capsicum* spp., the use of capsaicin as an additive in food for medicinal and other beneficial purposes requires continued monitoring of the potential harmful effects of heavy metals (also known as trace elements) accumulation in pepper fruits and other edible plants when SS or any other soil amendment is used in growing plants. The storage of great variety of molecules in animal and plant cells and the mechanism of this storage or bioaccumulation allow living organisms to accumulate nutrients and essential minerals. During this process, plants and animal cells can also absorb and accumulate harmful substances from the soil solution such as heavy metals. There is limited information on heavy metal (such as Cd, Ni, and Pb) in soil amendments and absorption by edible plants. Presently, heavy metals in the food chain are one of the pollutants of most concern around the world [89]. However, reducing the accumulation of heavy metals in edible plants can be achieved using biochar. Survey of current adsorbents indicated that the large surface area of activated carbon (ranging from 500 to 2000 $m^2$ $g^{-1}$) makes it a perfect candidate for heavy metals adsorption. Results indicated that biochar effectively reduced the total amount of nitrate, ammonium, and phosphate in leachates by 34, 35, and 21%, respectively, relative to native soil alone [90]. Biochar adsorption of ammonia decreases $NH_3$ and $NO_3$ losses during composting and after manure applications and offers a mechanism for developing slow release fertilizers [91]. Antonious et al. [14] revealed that total metal content of soil and/or soil amendments is not a good predictor/

indicator of the metal concentrations in plants due to the fact that only a fraction of the total metals is available for plant uptake.

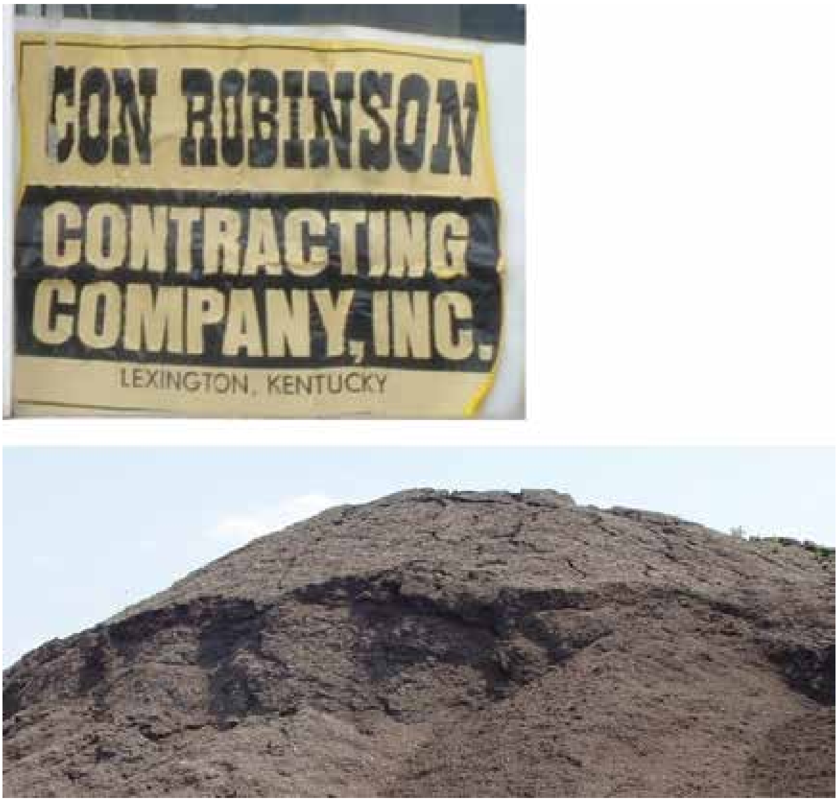

**Figure 4.** Yard waste made from yard and lawn trimmings, and vegetable remains obtained from Con Robinson Contracting Company, Inc. Lexington, Kentucky, USA.

Municipal SS, CM, horse manure (HM), and yard waste (YW) compost (**Figures 1**, **2**, **3**, and **4**) provide amendments useful for improving soil structure and nutrient status. However, soil amendments contain heavy metals that may potentially affect soil microbes and the enzymes they produce. Accordingly, it is important to monitor $NH_3^+$, $NO_3^-$, phosphate, and trace elements (Ni, Pb, Cd) concentrations in native soil amended with SS, CM, and their transport into runoff, seepage water, and potential bioaccumulation in edible plants at harvest [92]. According to the USEPA Part 503 Biosolids rule, 10 elements (As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, and Zn) in SS applied to soil are regulated [93]. The USEPA has defined limits for clean sludge in terms of its trace elements content (Zn 1400, Cu 1500, Ni 420, Cd 39, Pb 300, Cr 1200, Mo 75 mg $kg^{-1}$) and reported that sludge could be added to agricultural soil if these elements are below the standard disposal limits [94]. However, total metal concentrations in SS or even native soil do not furnish sufficient information regarding the potential availability of elements for plant uptake. Antonious et al. [14] found that the total concentration of each metal in the soil was significantly greater than concentration of metal ions available to plants. Accumulation of trace elements in plants grown in biosolids (sewage sludge) varied among plant species [92] and even among accessions of the same species [95]. Intake of trace elements-contaminated vegetables and fruits may pose risk to human health. Application of SS and CM to agricultural soils is based on crop requirements of NPK that are usually applied with a maximum application rate of 15 t year$^{-1}$ [14]. In fact, there is no specific rate for animal manure application to soil, but the rate of application is proposed through good agricultural practices of fertilizer use. **Table 2** shows the bioaccumulation factor (BAF) of seven heavy metals from soil into pepper fruits grown under four soil management practices at Kentucky State University. BAF is defined as the concentration of metal in plant tissue divided by the concentration of the same metal in soil, expressed on dry weight basis. According to Antonious et al. [95], BAF values less than one are desirable and represent levels that do not pose human health hazards. Results revealed that BAF values in pepper fruits ranged 0–0–0.01 for Cr; 0.1–0.4 for Ni; 0.7–2.0 for Cu; 1.0–1.4 for Zn; 0.9–1.2 for Mo; 0.7–1.0 for Cd; and 0.2–0.8 for Pb.

| Heavy metal | Treatment | μg g$^{-1}$ fresh fruit | BAF |
|---|---|---|---|
| **Cr** | YW | 0.002 ± 0.001 | 0.100 |
| | SS | 0.001 ± 0.001 | 0.098 |
| | NM | 0.000 ± 0.000 | 0.000 |
| | CM | 0.00 ± 0.000 | 0.000 |
| **Ni** | YW | 0.015 ± 0.010 | 0.353 |
| | SS | 0.013 ± 0.001 | 0.198 |
| | NM | 0.012 ± 0.003 | 0.115 |
| | CM | 0.012 ± 0.008 | 0.329 |
| **Cu** | YW | 2.980 ± 0.930 | 1.976 |
| | SS | 1.113 ± 0.120 | 1.081 |
| | NM | 0.864 ± 0.235 | 0.750 |
| | CM | 1.625 ± 0.964 | 1.523 |
| **Zn** | YW | 2.015 ± 0.880 | 1.388 |
| | SS | 1.751 ± 1.002 | 1.253 |
| | NM | 1.581 ± 0.600 | 0.995 |
| | CM | 1.595 ± 0.085 | 1.053 |
| **Mo** | YW | 0.021 ± 0.001 | 0.901 |
| | SS | 0.015 ± 0.002 | 1.198 |
| | NM | 0.009 ± 0.006 | 0.869 |
| | CM | 0.017 ± 0.009 | 1.217 |
| **Cd** | YW | 0.014 ± 0.001 | 1.132 |

| Heavy metal | Treatment | $\mu g\ g^{-1}$ fresh fruit | BAF |
|---|---|---|---|
| | SS | 0.014 ± 0.007 | 1.007 |
| | NM | 0.012 ± 0.004 | 0.731 |
| | CM | 0.012 ± 0.006 | 0.876 |
| **Pb** | YW | 0.016 ± 0.009 | 0.316 |
| | SS | 0.037 ± 0.001 | 0.808 |
| | NM | 0.012 ± 0.005 | 0.222 |
| | CM | 0.035 ± 0.012 | 0.765 |

Each concentration in the table is an average of three replicates ± standard error.
YW = yard waste; SS = sewage sludge; CM = chicken manure; and NM = no-mulch bare soil.

**Table 2.** Concentrations and bioaccumulation factor (BAF) of seven heavy metals in pepper (*Capsicum annuum* L.) fruits of plants grown under four soil management practices.

### 6.1. Quantification of trace elements (Ni, Pb, Cd) in soil

To monitor the concentration of trace elements in soil following the incorporation of soil amendments, samples collected from field plots should be oven-dried at 105°C and ground manually with a ceramic mortar and pestle to pass a 1 mm sieve. Ten ml of concentrated nitric acid ($HNO_3$) is added to each 1-g of sieved dry powder, and the mixture is allowed to stand overnight and then heated for 4 h at 125°C on a hot plate. This mixture should be diluted with double-distilled water (50 mL) and filtered through filter paper No.1 before quantification of heavy metals. Mehlich-3 extractable Cd, Ni, and Pb can be determined in soil extracts using inductively coupled plasma (ICP) spectrometer [7, 14, 96, 97].

### 6.2. Quantification of trace elements (Ni, Pb, Cd) in edible plants at harvest

For the determination of trace elements in plant tissues, fruit samples of the growing plants of comparable size are collected at random, washed with deionized water, and dried in an oven at 65°C for 48 h. The dried samples should be grounded manually with ceramic mortar and pestle to pass through 1 mm sieve. Bioavailability of heavy metals can be defined as the total metals available in the soil, whereas the bioaccumulation factor (BAF) is defined as the ratio of the metal in the plant divided by total metal in the soil [98]. As described earlier, BAF values below 1 are desirable and present levels that do not pose human health hazards, while BAF values >1 would be less favorable. Assessing the bioavailability and speciation of trace elements in native soil and soil mixed with organic amendments is crucial to determining the environmental impact of contaminated soils.

## 7. Antibiotics and hormones

Sewage sludge (SS) obtained from waste water treatments plants contains organic matter and nutrients that, when properly treated, can be a valuable and safe resource for agriculture production systems [97, 99, 100]. Kentucky State University has been a pioneer in the use of municipal SS for land farming. Extensive work has been conducted by Antonious for more than 20 years. Composted municipal SS was proven to be a valuable fertilizer for many vegetable crops, including potatoes, peppers, broccoli, squashes, tomatoes, eggplants, onions, melons, cabbages, kales, and collards. After monitoring the bioaccumulation of heavy metals in plants grown in native soil mixed with municipal SS, data revealed that most of the heavy metals concentrations in edible plants were below their permissible limits and the plants were safe for human consumption. One of the outputs of this work was the transfer of this technology to the organic growers. Currently, municipal SS is commercially available as an organic base fertilizer known as Louisville Green (www.louisvillegreen.com). The intensive research carried out at KSU revealed the safety of SS as nutrient-rich materials resulting from the treatment of SS at the Metropolitan Water Plant Facility in Louisville, KY, (**Figure 1**).

There is an emerging concern regarding the impact of endocrine disrupting compounds (EDCs) in reclaimed water and biosolids [101]. EDCs are exogenous agents (agents from outside the organism or system) that have the potential to interfere with the production, release, transport, metabolism, binding, or elimination of the natural hormones responsible for the body regulation of developmental processes [102]. EDCs could be one or more of the following chemicals: pesticides, plasticizers, natural chemicals found in plants (phytoestrogens), pharmaceutical products, or hormones that are excreted in animal or human waste. Natural and synthetic estrogens are some of the most potent EDCs found in municipal wastewater. EDCs have been attributed as a cause of reproductive disturbance in humans and wildlife. Human exposure to these chemicals in the environment is a critical concern with unknown long-term impacts [103].

The group of molecules identified as endocrine disruptors is highly heterogeneous and can be classified into several categories, such as hormones (natural and synthetic estrogen or steroids), pharmaceutical and personal care products, industrial chemicals, pesticides, combustion byproducts, and surfactants [104]. There is an increasing evidence that EDCs poses a health risk in humans and animals. EDCs have been associated with adverse effects on reproduction, breast development and cancer, prostate cancer, neuroendocrinology, thyroid, metabolism and obesity, and cardiovascular endocrinology [102]. The primary source of EDCs is municipal SS. Other sources include industrial manufacturing processes and agricultural waste [105]. Wastewater treatment plants are generally not designed for the removal of trace organic compounds (i.e., detected concentration occurs at nanograms per liter) such as pharmaceuticals and potential EDCs [105]. The treatment efficiency of most pharmaceuticals and personal care products was as low as 35% [104]. Variations in wastewater treatment processes and operational conditions are generally regarded as the reason for fluctuations in removal efficiencies and effluent concentrations [101]. EDCs removal methods fall into three categories: physical removal, biodegradation, and chemical advanced oxidation. Biodegradation is the primary removal mechanisms for EDCs in activated sludge systems, which are commonly used biological treatment techniques for municipal wastewater treatment. About 90% of natural steroids are degraded in the activated sludge system [103].

Other technologies have been studied to remove the remaining EDCs. These technologies include chemical removal, activated carbon, chlorination, ozonation, ultraviolet (UV) irradiation, and membrane separation [101]. Treatment plants are seeking viable alternatives to alleviate concerns over cost, energy consumption, and brine disposal [105]. The use of ozone is a unique option because it is a highly effective oxidant for removing the majority of trace organic contaminants, particularly the steroid hormones, and because it has potential for reduced energy and chemical requirements [106]. Despite the effectiveness of ozone, there are fewer than 10 recycled water facilities (RWF) in the US that currently use ozone [105]. Some plants use UV treatment alone which has not shown to be an economically reasonable option for removing estrogens from wastewater. The application of advanced oxidation processes such as photo-oxidation which combines UV irradiation with ozone has achieved a high removal efficiency of EDCs [102]. EDCs are ubiquitous compounds with small molecular mass (<1000 Daltons), present in the range of ng $L^{-1}$. These compounds are biologically active, and their trace level concentrations make the detection and analysis procedures very challenging [104]. Highly sensitive measurements are necessary, including chemical monitoring, such as liquid chromatography-tandem mass spectrometry, gas chromatography-tandem mass spectrometry, high-performance liquid chromatography, and sensitive bioassay [103]. Determining the presence of EDCs in reclaimed water and municipal SS is important, because these contaminants may be introduced into the food chain through bioaccumulation. The use of SS and reclaimed water in agriculture is expected to improve rural communities and provides food crops that are consumer safe with little or no contaminants. Eleven EDCs (atrazine, bisphenol A, linuron, 4-nonylphenol, butylbenzyl phthalate, diethylhexyl phthalate, 17β-estradiol, 17$\alpha$-ethynylestradiol, estrone, octylphenol, and triclosan) should be monitored prior to land application. In addition, contaminant metals (arsenic, cadmium, lead, mercury, nickel, and zinc) should be also monitored. Data of this type of research are critical for understanding the behavior and potential accumulation of EDCs and heavy metals within the particular food, when reclaimed water and biosolids [107] are used for growing edible plants.

## 8. Microorganisms and soil enzymes

Government agencies, research scientists, farmers, and all citizens are looking for a healthy environment. Soil microorganisms are present in low amounts; however, they have a significant role in nutrient recycling to maintain the NPK, the main plant nutrients at the required level. Microorganism's biomass is often related to crop type, soil type, and landscape [108, 109]. Soil fungi usually constitute 75–95% of the soil microbial biomass and when we include bacteria, they are responsible for about 90% of the total energy flux of organic matter mineralization in soil [110]. Among the main groups of microorganisms living with plant roots are saprotrophic fungi and mycorrhiza. Urease, invertase, phosphatase, cellulose, dehydrogenase, and amylase are among enzymes secreted by soil microorganisms (bacteria, fungi, protozoa, algae). These enzymes are responsible for degrading complex forms of organic matter and xenobiotics in soil and water ecosystems. Polysaccharides (sticky substances) are also produced by soil microorganisms. These sticky substances play a significant role in sticking and adhering soil particles together and help the soil to resist erosion that can reduce agricultural productivity [111].

**Figure 5.** Kale and collard plants grown in soil amended with sewage sludge applied at 15 t acre$^{-1}$ at Kentucky State University Harold Benson Research and Demonstration Farm (Franklin County, KY, USA).

**Figures 5** and **6** revealed that kale, collard, and pepper were successfully grown in SS and CM amended soils that contained greater soil urease and invertase activities. On the contrary yard waste (**Figure 4**) used at KSU Research Farm revealed that, its application in spring broccoli did not alter soil urease or invertase activities to any appreciable extent (data not shown).

SS increased soil urease and invertase activity (**Figure 7**). Urease is an enzyme that depends on Ni for its activity [112]. Accordingly, Ni in SS might be the cause of elevated urease activity. This increase could be due to the presence of urea, the substrate of the enzyme urease. SS obtained from municipal plants contains great amounts of enzymatic substrates [113]. SS used at KSU for growing many vegetable crops including broccoli contained 1.2 μg Ni g$^{-1}$ dry soil. However, broccoli plants showed normal growth in the field without any apparent symptoms of Ni toxicity or deficiency. Results indicated that the addition of SS to native soils has increased total crop marketable yield compared to no-mulch native soils (data not shown). Indicating that incorporation of organic materials, such as municipal SS, into soil promotes microbiological activity. Microbial activity and soil fertility are closely related because it is through the biomass that the soil mineralization of the important organic elements (C, P, and N) occurs. Accordingly, soil biological monitoring is a potential and sensitive indicator of soil ecological stress for early restoration. **Figure 7** revealed that soil invertase and urease activities were increased by 89 and 47%, respectively, in SS compared to native soil.

**Figure 6.** Hot pepper plants grown in soil amended with chicken manure applied at 15 t acre$^{-1}$ at Kentucky State University Harold Benson Research and Demonstration Farm (Franklin County, KY, USA).

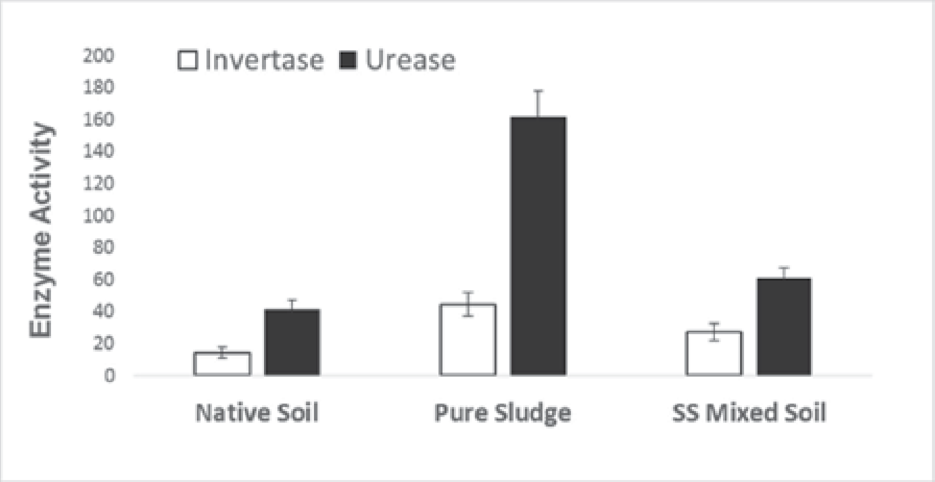

**Figure 7.** Invertase activity expressed as mg glucose released g$^{-1}$ dry soil and urease activity expressed as mg $NH_4$–N released g$^{-1}$ dry soil h$^{-1}$.

Tabatabi and Bremner [114] used a simple method for the quantification of urease activity in soil. In their method, they used a few grams of soil (about 5 g) and added 10 mL of 0.1 M phosphate buffer in a volumetric flask kept in water bath at 30°C for 1 h to allow the soil temperature to equilibrate. The liberated $NH_4^+$ ions were determined by the selective electrode method [115]. For standardization, a series of standard solutions of $NH_4$ Cl covering the concentrations of 0.1–100 μg $NH_4$–N mL$^{-1}$ of water was prepared. In this method, urease activity was expressed as mg $NH_4$–N released $g^{-1}$ dried soil during the 1 h incubation at 30°C [17]. For invertase quantification in soil, the method described by Balasubramanian et al. [116] was used. For standardization, a calibration curve was obtained using analytical grade glucose in the range of 10–50 μg $mL^{-1}$ glucose standards.

Addition of SS, as a source of organic matter, to agricultural soil reduced the C/N ratio (**Table 3**). Kizilkaya and Bayrakli [117] added nitrogen as ammonium sulfate, $(NH_4)_2SO_4$, to reduce the C/N ratio in soil from 9:1 to 6:1 and 3:1. This resulted in a rapid increase in soil enzymatic activities. Investigators [118, 119] reported that reducing the C/N ratio is indicative of a high OM decomposition rate. On the contrary, OM with high level of C/N ratios has relatively low rates of decomposition and causes low rates of N-mineralization [120] in soil.

| Soil parameters | Sewage sludge incorporated with native soil | Yard waste incorporated with native soil | Native soil |
|---|---|---|---|
| % N | 0.39 a | 0.32 a | 0.15 b |
| % P | 0.31 a | 0.24 a | 0.18 a |
| % K | 0.23 a | 0.28 a | 0.25 a |
| % C | 3.7 a | 3.8 a | 1.6 b |
| % Organic matter | 3.3 a | 7.6 a | 2.7 b |
| C/N ratio | 9.2 c | 11.9 b | 17.7 a |
| pH | 8.5 a | 7.3 b | 6.9 b |

Statistical comparisons were done between three soil management practices (each replicated six times) for each soil parameter. Values in each row accompanied by the same letter are not significantly different ($P > 0.05$) using Duncan's multiple range test.

**Table 3.** Soil properties in the rhizosphere of broccoli plants grown in native soil amended with sewage sludge and soil amended with yard waste at KSU Harold Benson Research and Demonstration Farm, (Franklin County, Kentucky, USA).

## 9. Conclusion

Organic waste used as fertilizer must be safe for the environment and wildlife, and safe for all who apply and consume the food product. The simultaneous use of recycled waste to enhance soil physical, chemical, and microbial conditions could also enhance soil fertility and crop yield. Organic farming, farming without chemicals, requires organic fertilizers. While such a definition is concise and clear, it is unfortunately untrue and misses out on several characteristics which are of fundamental importance. All materials, living or dead, contain chemical compounds; therefore organic farming utilizes chemicals. The United States Department of Agriculture (USDA) has framed a handy definition of organic farming: “Organic farming is a production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, and growth regulators.” The potential health hazards of pesticide residues, nitrates, and phosphates, resulting from conventional agriculture are now receiving attention.

Thirty percent of US streams have high levels of N and P contamination and drinking water violations due to nitrates and phosphates that have been doubled in the last 8 years. Organic wastes are usually rich in carbon and nitrogen, and their addition increases the soil content of labile carbon, accelerates the activity of soil microbes, increases nitrification and denitrification rates. With the growing interest in recycling organic amendments, it is important to monitor the activity of urease, invertase, and phosphatase since these three enzymes play a significant role in the soil N, C, and P cycles, respectively. In addition, the activity of these three enzymes and other soil enzymes can be used as a direct indicator of soil health and soil microbial population and activity in the rhizosphere of growing plants (a zone of increased microbial and enzyme activity where soil and root make contact). With increasing emphasis on fertility sustainability and environmental friendliness, restoration of soil microbial ecology has become important. In agricultural practice, composting of soil with sewage sludge, chicken manure, or yard waste provides an organic amendment useful for improving soil structure and soil nutrient status and generally increases soil organic matter and stimulates soil microbial activity. In the US, about 11.4 million tons of poultry litter was produced and about 90% of this amount was used as fertilizer in agricultural production [121]. It has been found that poultry litter contains many essential plant nutrients (N, P, K, S, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn) and has been reported as excellent fertilizer [122]. It is expected that significant chicken manure generation will become available in increasing quantities because of the increasing growth in the poultry industry. In addition, as more sewage sludge treatment districts turn to composting as a viable means of sludge stabilization, sewage sludge will also become available in increasing quantities. Sewage sludge and chicken manure contain significant amounts of trace elements that may impact soil microorganisms and the enzymes they produce by blocking of either the enzyme or substrate when present in excessive concentrations. Trace elements are among the major contaminants of food supply. They are not biodegradable, have long biological half-lives, and have the potential for accumulation in edible plants grown under this practice [123] that requires environmental measurement and mitigation [124]. Trace elements may also accumulate in the different human and animal body organs leading to potential adverse effects on human health. The rate of release of trace elements from sewage sludge into soil solution and subsequent uptake by plants could also result in phytotoxicity and/or bioaccumulation. Regarding the use of horse manure as organic fertilizer, typically, a ton of horse manure contains 11 pounds of N, 2 pounds of P, and 8 pounds of K [125]. Horse manure contains about 60% solids and 40% urine [126]. During cleaning, soiled bedding removed with the horse manure may account for another 8–15 pounds of waste per day. The volume of soiled bedding removed during cleaning is almost twice the volume of manure removed but varies widely depending on management practices. As described earlier, field application of horse manure is also based on fertilizer needs of a particular crop. The approximate fertilizer value of manure from bedded horse stalls based on its dry matter content, which is about 46%, is 4 lb ton$^{-1}$ ammonium-N, 14 lb ton$^{-1}$ total N content, 4 lb ton$^{-1}$ $P_2O_5$ (phosphate), and 14 lb ton$^{-1}$ $K_2O$ (potash), whereas the fertilizer value of horse manure at 20% moisture without bedding is approximately 12–5–9 lb ton$^{-1}$ ($N–P_2O_5–K_2O$). Overall, organic amendments from animal manure are excellent fertilizers. However, there is an emerging concern regarding the impact of endocrine disrupting compounds (EDCs) in reclaimed water and sewage sludge. Most livestock grown in the US and worldwide are raised in large-scale concentrated animal-feeding operations. This high population densities require heavy use of antibiotics that can cause severe local soil, air, and water pollution.

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Chapter 8

# Physicochemical Properties of a Red Soil Affected by the Long-term Application of Organic and Inorganic Fertilizers

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Additional information is available at the end of the chapter

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## Abstract

Red soils are widespread throughout subtropical and tropical regions and are the most important resources for grain production in South China. Application of chemical fertilizers alone or chemical fertilizers combined with organic amendments is commonly practiced to improve physicochemical properties and fertility for red soils. This chapter summarizes the findings of a 22-year long-term field experiment conducted in the red soil region of south central China. Changes in soil pH, soil organic matter (OM), nitrogen (N), phosphorus (P), and aggregate distribution and stability as affected by the long-term fertilization treatments were examined and discussed. Combined application of chemical fertilizer and rice straw or pig manure significantly increased soil pH in the first 7 years, but soil pH decreased linearly at a rate of 0.04–0.07 unit yearly since then. Soil total N and total P content significantly increased during the long-term fertilization, and the effects of pig manure addition on N and P build-up were greater than that of rice straw addition. In contrast, soil total potassium (K) contents significantly decreased by the long-term fertilization. There was a significant difference between the effect of rice straw addition and pig manure amendment on various aggregate size distribution in the red soil.

**Keywords:** red soil, soil physicochemical properties, rice straw, pig manure, aggregate structure

## 1. Introduction

Red soil, which is widespread throughout subtropical and tropical regions, is the most important grain production base in South China. Red soils are naturally poor in physical conditions and are also characterized by low pH, cation exchange capacity (CEC), and fertility. Red soil also has low concentrations of P in soil solution and results in frequent P deficiency of plants [1]. The improvements of soil fertility especially plant available P level, soil pH, soil structure, and water-holding capacity in the red soil region are always a major challenge. The application of chemical fertilizer alone, and that combined with organic fertilizers, such as crop residues and farmyard manure, are two common approaches to improving soil quality for grain production [2–5]. Rice (*Oryzasativa* L.) is the main cereal crop in the red soil region. Returning rice straw containing C, N, P, K and microelements to the soil (both used as a surface cover and incorporated) has been extensively shown to increase organic matter and nutrient contents resulting in improved soil physical, chemical and biological characteristics. Crop residue is returning not only can increase crop yields, but it also can enhance soils' resistance to wind and water erosion [6, 7]. Animal manures, such as pig manure with ample N, P and K, are valuable resources to supply the needed plant nutrients and organic matters [2, 4, 5, 8, 9].

Therefore, rice straw and fresh pig manure are commonly used as organic amendments in the red soil region of China. When combined with chemical fertilizers, those organic amendments could effectively regulate soil physicochemical properties and soil fertility. Many researchers have proven that long-term application of organic-inorganic fertilizers significantly increased soil water supply capacity, promoted soil nutrient recycle and distribution, improved soil aggregate structure [2]. Therefore, in this chapter, the changes or fluctuations of soil acidity, soil organic matter, soil NPK fertility, and soil aggregate structure due to the combined application of chemical fertilizer and rice straw or pig manure in a long-term (1988–2009) experiment were summarized. Lessons learned can be used to improve nutrient management so that crop yields are optimized and the impact of food production on the environment is minimized.

## 2. The nature and properties of red soils

### 2.1. Distribution of red soils in southern China

Red soils are mainly distributed in the tropical and subtropical zone all over the world, which occupy around $6.4 \times 10^9$ ha, accounting to about 45% of the world land area. In addition, most of the red soils are distributed in the developing countries. The population living in the red soil regions is about 2.5 billion, roughly 48% of the global population [10]. Various types of yellow or red soils collectively known as red soil series, such as laterite, laterite red soil, red soil, and yellow soil (equivalent to ferrisol or ultisol [11]), are widespread in southern China. Scattered patches of similar soil are also seen in south central China. The total area of red soil in China is about $2.18 \times 10^8$ ha, covering about 21.8% of the total land area, or 28% of the farmland area. However, the population in the red soil series region accounts for 40% of the overall national population [10, 12] due to its warm and humid climate.

## 2.2. Basic properties of red soils

Rich in iron-aluminum (hydr)oxides with strong fixation capacity of phosphate, low pH, and organic matter content, and poor nutrient availability are the main yield-limiting factors for the red soils. According to the results of the second national soil census data, the fertility of major red soils was moderate or poor (**Tables 1** and **2**). Soil phosphorus (P) availability of red soils was considered seriously deficient. These serious P-deficient red soils cover most of the farmland in the region. Soil potassium (K) of the red soils is not as bad as P by 26.3 and 13.6% of the red soil area considered moderate and seriously deficient, respectively. Soil nitrogen (N) status of the red soils is situated between the P and the K with most in the moderate and serious deficient category [12, 13].

| Soil type | Nutrition level (Code) | Organic matter | Total N | Total P | Total K | Available P | Available K |
|---|---|---|---|---|---|---|---|
| | | g kg$^{-1}$ | | | | mg kg$^{-1}$ | |
| Natural soil | Fertile (A) | >35 | >1.75 | >1.0 | >30 | >10 | >150 |
| | Mild deficiency (B) | 25–35 | 1.25–1.75 | 0.6–1.0 | 20–30 | 5–10 | 100–150 |
| | Moderate deficiency (C) | 15–15 | 0.75–1.25 | 0.2–0.6 | 10–20 | 2.5–5 | 50–100 |
| | Severe deficiency (D) | <15 | <0.75 | <0.2 | <10 | <2.5 | <50 |
| Upland soil | Fertile (A) | >20 | >1.5 | >1.0 | >30 | >10 | >150 |
| | Mild deficiency (B) | 15–20 | 1.0–1.5 | 0.6–1.0 | 20–30 | 8–10 | 100–150 |
| | Moderate deficiency (C) | 10–15 | 0.5–1.0 | 0.2–0.6 | 10–20 | 5–8 | 50–100 |
| | Severe deficiency (D) | <10 | <0.5 | <0.2 | <10 | <5 | <50 |

**Table 1.** Evaluation standard of soil fertility status of the hilly upland red soil in southern China [12].

| Province (District) | Organic matter | Total N | Total P | Total K | Available P | Available K |
|---|---|---|---|---|---|---|
| | g kg$^{-1}$ | | | | mg kg$^{-1}$ | |
| Hunan | 20.6 (A$^{*}$) | 1.14 (B) | 1.03 (B) | 20.0 (B) | 8.2 (B) | 98.7 (C) |
| Jiangxi | 20.9 (A) | 1.03 (B) | 0.55 (C) | 12.4 (C) | 17.6 (A) | 91.0 (C) |
| Zhejiang | 19.8 (B) | 1.06 (B) | 0.56 (C) | 11.1 (C) | 6.9 (C) | 105.5 (B) |

$^{*}$ Capital letter in the bracket represents soil with different fertility level. A, fertile; B, mild deficiency; C,: moderate deficiency; D, severe deficiency.

**Table 2.** Average content of various soil nutrients and their corresponding soil fertility level in the upland red soils from Hunan, Jiangxi, and Zhejiang provinces, China [12].

## 3. Long-term application of fertilizers

### 3.1. Commonly used organic fertilizers in the red soil region

Commonly used organic fertilizers in the red soil region come mainly from green manure, farmyard manure, and crop residues (returned and left in place). In this area, rice and rapeseed are the main field crops covering 55 and 11% of the total cropped area, respectively. The amount of rice straw and rapeseed stalk account for 70–75% and 8.5–11% of the total crop residues in this region [14]. Double rice (two rice crops per year) and winter rapeseed is the main cropping pattern producing 9500–1200 kg hm$^2$ straw yearly, but the rapeseed only yield about 1660–6900 kg hm$^2$ residue yearly. Dry land crop straw is mainly used as livestock feed and cooking fuel, and only small portion is returned to the field. Radish (*Raphanus sativus*) and milk vetch (*Astragalus sinicus* L.) are also used as the main winter green manure which produced about 10,500–15,000 kg hm$^2$ fresh biomass yearly. Pig and cattle manure were the main livestock manures in the region [14]. Crop, especially rice, above and below ground residue is another important source of soil organic matter. The nutrient contents of major organic materials used in the typical red soil region, Yujiang Country, Jiangxi Province, China, are presented in **Table 3**

| Type | C | Total N | C/N ratio | Total P | Total potassium |
|---|---|---|---|---|---|
| | g kg$^{-1}$ | | | g kg$^{-1}$ | |
| Cattle dung | 404.1 | 18.82 | 21.5 | 7.19 | 4.37 |
| Pig dung | 368.4 | 24.36 | 15.1 | 9.36 | 2.80 |
| Milk vetch | 440.2 | 30.82 | 14.3 | 3.65 | 26.02 |
| Radish | 429.6 | 23.41 | 18.4 | 2.82 | 18.12 |
| Peanut straw | 435.6 | 12.48 | 34.9 | 0.84 | 12.61 |
| Cole stalk | 467.4 | 5.22 | 89.7 | 0.46 | 15.72 |
| Rice straw | 432.4 | 10.89 | 39.7 | 0.55 | 19.95 |
| Wheat straw | 457.5 | 6.12 | 74.8 | 0.66 | 7.90 |
| Rice root | 306.2 | 8.38 | 36.5 | 1.23 | 3.57 |
| Wheat root | 329.6 | 6.34 | 52.1 | 0.89 | 4.05 |

**Table 3.** Nutrient contents of major organic materials used at the Yujiang Country, Jiangxi Province, a typical red soil region [14].

### 3.2. Long-term fertilization experiment on a typical red soil

The Red Soil Ecological Experiment Station, constructed in 1985 by the Institute of Soil Science, Chinese Academy of Sciences (ISSAS), is located in Liujia Zhan, Yujiang County, Jiangxi Province, China (28°15′20″ N, 116°55′30″ E). It is one of the key laboratories for studying the ecology of red soils in China. Red Soil Ecological Experiment Station is dedicated to finding solutions to ecologically sound and environmentally friendly use of the red soil resources. It primarily explores (1) the structure, function, and productivity of red soil agricultural ecosystems; (2) the characteristics of material cycling and energy transformation between red soils and the environment; and (3) the relationships among regional resources, the environment, and the economy, in order to provide a scientific basis for sustainable development of the region.

In order to understand the changes of soil physicochemical properties, soil fertility, and the sustainable utilization of red soil as affected by the combined application of organic–inorganic fertilizers and the response of the cultivated crops to those changes and variations, a long-term fertilization experiment was established at the station in 1988. The experiment had a completely randomized block design with five fertilizer treatments and three replications. Each plot was 33.3 $m^2$. In this chapter, three relevant treatments were chosen for discussion. Those treatments were: NPK (control, CK), CK plus rice (*Oryza sativa* L.) straw (RS) and CK plus pig manure (PM). The crops grown during the experiment before 1995 included peanut (*Arachis hypogaea* L.) in Season 1 (April–August) and cole (*Brassica napus* L. *var. napus*) in Season 2 (September–December). Since 1995, peanut was planted in Season 1 and the land was followed in Season 2. The sources of chemical fertilizers for the experiment were urea for N, KCl for K, and Ca-Mg phosphate for P. The amounts of chemical fertilizers applied to the plots every year were as follows: N, 60.0 kg $hm^{-2}$; $P_2O_5$, 19.65 kg $hm^{-2}$; K, 58.85 kg $hm^{-2}$. At the same time, 3000 kg $hm^{-2}$ of rice straw (dry weight) and 30,000 kg $hm^{-2}$ of fresh pig manure were added to the Treatment RS and PM in addition to chemical fertilizers, respectively. Nutrient contents of the rice straw and pig manure on dry weight basis were as follows: organic carbon, 376.0 and 265 g $kg^{-1}$; total N, 6.7 and 36.5 g $kg^{-1}$; total P, 2.0 and 23.0 g $kg^{-1}$; Total K, 23.0 and 52.0 g $kg^{-1}$; C/N ratio, 56.1 and 7.26, respectively.

The test site is in the typical middle subtropical region, with a mean annual rainfall of 1785 mm (ranging 1040–2550 mm) during the past 22 years, a mean annual temperature of 17.8°C (ranging 16.1–18.9°C), and the frost-free days per year ranged from 240 to 300 days. The soil at the site is a typical udic ferrosol [11] with kaolinite red clay parent material of Quaternary age. Before the long-term experiment, the land was a gently sloping hill covered with herbaceous vegetation with no history of crop production, and partial chemical and physical characteristics of the soils tested in 1988 were as follows: pH, 4.65; soil organic carbon, 3.71 g $kg^{-1}$; TN, 0.34 g $kg^{-1}$; TP 0.53 g $kg^{-1}$; 10.6 g $kg^{-1}$; C/N ratio, 10.9.

## 4. The effects of organic-chemical fertilizer application on soil properties

### 4.1. Soil acidity

Soil pH had a significant increasing trend during the long-term organic–inorganic fertilization (**Figure 1**) from 1988 to 1995, but it gradually decreased since then. There was a significant negative linear correlation between soil pH and cultivation time after the seventh year of the experiment. This suggests that initial fertilization raised pH in the very acidic soil to near neutral, but continuous fertilization led to further soil acidification. The mechanism of pH changes remains to be explored. The CK treatment decreased soil pH by 0.07 yearly, while addition of rice straw and pig manure decreased the pH by 0.05 and 0.04 yearly, respectively (**Figure 1**). At the rate of pH decline, soil acidity of the upland red soil would reach the original soil condition in 1988 after 30 (CK and RS) or 48 (PM) years of cultivation. In addition, soil pH of RS treatment decreased faster than that of the PM treatment after the seventh year.

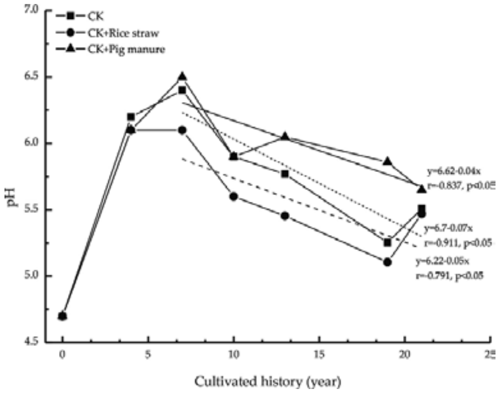

**Figure 1.** Changes of soil pH in the upland red soil as affected by the long-term application of organic–inorganic fertilizers (1988–2009).

## 4.2. Soil organic matter

Compared with the CK, addition of rice straw and pig manure significantly increased soil organic matter contents in the upland red soil during the long-term fertilization (**Figure 2**) study. The rice straw treatment had a higher accumulation rate of organic matter (0.34 g $kg^{-1}$ yearly) than the pig manure treatment (0.14 g $kg^{-1}$) probably due to the higher amount of C and C/N ratio of the former.

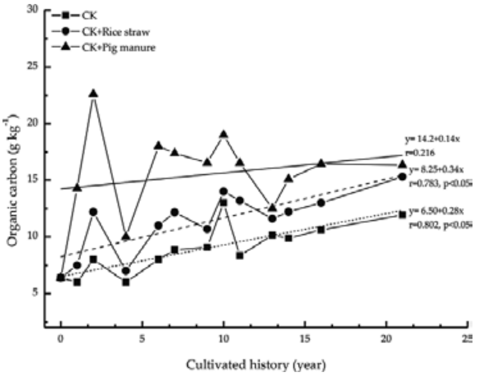

**Figure 2.** Changes of soil organic matter in the upland red soil as affected by the long-term application of organic–inorganic fertilizers (1988–2009).

### 4.3. Soil nitrogen, phosphorus, potassium

Compared with the initial value in 1988, soil total nitrogen (TN) showed a significant increasing trend during the long-term fertilization (**Figure 3**). Both RS and PM treatments significantly increased soil TN content compared to the CK, but PM treatment was more effective in the accumulation of TN in the upland red soil.

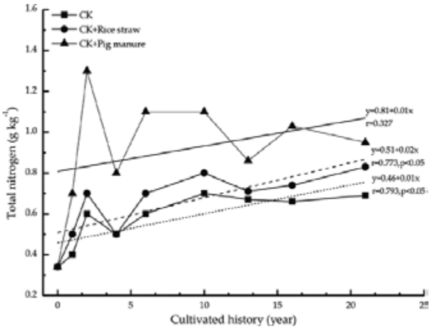

**Figure 3.** Changes of soil total nitrogen in the upland red soil as affected by the long-term application of organic–inorganic fertilizers (1988–2009).

The upland red soil in this study is a typical acidic soil and characterized by its strong P-fixation capacity and low P availability due to the high content of aluminum and/or iron oxides, which can convert P in the soil solution to water-insoluble Fe-Al-P. Compared with the CK, rice straw addition was not significant in improving soil total phosphorus (TP) and Olsen-P, but the addition of pig manure did significantly increase soil TP and Olsen-P by 28.4–116.7% and 292.6–731.8%, respectively (**Figure 4**). There is a significant positive correlation between soil TP content and the year of cultivation ($r = 0.843$, $p < 0.05$). Based on this relationship, the soil TP content in the PM treatment was increased by 30 mg kg$^{-1}$ yearly.

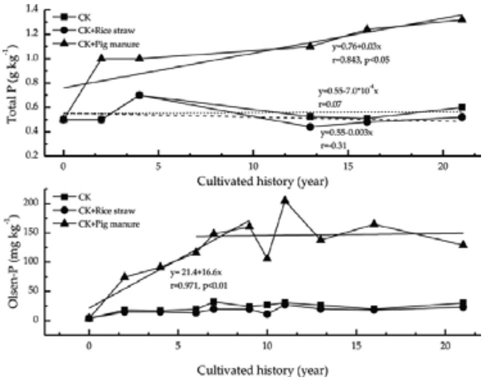

**Figure 4.** Changes of soil total phosphorus (P) and Olsen-P in the upland red soils as affected by the long-term application of organic–inorganic fertilizers (1988–2009).

During the long-term fertilization study, soil potassium (K) in the upland red soil significantly decreased at the rate of 50 or 60 mg $kg^{-1}$ yearly and there was no significant difference among the three treatments (**Figure 5**). Compared with their initial values in 1988, soil total K was reduced by 1.9–12.0%, 2.8–9.3% and 0.9–14.0% in the CK, RS, and PM, respectively (**Figure 5**). The K reduction is probably due to plant uptake and removal or leaching losses.

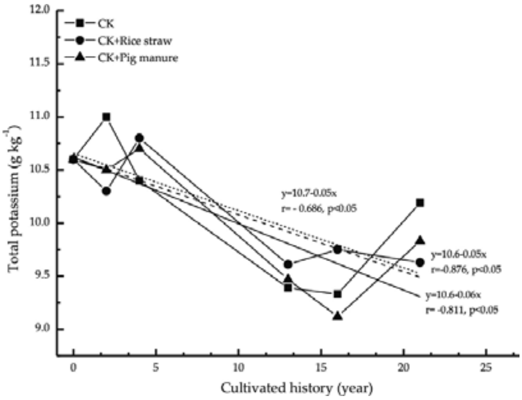

**Figure 5.** Changes of soil total potassium (K) in the upland red soil as affected by the long-term application of organic–inorganic fertilizers (1988–2009).

### 4.4. Soil aggregate structure

Soil aggregate formation and stability are the results of complex interactions among biological, chemical, and physical processes in the soil, which are also important determinants of soil fertility and productivity. Distribution or/and redistribution of various sized soil aggregates in the upland red soil could be significantly affected by the long-term application of organic–inorganic fertilizers (**Figure 6**, [15]). The distribution of dry-sieved aggregates (DSA) in the upland red soil showed similar trends among CK, RS, and PM in the order of (>5) >1–0.25 > 5–2 > 0.25–0.05 > 2–1 > (<0.053 mm), whereas the distribution of water-stable aggregates (WSA) was in the order of 0.25–0.053 > 1–0.25 > (>5)> (<0.053) > 5–2> 2–1 mm (**Figure 5**). Compared with the CK, addition of rice straw and pig manure significantly increased the proportion of DSA in 1–0.25, 0.25–0.053, and <0.053 mm fractions. No change in the 2–1 mm fraction, however, was detected. Addition of rice straw significantly decreased the proportion of WSA in the >5 mm fraction and increased the proportion of WSA in 2–1 and 1–0.25 mm fractions but did not affect the 1–0.25 mm aggregate fraction (**Figure 6**). Evidently, there was a significant difference between the effect of rice straw addition and pig manure amendment on aggregate size distribution in the upland red soil.

Overall, 86.9–93.8% of DSA and 47.4–50.0% of WSA were in the macro-aggregate (>0.25 mm) fraction in the upland red soil after 22 years of combined application of NPK fertilizer and organic amendments (**Figure 6**). Compared with the CK treatment, addition of rice straw and pig manure increased the proportion of the >0.25 DSA fraction by 4.9 and 7.9%, respectively;

and only the addition of pig manure significantly increased WSA by 5.9% in the >0.25 mm fraction (**Figure 7**).

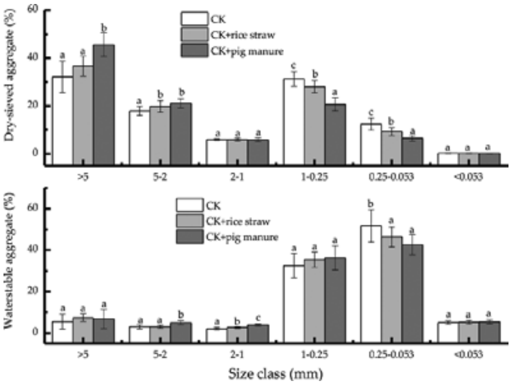

**Figure 6.** Distribution of dry-sieved aggregates (DA) and water-stable aggregates in the upland red soil impacted by long-term fertilization (1988–2009). Same lowercase letters indicate no significant difference at $p < 0.05$ between different fertilizer treatments.

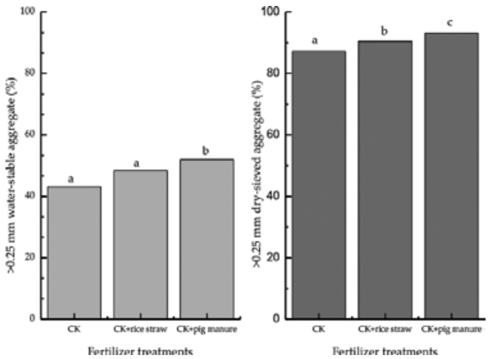

**Figure 7.** Proportion of macro-aggregate (>0.25 mm) in the upland red soil impacted by the long-term fertilization treatment (1988–2009). Same lowercase letters indicate no significant difference at $p < 0.05$ between different fertilizer treatments.

## 5. Sustainable development and nutrient management of red soil

Red soils are usually low in pH, nutrients, and organic matter content, and difficult to cultivate because of its poor physical condition and low water-holding capacity. Therefore, the productivity of the red soils is low under natural conditions. Most farmers have livestock or poultry production on the side in the red soil region, and they use the grain to feed the animal and use the manure to improve soil productivity. Although combined application of chemical fertilizer and rice straw or pig manure was an effective method to improve soil fertility (**Figures**

**3** to **5**), soil macro-aggregates (**Figures 6** and **7**), and soil organic matter content (**Figure 2**), it could result in N and P accumulation and soil acidification in the long term (**Figure 1**). The accumulation of P in the soil may result in high risk of P losses to the environment [16]. Therefore, it is imperative to monitor nutrient balance when large quantities of organic amendments are used. This study showed that it only took 4–5 years of applying phosphate fertilizer alone, 2 years when organic-chemical fertilizers were applied simultaneously to increase Olsen-P to over 20 mg P $kg^{-1}$ (sufficiency level) (**Figure 4**). When soil P is sufficient for plant growth, continuous application of P fertilizer including organic manure not only hurt farmer's economic return but also contributing to non-point source pollution. Therefore, better nutrient management strategy needs to be developed and disseminated to farmers in the region in order to prevent potential environmental risk from crop production.

Better soil quality is generally associated with greater concentrations of soil organic matter and a plentiful supply of essential mineral elements. Thus, the recycling of organic matter and mineral elements from crop residues and animal manure to soil often benefits agricultural sustainability. Red soil production under long-term fertilization is perceived to be environmentally friendly, but P accumulation and acidification in the PM may lead to faster degradations of soil quality than in the CK and may be an important cause of eutrophication in water bodies (**Figures 1** and **4**).

Soil acidification is an important cause of soil degradation. Generally, soil acidification is a slow process under natural conditions over hundreds to millions of years, because soils are strongly buffered by ion exchange reactions, the weathering of soil minerals, and interactions with aluminum and iron in the acidic range [17]. However, this process in red soils is accelerated by the addition of crop straw and animal manure; and soil acidification appeared only after 7 cultivation years (**Figure 1**). Generally, strategies for the application of organic manure have been based on meeting crop N needs to maximize plant growth and minimize nitrate loss by leaching, a potential groundwater contaminant [18]. Guo et al. (2010, [19]) showed that severe soil acidification in China's croplands occurred, and attributed it to the combination of high-N fertilizer inputs, plant uptake, and removal of base cations from soils, and acid deposition, with the dominant effect from the overuse of N fertilizer. But, soil is a mixture of acid/base systems, so there are many causes of soil acidification in addition to N-induced acidification.

Most of organic manures often contain significant amounts of low-molecular-weight organic acids due to decomposition of large quantities of organic matter input, which could be another reason for the accelerated P accumulation and soil acidification [16]. Many organic acids contain carboxyl and hydroxyl groups, and possess negative charge, which strongly competes for the adsorption sites with phosphate [20, 21]. Manure can also change soil pH and thus alter soil P availability. Guo et al. (2010, [19]) pointed out that soil could be acidified by the excessive application of farmyard manure. But the mechanisms of manure-induced P accumulation and P accumulation-induced soil acidification still need further investigation [18], especially in the red soil with strong P fixation capacity. Maintaining stable levels of production and quality under fertilization without compromising economic profitability or the environment is an important guarantee of agricultural sustainability. Therefore, new strategies of using both commercial fertilizers and animal manure must be established to prevent P buildup and to minimize acidification in the red soil region.

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Chapter 9

# An Overview of the Studies on Biochar Fertilizer Carried Out at the Beginning of the Twentieth Century in Japan

Naoki Moritsuka and Kaori Matsuoka

Additional information is available at the end of the chapter

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## Abstract

Biochar is a recently coined term for charred organic matter used as a soil amendment. Although the term is relatively new, the substance has been used for a long time throughout the world, including Japan. After we read a Japanese book entitled *Nibai Shukaku Tenri Nouhou (How to Double Crop Yield by Almighty Farming System)* originally published in 1912, we found that there were conflicting opinions between the author (Mr. Katsugoro Oyaizu) and soil scientists of the time (Dr. Gintaro Daikuhara and others) on the benefits of the use of biochar fertilizer. Previous publications on this topic have been written in Japanese from a sociological viewpoint. By referring to the literature published at the beginning of the twentieth century in Japan, we attempt to shed light on the conflict between traditional knowledge of biochar fertilizer and new concepts of soil science imported from the Western countries. We also describe briefly the socioeconomic impacts on the use of biochar fertilizer in the later generations.

**Keywords:** agricultural chemistry, biochar fertilizer, Japan, Meiji and Taisho periods (1868–1926), modernization

## 1. Introduction

"Biochar" is a recently coined term used to denote a carbon-rich product obtained when biomass, such as wood, manure or leaves, is heated with little or no available air. It is similar to the term "biosolid," which is used to describe treated sewage sludge for agricultural use. The term biochar only applies to the material used as a soil amendment and is distinguished from charcoal used for fuel or as a reductant [1].

In Japan, material containing biochar has long been used as a fertilizer to improve soil quality and to increase crop production. For example, it is described as *yaki-goe* (burned fertilizer) in *Nogyo Zensho* (an encyclopedia of agriculture), which is one of the earliest agricultural textbooks called *nosho* and was written by Yasusada Miyazaki and published in 1697 during the Edo period (1603–1868). After reading the book *Nibai Shukaku Tenri Nouhou (How to Double Crop Yield by Almighty Farming System)* published in 1912 [2], we also found that there were conflicting opinions between the author and scientists who doubted the benefits of the use of biochar as a fertilizer.

Mr. Katsugoro Oyaizu, an agricultural extension worker, wrote the book and strongly advocated the use of *kuntan hiryo* (biochar fertilizer) for various crops [3]. Oyaizu was born in 1847 in Aichi Prefecture. In about 1880, he began to conduct agricultural experiments and promoted the use of heated soil as a fertilizer. From 1887 to 1888, he served as an agricultural extension worker in Aichi Prefecture. Based on his knowledge of heated soil fertilizer, he proposed a method to produce biochar fertilizer to the government in 1900 and summarized it in his book, which became a bestseller.

Dr. Gintaro Daikuhara was one of the scientists who disagreed with Oyaizu on the effects of biochar fertilizer. Daikuhara was born in 1868 in Nagano Prefecture, and thus was about 20 years younger than Oyaizu. A year after graduating from the College of Agriculture, Tokyo Imperial University (the predecessor of the University of Tokyo) in 1894, he joined the staff of

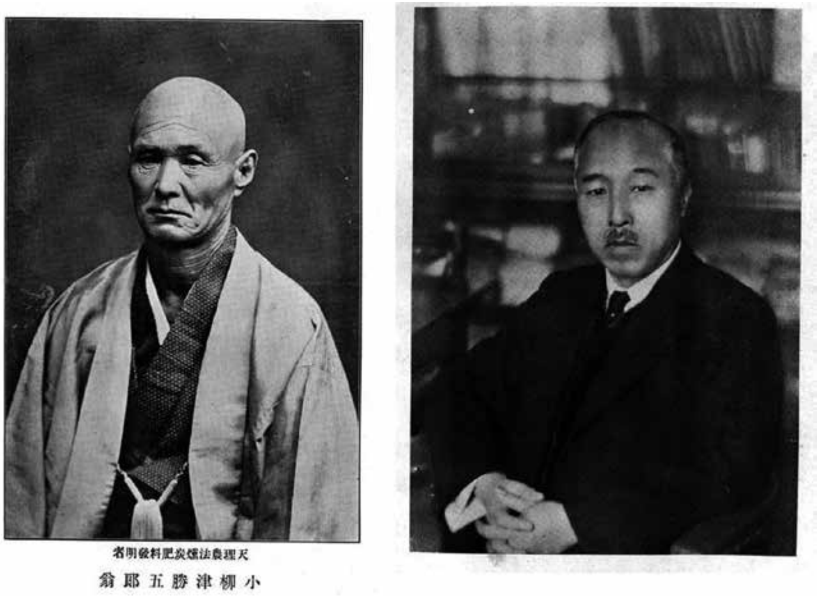

**Figure 1.** Photographs of Katsugoro Oyaizu (left) and Gintaro Daikuhara (right). The photo of Oyaizu is reprinted from his book [2], and the photo of Daikuhara is reprinted from a collection of his papers [11]. The dates of photographs are not known. Reproduced from our study [12] with permission from the Japanese Society of Soil Science and Plant Nutrition.

the Imperial Agricultural Experiment Station where he served as an agricultural chemist for 27 years [4]. His publications on soil acidity [5] and a new method of its determination [6] attracted worldwide attention [7]. He also wrote textbooks on soil science in Japanese [8, 9], which contributed to establishing the basis of modern soil science in Japan [10].

**Figure 1** shows the photographs of Oyaizu and Daikuhara. Both of them studied the effects of biochar fertilizer, but reached different conclusions. This episode has been written by several researchers, mainly by historical sociologists (for example, see [3, 12–14]). But these reports are available in Japanese only, and they are often written from a sociological viewpoint with limited insight to the experimental data.

In this chapter, we introduce these two agronomists and their thoughts on the use of biochar fertilizer with a parallel focus on the growth of modern soil science and fertilizer market in Japan. By referring to the publications of Oyaizu and Daikuhara et al, we attempt to unveil the conflict between traditional knowledge of biochar fertilizer and new concepts of soil science that had been imported from the Western countries in the early twentieth century. We also describe briefly the socioeconomic impacts on the use of biochar fertilizer in the later generations.

## 2. The fertilizer market in the beginning of the twentieth century in Japan

The Meiji period started in 1868 and ended in 1912. During this period, the Japanese people eagerly took in new ideas about culture and civilization from the Western countries. It was towards the end of the Meiji period when Oyaizu and Daikuhara crossed paths. At that time, new developments in agricultural technology and transportation had increased the demand for fertilizer. To meet the demand, new types of commercial fertilizers such as soybean meal, animal bone meal and chemical fertilizers were being imported into Japan [15].

In spring of 1896, Mr. Yasuie Suzuka, who was the president of fertilizer trading company, was taught by Dr. Jun Sawano, who was the first director of the Imperial Agricultural Experiment Station, that ammonium sulfate was a promising fertilizer and worth importing. Suzuka then imported 5 tonnes of ammonium sulfate for the first time from Australia [15]. In the beginning, however, it was difficult to sell the ammonium sulfate "medicine" to fertilizer dealers [16]. To ensure the quality of ammonium sulfate and enlarge its market, Suzuka relied on fertilizer testing services offered by the Imperial Agriculture Experiment Station [15].

Fertilizer testing services had been available since the founding of the station in 1893 [17]. The Fertilizer Regulation Act was put into operation in 1901, and the number of requests increased rapidly. The number of employees at the station increased, and a method for fertilizer analysis was established. The book *Standard methods in agricultural chemistry* [18] identified the following properties to be tested: moisture, ash, organic matter, nitrogen, phosphorus and potassium. The concentrations of nitrogen, phosphorus and potassium in a fertilizer were regarded as the most important properties.

From the 1900s to the 1930s, the ammonium sulfate market in Japan shifted from importation to domestic production. In 1902, sales of commercial fertilizers was largest for soybean meal (28% of the total amount), which was followed by fish meal of herring and sardine (27%), chemical fertilizers (14%) and rapeseed oil cake (13%) [19]. Chemical fertilizers included superphosphate, ammonium sulfate and sodium nitrate, but the percentage of their sales to the total amount was slightly more than 10%.

In the 1910s, the Haber-Bosch process of ammonia synthesis was invented in Germany. The rapid German commercialization of the Haber-Bosch process, however, was not followed by a similarly rapid conquest of the world fertilizer market [20]. In 1913, Chile, possessing huge deposits of sodium nitrate, was the largest producer of nitrogen in the world (56.5%) and Germany was the second (15.6%) [21]. At that time, Japan accounted for only 0.5% of world production. As shown in **Figure 2**, production increased rapidly thereafter. By 1934, Japan accounted for 10.5% of world production and had become the third largest producer in the world after Germany (23.5%) and the United States (13.0%).

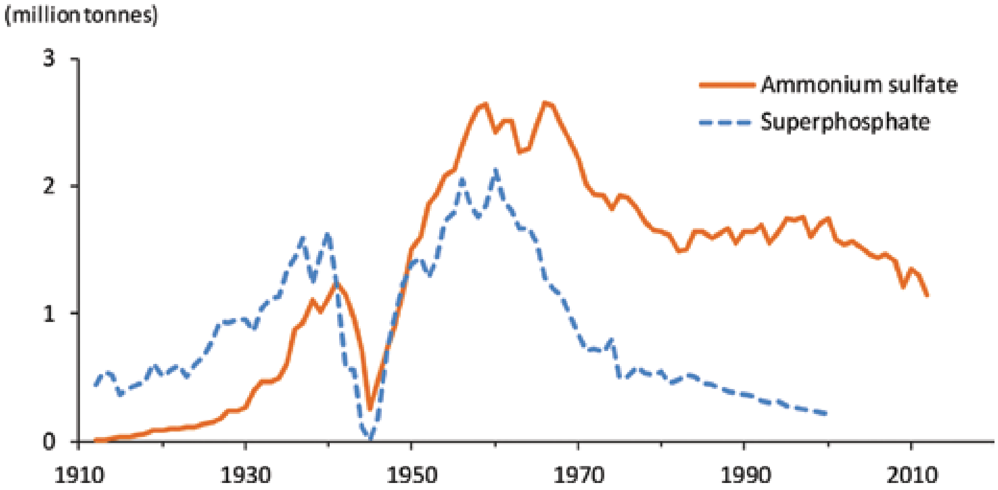

**Figure 2.** Annual production of ammonium sulfate and superphosphate in Japan during the Taisho (1912–1926), Showa (1926–1989) and Heisei (1989–present) periods. The values were derived from data in several fertilizer handbooks published by the Association of Agriculture and Forestry Statistics, Tokyo.

## 3. The conflict between Oyaizu and Daikuhara on biochar fertilizer: ca. 1890–1912

The conflict between Oyaizu and the scientists began in about 1890. A year after Oyaizu published a leaflet on methods to produce fertilizer by heating soil [22], Dr. Oskar Kellner, a German agricultural chemist who had been invited to teach agricultural chemistry at the Komaba School of Agriculture (the predecessor of Faculty of Agriculture at the University of Tokyo) reported that production of such fertilizer was worthless [23]. His comments were based on an analysis by Dr. Max Fesca, a German agronomist who also taught agronomy at the Komaba School of Agriculture.

The article appeared in 1887 began by noting that heated soil fertilizer had already been reported as having a number of merits in *Nogyo Moukun* (agricultural textbook) published by Shosaku Itoh in 1840. Kellner said, "Someone appeared recently who engages in the production and extension of heated soil fertilizer, but the outcome is not always satisfactory". By "someone" Kellner clearly meant Oyaizu, although his name was not used. Kellner pointed out that, in order to evaluate the benefits of heated soil fertilizer, it is necessary to examine the effect of heating treatments on the content of fertilizer elements in the soil. Analytical results were provided by Fesca. A soil sample was treated with heat followed by addition of an ammonia solution. The soil samples (untreated soil, heated soil and heated soil treated with an ammonia solution) were subjected to extraction with about 10 mol $L^{-1}$ hydrochloric acid, and the concentration of fertilizer elements in the extracts was determined. The concentration of nitrogen in the heated soil was less than half of that in the original soil. Volatilization of ammonia during the heating and watering treatments was indicated. The concentrations of phosphorus and potassium were not different among the samples. It was concluded from these results that the use of heated soil fertilizer was neither effective nor economical.

Years later, Oyaizu recalled this event [2] and wrote, "Mr. Fesca, a German specialist employed by the Ministry of Agriculture and Commerce, reported that heated soil fertilizer is of no value. My business suffered a setback."

Some of the Japanese agronomists who were taught by Kellner and Fesca also criticized the leaflet written by Oyaizu [3]. It is likely that Daikuhara was also influenced by these German teachers. From 1897 to 1990, Daikuhara carried out an extensive survey on heated soil fertilizer at the Kinai Branch of the Imperial Agricultural Experiment Station in Osaka Prefecture [7].

In a report entitled "Studies on heated soil fertilizer" [24] and published in 1901, Daikuhara wrote, "The effects of heated soil fertilizer have been experienced by local farmers and recorded in old books." He reported that heated soil fertilizer was still being produced in 10 of Japan's 47 prefectures and that it was frequently used in Hyogo, Hiroshima, Oita and Tottori Prefectures. Unlike some of his colleagues, however, Daikuhara did not completely agree with the conclusions of Kellner and Fesca. He wrote, "Mr. Fesca used strong hydrochloric acid for extracting soil samples. This method is not suitable to evaluate the content of soil nutrients available to crops. I agree with his comment on the loss of nitrogen by volatilization. For the other elements, however, the content of plant-available fraction must have been influenced by the heating treatments. This could be revealed by extracting samples with dilute acid solutions instead of strong hydrochloric acid."

Daikuhara prepared heated soil samples by heating soil in a pan for 20 minutes. For the analyses, he used 1% solution of hydrochloric acid, oxalic acid and citric acid as well as strong hydrochloric acid solution. He also conducted plant growth experiments several times (**Figure 3**) and obtained new findings. Most importantly, he found that the contents of nitrogen, phosphorus and potassium extracted by dilute acid solutions were increased in the heated samples, whereas the whole contents of nitrogen and organic matter were decreased. In this way, he determined the fraction of soil nutrients available to plants and succeeded in obtaining soil analytical results which agreed with crop growth better than before.

| Treatments | H | HC | O | OC |
|---|---|---|---|---|
| Plant length (cm) on April 22 | 41.8 | 42.1 | 22.1 | 37.6 |
| Relative grain yield (%) at harvest | 76 | 100 | 11 | 33 |

**Figure 3.** Barley grown with heated soil fertilizer and/or chemical fertilizer (N, P and K) in Daikuhara's first preliminary experiment [24]. The photo and the data are cited from a collection of his papers [11]. The photo was taken in the middle of April 1898 at the Kinai Branch of the Imperial Agricultural Experiment Station, Osaka Prefecture. The four treatments in the picture are heated soil without chemical fertilizer (H), heated soil with chemical fertilizer (HC), original soil without chemical fertilizer (O) and original soil with chemical fertilizer (OC). This preliminary experiment was carried out in the open system using an earthen pipe as a pot and a sandy soil as a growth medium. The growth and yield of barley in the OC treatment were better than those of the O treatment, indicating that the growth was limited by fertilizer elements. Furthermore, the growth and yield in the H and HC treatments were better than those of the OC treatment. If we assume that the growth of barley was limited by nitrogen, then the inhibition of nitrification by soil heating (for example, see [25]) and the prevention of downward leaching of very mobile nitrate in a sandy soil may be responsible for the better growth in the H and HC treatments in addition to the enhanced mineralization of soil organic nitrogen by heating [26].

Daikuhara was the first Japanese scientist to use a dilute acid solution to extract nutrients from soil. According to his study [27], he had been impressed by pioneering work by Dr. Bernard S. Dyer published in 1894 [28]. Dyer was an agricultural chemist in the United Kingdom who proposed the use of a solution of 1% citric acid to determine whether a soil is in need of phosphate fertilizer. Dyer wrote in his paper, "It may be said to have been pretty widely recognized that some very much weaker solvent than strong mineral acid ought to be used in soil analyses, if these are to be of much use as indications of the proportion of available mineral plant food."

Although the overall results indicated that heated soil fertilizer can be valuable, at the end of the report, Daikuhara added the following supplementary note: "The heated soil fertilizer analyzed in this report is different from the so-called new fertilizer composed of a mixture of charred straw and human waste. Currently, someone is encouraging the use of this fertilizer.

But its value is not worth describing at all." Again, this "someone" was Oyaizu, and the "new fertilizer" referred to biochar fertilizer.

What was the difference between heated soil fertilizer and biochar fertilizer? Both fertilizers are prepared by smoldering organic matter. In the case of heated soil fertilizer prepared in the fields, plant residues such as tree branches, mulberry branches and rice husks are covered with soil, and a mound of soil is prepared. After setting fire to plant residues in the mound, the surrounding soil is treated with heat for several days (**Figure 4**). The product thus prepared is a mixture of heated soil, ash and charred materials. The heated soil fertilizer that Oyaizu recommended in the leaflet [22] was produced in a similar way but in a kiln. The main ingredient was rice stubble and soil together with rice straw as a fuel. After smoldering these materials, other ingredients (sulfur, human waste, salt and fish meal) were added to harmonize the composition of fertilizer.

**Figure 4.** A mound of soil smoldered for the production of heated soil fertilizer. The picture is reprinted from a leaflet of an Okuno-type apparatus to prepare heated soil, which was produced and sold by Marukome Shokai. The date of photograph is unknown, but it is probably from about 1935. Although buried and unseen in the picture, this apparatus made of iron was the most popular type before the Pacific War (1941–1945). The production of the apparatus was restricted severely by the shortage of iron during the war [29].

To prepare biochar fertilizer, Oyaizu recommended preparing a hole with a depth of about 1.4 m and a diameter of 1.8 m [2]. A bundle of rice straw is then set on fire and thrown into the bottom of the hole. Subsequently, additional plant residues of any kind are thrown into the hole several times until white smoke emerges without a flame. After a few hours of smoldering, diluted human waste is poured onto the charred materials. The last step can be omitted when green grasses are used as an ingredient.

Because heated soil fertilizer contains heavy soil and biochar fertilizer does not, these materials are expected to be different in terms of the weight per unit volume. However, what Daikuhara wanted to say in the above note was not such a scientific matter but his opinion that biochar fertilizer cannot become as valuable as heated soil fertilizer.

Oyaizu was angry at the reaction by scientists, and wrote [2], "In 1900, I submitted the outcomes from experiments to the Ministry of Agriculture and Commerce, so that biochar fertilizer would be produced widely. As a result of the analysis by the authorities concerned, however, the biochar fertilizer was regarded as valueless in the same way as the heated soil fertilizer I proposed before. The authorities and scientists rejected all of my results. This event disappointed me very much." The Imperial Agricultural Experiment Station was the "authorities concerned" to which Daikuhara belonged at that time.

In such circumstances, Oyaizu was supported by Mr. Masayoshi Inoue, a bachelor of agriculture who had made efforts to support the use of biochar fertilizer. Inoue wrote [30], "So-called agronomists did not pay any attention to biochar fertilizer on the grounds that it contained little nitrogen, phosphorus and potassium." Inoue also referred to Daikuhara's report on heated soil fertilizer and complained, "On heated soil fertilizer, a certain doctor at the Imperial Agricultural Experiment Station in Tokyo published a report. But he focused on the effect of soil heating only. Charred materials present in the heated soil fertilizer were not described at all." The "certain doctor" is Daikuhara. He had moved from Osaka to Tokyo in 1903 [7]. As noted above, he prepared a heated soil sample in a manner different from local farmers, and there was no biochar in his samples.

Why did Oyaizu believe in biochar fertilizer, despite the criticism from the agronomists? This is probably because he had conducted field trials by himself and with many farmers and found empirically that the application of biochar fertilizer improved crop productivity (**Figure 5**). In his book [2], he wrote, "In order to support the growth of living crops, it is necessary to apply a living body to them." and "Biochar fertilizer is a living body which provides the following functions; 1) absorption and preservation of heat, 2) absorption of moisture, 3) absorption of nitrogen, 4) effects on sterilization, and 5) effects on soil quality." According to the traditional classification of fertilizers by Nobuhiro Sato in the Edo period, "a living body" is a type of fertilizers originating from living creatures (mainly animals) such as human waste, horse dung and fish meal. Although Oyaizu had written that he was impressed by the thoughts of Sato and the biochar fertilizer he proposed did contain human waste, it is not clear whether Oyaizu followed the traditional definition precisely. The term "a living body" was omitted by his son, when the book was revised and enlarged in 1915 [31]. We can at least safely say that Oyaizu considered that the effect of biochar fertilizer was not limited to the contents of fertilizer elements, but it extended to its capacity to absorb heat, water and nitrogen.

**Figure 5.** *Mugi* (probably barley) grown at the Imperial Agricultural Experiment Station (Nishigahara, Tokyo) in 1912, with an aid of a political party "*Dai-Nippon Koudou Kai*" to Oyaizu. *Mugi* is a general Japanese term including wheat and barley. The picture is reprinted from the book revised by Oyaizu's son [31]. Plants on the right were grown with the application of biochar fertilizer, whereas plants on the left were grown by the conventional method. The Oyaizu group and the Experiment Station began collaborating on the cultivation of *mugi* with biochar fertilizer in 1910, but few quantitative results were reported by the station [2].

In the first chapter of his book [2], Oyaizu expressed his viewpoint as follows, "It is evident that Western science, however progressive it is, is not a universal truth. I cannot understand why so-called scientists in our country are eager to copy it. Originality is most important, because we, Japanese, have traditional knowledge of agriculture." A year after the book was published, Oyaizu passed away. Following the wishes he expressed, the coffin was filled with biochar fertilizer [3].

It should be noted that we have interpreted Oyaizu's words as we understand them. His intended meaning might have been different from our interpretation. Although we had trouble in understanding parts of it, his book starts with the theory of Yin and Yang, the Chinese philosophy that has influenced the development of science in Asia, including Japan (the so-called Eastern science) (**Figure 6**). Harmonization of fertilizer according to the Yin and Yang theory was regarded as critically important in *Nogyo Zensho* by Miyazaki; for example, *yaki-goe* (burned fertilizer) should be used in combination with *mizu-goe* (liquid fertilizer containing human waste) for the harmonization of Yin and Yang in order to support the growth and ripening of crops by the power of Yin and Yang, respectively [32]. Therefore, it is not an exaggeration to say that the conflict between Oyaizu and Daikuhara was representative of the larger struggle between the two established philosophies, one from the East and the other from the West.

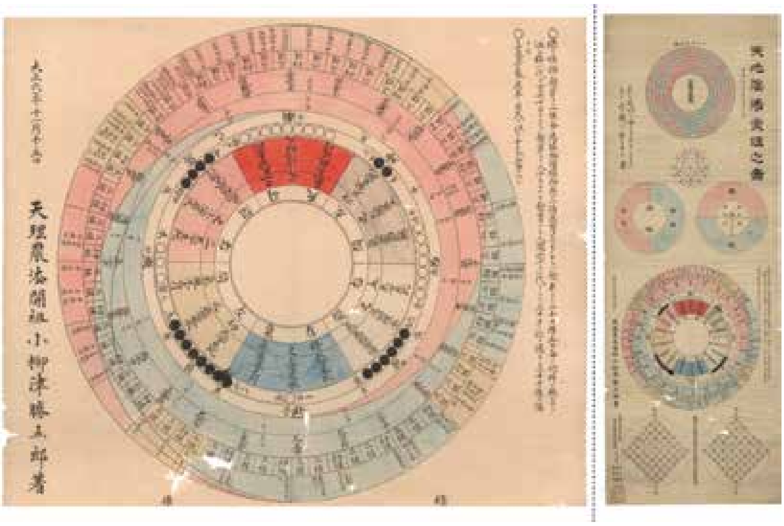

**Figure 6.** A circular crop calendar proposed by Oyaizu and printed in 1917 after his death (left). In the circle, summer and winter are depicted in red and blue colors, respectively, and the seasons rotate clockwise. Field management is described in the inner peripheral part. This calendar is designed to inform farmers on the suitable seasons for each field management practice in the double cropping of rice and *mugi*. It is a part of the schematic diagram called "Harmonization of Yin and Yang" with the length and width of 105 cm and 39 cm, respectively (right). The contents of the calendar are also explained in Oyaizu [31].

## 4. Additional descriptions of biochar fertilizer by Daikuhara: ca. 1912–1920

As noted above, Oyaizu proposed a method to produce biochar fertilizer to the government in 1900. The Imperial Agricultural Experiment Station began experiments on biochar fertilizer in 1908. As a response to Oyaizu's popular book, a comment of the station's director (Dr. Yoshinao Kozai) appeared in a newspaper *Jiji Shinpou* on July 25, 1912. In the article, Kozai expressed his personal opinion. He said, "It is great if the proposed biochar fertilizer can increase the crop yield by 20–30%," but also added, "It is premature to make a judgement, because the results from our station are not yet satisfactory and the research is still ongoing."

A year later, a comment of the chief of agriculture department in the Ministry of Agriculture and Commerce (Mr. Hitoshi Douke) also appeared in a newspaper *Tokyo Asahi Shinbun* on November 27, 1913. In the article, Douke said, "We have compared biochar fertilizer with conventional fertilizer for 4 years at several stations, including Nishigahara (**Figure 5**). But we could not find such a big effect as has been reported by some advocators." He continued, "Growing crops with biochar fertilizer requires labor several times more than that with conventional fertilizer. It is premature to say that biochar fertilizer is excellent only from the data of the crop yield."

In April 1915, two years after the death of Oyaizu, Daikuhara gave a lecture on soil and fertilizer at Kanagawa Agricultural Experiment Station [33]. In reference to biochar fertilizer, he said, "Biochar fertilizer has been promoted in the past few years. But it should be kept in mind that similar fertilizer had been produced for a long time before it attracted public attention." He introduced several examples of traditional biochar fertilizers in Japan and continued, "Biochar fertilizer is only charred organic matter. It does not have any special function. If I need to say more, biochar fertilizer will improve soil physical properties to some extent. Potassium and phosphorus in the ingredients will be solubilized during the process of production. Some of the nitrogen in biochar fertilizer will be available to crops, but the nitrogen supplied from biochar fertilizer should be ascribed to human waste added to the charred materials."

In the lecture, he noted that the contents of nitrogen, phosphate and potassium in biochar fertilizer were 0.6–0.7%, 0.3% and 0.6%, respectively. He also presented results from a pot experiment in which barley was grown as a test plant. He suggested that decomposable organic matter such as rice straw should be used for the production of compost, whereas slowly decomposable organic matter such as fallen leaves might be more suitable in production of biochar fertilizer. The lecture ended with the following statement, "Furthermore, if we continue to apply a fertilizer with low organic matter content, it is apparent that soil organic matter will decrease gradually and soil fertility will be depleted." He emphasized that about half of the organic matter was lost during the production of biochar fertilizer.

Daikuhara's view of biochar fertilizer had become slightly more positive over time. Inoue stated [30], "In past years, a certain doctor visited Chiba Prefecture. He was surprised very much to see the vigorous growth of crops to which biochar fertilizer had been applied." Again, "a certain doctor" referred to Daikuhara. In February 20, 1908, Daikuhara visited Mr. Yajima Chiba together with Mr. Tadaharu Kato, who was a principal of Mobara Agricultural School. Chiba explained how to grow *mugi* (a general Japanese term including wheat and barley) with biochar fertilizer [34].

Did Daikuhara pay attention to the rate of decomposition of biochar or compost after their application to soil? Now it is well known that biochar is stable in the environment and decomposes very slowly in soil. In the first volume of his textbook entitled, *Dojyogaku Kougi (Lectures on the Science of Soils*) [8], he cited his own results [24]. He wrote, "There is no doubt that the heating treatment not only affects soil microbes but also accelerates the breakdown of soil components". To support this, he referred to partial sterilization, a phenomenon discovered around 1900. Daikuhara paid attention to works by Sir Edward John Russell and his colleagues in the United Kingdom. Darbishire and Russell [35] demonstrated that partial sterilization of soil by heating to 100°C leads to a marked increase in the amount of oxygen absorbed by microorganisms of the soil. Absorption of oxygen by microbes and the release of carbon dioxide from soil are essentially two sides of the same coin. It follows that Daikuhara knew about the microbial decomposition of organic matter when he made his lecture on biochar fertilizer in 1915. But it is unlikely that he was aware of the very slow decomposition of biochar in soil.

In the second volume of his textbook [9], Daikuhara stressed the importance of the maintenance and improvement of soil fertility. At that time, the three fertilizer elements (nitrogen, phosphorus and potassium) were known to the public, and the effects of chemical fertilizer were exaggerated. Daikuhara criticized the crowd of "three-element admirers," possibly in a similar frame of mind that Oyaizu had toward Daikuhara. He wrote that soil fertility is controlled by various factors, including chemical factors such as the contents of nutrients and organic matter, and also physical factors such as aggregate structure, soil depth and moisture content. He emphasized that application of three elements in the form of chemical fertilizer was not the only solution and that application of organic matter and lime to soil is indispensable, considering the climate, soil type and farm management in Japan. His thoughts had become more holistic. This may have been related to his extensive research activities, especially on the denitrification after application of sodium nitrate to paddy soil [36] and on the acidification of soil after application of potassium salt [5].

Part of his wishes was realized by younger soil scientists, including Dr. Matsusaburo Shioiri. In Konosu experimental field of the Imperial Agricultural Experiment Station, the longest field experiment in Japan was started from 1925 with the aim to evaluate the effect of application of organic and chemical fertilizers on the yield of rice and wheat and the fertility of soil [17]. It was about 80 years after the world's longest field experiment had been launched by the Rothamsted Experimental Station in the United Kingdom [20].

In 1921, Daikuhara was appointed as a professor at Kyushu Imperial University. Two years later, he was appointed as the Director of the Agricultural Experiment Station in the Province of Korea. After this, he became the President of Kyushu Imperial University and Doshisha University. While working actively as the President of Doshisha University, he passed away in 1934 [4]. His series of textbooks on soil science was to be composed of three volumes, but the final volume remained unpublished [10].

## 5. The use of biochar fertilizer during and after the Pacific War in Japan: ca. 1940–present

From the present perspective, we feel that both Oyaizu and Daikuhara devoted themselves to improving Japanese agriculture from a holistic viewpoint. In the last section, we briefly examine the influence of their achievements on later generations.

In December 1941, Japan triggered the Pacific War. During the war, the production of ammonium sulfate decreased sharply from 1.24 to 0.24 million tonnes per year (**Figure 2**). The production of superphosphate also decreased to as little as 0.01 million tonnes per year. These sharp decreases were because imports of the key ingredients were stopped by the war and also because the ammonia produced by the fertilizer industry was converted to nitric acid due to the critical demand for the production of explosives [21]. In addition, the production of commercial organic fertilizers such as rapeseed oil cake and fish meal also decreased during the war.

Because of the shortage of all commercial fertilizers, the government encouraged farmers to produce traditional homemade fertilizers, including heated soil fertilizer [37] and biochar fertilizer. Dr. Shingo Mitsui, an outstanding soil scientist, began to reevaluate and extend the findings of Daikuhara [38]. When he began these experiments in 1939, the use of heated soil fertilizer was almost extinct in Japan [26].

In a textbook of fertilizers published in 1942 by Dr. Hideo Misu [39], the biochar fertilizer proposed by Oyaizu was described as one of 178 types of fertilizer. The composition of the product (38–51% moisture, 0.74–1.06% nitrogen, 0.28–0.70% phosphate and 0.63–0.85% potassium) was reported to vary depending on the type of ingredients used for smoldering and the amount of human waste mixed in. This fertilizer was thought to have small direct effects from the fertilizer elements and some indirect effects from the charred carbon. In contrast to the previous negative descriptions of biochar fertilizer by scientists, a short biography of Oyaizu was published as an independent book chapter (at least twice in 1938 by Sakurai and 1941 by Iyoda), in which he was described as one of the great agricultural experts.

In August 1945, the war came to an end. A year after, Oyaizu's book [2] was republished again with some revisions [40]. It was more than a generation after the original was published. The title of the book was shortened by removing the word *Tenri* (almighty), and the first chapter was simplified by omitting the theory of Yin and Yang. These revisions suggest strongly that the Japanese people at that time accepted Western knowledge and that the theory of Yin and Yang proposed in the original was regarded as out of place or out of date.

In 1945, the capacity of factories in Japan to produce ammonium sulfate was only about 10% of the capacity in 1941 because of the insufficient maintenance of the equipment and also because of the air raids during the war [41]. After the war, the government put an emphasis on the production of ammonium sulfate [41]. Its production level had recovered by 1949 and peaked in 1959 and 1966 (**Figure 2**). During the period of rapid industrial growth, Japan's farming systems were modernized with the use of agricultural machines, pesticides and fertilizers. Traditional fertilizers, which required time and labor to produce, became to be regarded as old-fashioned. The textbook written by Misu was revised and republished in 1949 [42]. In the revised book, biochar was described more specifically as *mokutan matsu* (wood charcoal powder) having several indirect effects on crop growth. At the same time, the term *kuntan* (biochar) disappeared together with Oyaizu's name.

In the 1970s, the domestic production of rice became sufficient to meet demand. The government started to pay farmers to reduce rice production by introducing the *gentan* policy in 1970. Things began to change. The public attention to food shifted from the quantity to the quality. The environmental pollution caused by industrial activities had come to be widely recognized. By that time, Itai-Itai disease and two outbreaks of Minamata disease had been identified and were known to be caused by the improper management of wastes containing toxic metals by large incorporations in Japan.

*Silent Spring*, written by Ms. Rachel Carson in the United States, focused on the detrimental effects of pesticides on the environment and became popular around the world after its publication in 1962. In Japan as well, the newspaper *Asahi Shinbun* published the serialized nonfiction novel, *Fukugou Osen (The complex contamination)* by Ms. Sawako Ariyoshi, in 1974. Her book [43] became a bestseller. In the book, Ariyoshi wrote that multiple contaminants at even trace levels may have cumulative or even synergetic effects on the environment and human health. She discussed several topics to make her readers aware that environmental pollution can be caused and suffered by everyone. One of the topics she described was the dark side of chemical fertilizer. Local farmers she interviewed said frequently that the "soil is dead," probably due to the application of chemical fertilizer in excess for a long period.

From the late 1970s to the early 1980s, several scientists, especially Drs. Sugiura, Kishimoto and Ogawa, intensively examined the function of biochar as a soil conditioner for afforestation (for example, see [44]). On November 26, 1986, the government designated biochar powder (precisely, wood charcoal powder) and vermiculite as soil amendments, which are effective to improve soil quality, especially water permeability. Nowadays, charred rice husk as a soil amendment mainly for potted flowers and kitchen garden is usually sold at commodity household stores, and it is occasionally produced by rice-growing farmers (**Figure 7**). In addition to this, growing concerns on global warming highlighted the very slow decomposition of carbon in biochar, thereby returning carbon from the air to belowground through the application of biochar to soil, i.e., biochar carbon sequestration [1].

**Figure 7.** Mounds of rice husk being smoldered in a rice field to produce biochar. A dark-colored soil beneath the mounds is a volcanic ash soil distributed widely in Japan. The photo was taken by the authors in Ibaraki Prefecture on August 30, 2014 soon after the harvest of rice. The Waste Management and Public Cleansing Law (revised) was put into operation on April 1, 2001. The revised law prohibited the open burning of wastes except for several cases including the burning or smoldering of agricultural wastes by farmers in a field of their own.

Various possible applications of biochar in relation to agroecosystem management have been evaluated by many scientists worldwide. For example, Fischer and Glaser [45] proposed co-composting of fresh organic matter and biochar during the composting process. Their idea is similar to Oyaizu's idea to harmonize the composition of biochar fertilizer by addition of human waste. Although we cannot go into detail here, we have also examined the effect of heating of sewage sludge on the mineralization of nitrogen [46] and the uptake of nutrients by a leafy vegetable [47]. These experiences led us to focus on the earlier works by Oyaizu, Daikuhara and Mitsui.

In addition to the agricultural studies, research has been carried out from the viewpoint of soil formation over the centuries. For example, *Terra Preta de Indio*, which is widespread in the Amazon basin, is a well-known anthropogenic soil whose high organic matter content is probably the result of the long-term input of biochar produced by pre-Columbian Indians [48] in combination with the stabilization of humic substances by aluminum and iron in the strongly weathered soils [49]. Likewise, the input of charred grassland plants that are highly stable in soil is hypothesized to be responsible for the formation of black, humus-rich volcanic ash soils distributed widely in Japan [50] (**Figure 7**).

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## Chapter 10

# C-$CO_2$ Emissions, Carbon Pools and Crop Productivity Increased upon Slaughterhouse Organic Residue Fertilization in a No-Till System

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Additional information is available at the end of the chapter

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### Abstract

The use of slaughterhouse organic residues (SORs) as a form of fertilization in no-till systems could be an alternative to promote their appropriate disposal. This chapter reports a study on a Haplic Cambisol (Inceptisol) regarding the influence of different rates of SORs applied isolated or together with synthetic mineral fertilizers (SMFs) for 5.5 years in a no-till system with diverse crop rotation. We evaluated crop productivity and several soil organic matter pools affected by the SOR and SMF combinations in a field experiment. In addition, a laboratory incubation experiment was performed with different rates of SORs to evaluate C-$CO_2$ emissions and C dynamics. The SOR applications provided significant increases in crop productivity, soil organic matter pools and C-$CO_2$ emissions. The SOR applications provided significant increases in crop productivity, soil organic matter pools and C-$CO_2$ emissions. The treatment with 50% SOR + 50% SMF was the best alternative to provide higher crop productivity, while the higher use of SOR promoted more increments in soil organic matter levels. Despite the increase in C-$CO_2$ emissions due to the use of SORs, higher C levels were observed as a function of SOR rates. We conclude that the application of SORs combined with SMFs represents an efficient strategy to reduce costs and increase C levels, providing agronomic and environmental benefits.

**Keywords:** Carbon sequestration, soil organic matter, conservation agriculture, global warming, greenhouse gases

# 1. Introduction

The world population is expected to reach more than 9 billion people by mid-century, creating enormous pressure over the global food supply. Concurrently in the food chain, meat production is an activity that causes the greatest environmental impact due to the inefficiency of the transformation of nonusable parts for direct consumption in reusable by-products [1]. Thus, the amount of waste to be recycled and reused for various purposes will increase significantly, negatively contributing to environmental sustainability due to its disposal in the environment in an inappropriate manner and thereby generating increased emissions of greenhouse gases (GHGs) [2-4]. This change may trigger an increase in the planet's average temperature by up to 5.8°C over the next 100 years [5]. It is estimated that alterations in soil management provide about 20% of the total emissions of greenhouse gases (GHGs) [6].

Global meat consumption is estimated to increase by 72% between 2000 and 2030, and much of this increase will be due to the consumption of poultry and pork [7]. Around 96 and 113 million tons of poultry and pork, respectively, are produced in the world. The Americas are responsible for 43.7% of the world's chicken production and 17.4% of pork production [8].

The United States accounts for 17% of the world's poultry meat production, China accounts for 13% and Brazil, becoming the third largest producer, accounts for 12%. China accounts for 60% of pork production, followed by the United States (10.5%), Russia (5.5%), Spain (3.9%) and Brazil (3.71%) [8]. The waste from slaughterhouses of poultry, pork and cattle has caused serious environmental consequences due to its improper disposal in the environment [2-4].

Poultry and pork production will generate 121 and 509 million tons of carbon dioxide, respectively, via carbon (C-$CO_2$) equivalent until 2020, with a prospective increase of 47% in 2030 [7]. The use of organic waste from the meat processing industry could increase the potential for soil carbon (C) drain and promote reduction in GHG emissions compared with industrial fertilizers derived from fossil fuels, thereby minimizing its environmental impact [9]. In addition to reducing its environmental impact, the organic waste produced in the slaughtering system is an organic fertilizer option for soil due to the presence of essential nutrients for plant growth and mainly due to its high content of organic matter, which acts positively on physical, chemical and biological soil properties, thus promoting plant development [10-12].

The use of industrial organic waste in combination with crop residues that return to the soil increases the C accumulation rate in the long term [13]. The C accumulation potential in the soil is governed by many factors, such as climate and soil type [14, 15], crop systems [16], soil management, including conservational systems [17, 18], and soil fertilization [19].

Thus, industrial organic waste presents several benefits regarding soil quality improvement and agronomic production increase [20]. However, the potential that these residues have to promote C compensation to the soil-plant-atmosphere system has been scarcely explored compared with the use of industrial mineral fertilizers.

## 2. Problem statement

The organic residues used in agriculture as fertilizers often originate from three main activities: agricultural, urban and industrial. Among agricultural residues, manure (cattle, porcine and poultry) is the most commonly used. With regard to the organic residues from urban activities, the products generated from composting of urban garbage and sewage sludge are the most used ones [21, 22]. Lastly, the waste generated in the food processing industry is the most used in the production of organic fertilizers.

The residues generated from chicken and poultry slaughterhouses have been causing serious pollution problems to the soil, surface water and groundwater. According to COWI Consulting Engineers and Planners AS [23] and Matos [24], 20% and 30% of chicken and swine weight, respectively, are considered inedible (blood, feathers, hairs, nails, fat, etc.). Part of the residues generated is destined for industrial purposes (e.g., animal food production), and approximately 20-22% are discarded in the environment.

Several reports have demonstrated the benefits of using organic fertilizers to the chemical properties of soil. According to Rasmussen and Collins [25], the use of organic fertilizers in agriculture aims to increase the soil organic matter content. The soil organic matter plus the clay soil content form an absorption complex that increases soil chemical properties. The complex, in this way, is capable of retaining the nutrients as nitrogen that would be eventually leached. Organic fertilizers also add micronutrients and macronutrients to the soil. Therefore, organic fertilizers increase the soil cation exchange capacity (CEC), provide better water retention, create complex toxic elements [26-29] and determine the biological and physical qualities of soil.

Marchesini et al. [30] reported crop yield increases provided by the use of organic fertilizers, which are more persistent despite presenting lower and slower effects compared with synthetic mineral fertilizers (SMFs). This could be due to their lower and progressive nutrient release and plant root system development.

However, it is important to emphasize that since agricultural soils can be considered a destination for residue waste, we must respect the limits imposed by legislation, avoiding overpowering the soil's capacity [29, 31, 32]. Although the practice can work in ameliorating soil conditions, it can also cause contamination, consequently affecting crop yield and quality [33].

Synthetic fertilizers that come from nonrenewable sources are commonly used in agriculture. In 2012, Brazil consumed more than 29 million tons of industrial fertilizers [34]. Therefore, lowering their use by replacement with organic residues in this way can positively contribute to environmental conservation.

In the search for more sustainable practices, correct management of organic residues in agriculture is an important process to promote their environmental, social and economic benefits. Exploring the potential of organic residues can promote their proper destination, increase soil quality and promote economic benefits.

In this light, the specific objectives of this research were (a) to assess the contribution of slaughterhouse organic residues (SORs) from poultry and porcine activities to carbon (C) alterations as well as (b) to study crop performance under a no-till system with organic residue applications with and without synthetic mineral fertilizers.

## 3. Materials and methods

The experiment was established in April 2009 at the State University of Ponta Grossa Farm (Fazenda Escola Capão da Onça) in the city of Ponta Grossa, Paraná, in southern Brazil (25° 05′ S and 50° 05′ W). The climate is classified as Cfb according to the Köppen system [19], with cold and humid winters and occasional frosts between May and July. The annual mean precipitation during the experimental period of 44 years is 1545 mm, with higher precipitation levels in the summer and no dry period defined. The mean maximum temperature is 24°C, and the minimum is 13.3°C. The soil is classified as Haplic Cambisol with medium texture, and it represents 27% of the region [35]. The results of the soil fertility analysis performed before the experiment were pH ($CaCl_2$, 1M), cation exchange capacity = 11.2 cmolc dm$^{-3}$, soil density = 1.35 Mg m$^{-3}$, total organic C = 11.9 g kg$^{-1}$, total organic nitrogen = 16.12 g kg$^{-1}$, available P = 38.1 mg kg$^{-1}$ and available K = 0.24 cmolc dm$^{-3}$.

The experimental design was of completely randomized blocks with six treatments and three replications. The following treatments were performed: $T_1$ = control, with no slaughterhouse organic residue (SOR) or synthetic mineral fertilizer (SMF) applications; $T_2$ = 100% SMF, with all plant nutrient supply applied via synthetic mineral fertilizer; $T_3$ = 100% SOR, with all plant nutrient supply applied via slaughterhouse organic residue; $T_4$ = 75% SOR + 25% SMF; $T_5$ = 50% SOR + 50% SMF; and $T_6$ = 25% SOR + 75% SMF. For $T_4$, $T_5$ and $T_6$, the rates of SORs applied were equivalent to 75%, 50% and 25% of the residues used in $T_2$, respectively. The rates of SMFs applied were based on the soil fertility analysis and the recommendation for the crops used in the region.

In $T_3$ (100% SOR), 94, 21 and 19 kg ha$^{-1}$ of N, P and K, respectively, were applied. These were equivalent to 2 Mg ha$^{-1}$ bio-fertilizer. The crop sequence from 2009 to 2012 was the alternation of crops used in the summer and winter seasons in the region.

Crop yield was determined by harvesting 5 m of the three central rows in the summer and winter season crops. The grains were submitted to a cleaning process to remove impurities and then dried for humidity correction. Grain weight was corrected to 14% humidity for beans and 13% for the other crops. The unit was converted to kg ha$^{-1}$ and then to Mg ha$^{-1}$. For black oat, we determined the dry mass production collecting two points with 0.17 m$^2$ at each plot. The soil total organic carbon (TOC) content was determined through the dry combustion method using an elementary C/N analyzer (TruSpec CN LECO® 2006, St. Joseph, EUA).

The results for content and the TOC stock in whole samples, the particle-size fractions and the SOM labile compartments were subjected to analysis of variance (ANOVA). The means that were significantly different from the *F* test were compared using the LSD test at 5% probability (*I* = 0.05) using SISVAR 5.1 [36].

# 4. Results and discussion

## 4.1. Crop response upon the use of slaughterhouse organic residues (SORs)

Combinations of 25% + 75%, 50% + 50% and 75 + 25% mineral fertilizer with organic fertilizer demonstrated significantly higher yields in several crop seasons compared with fertilization with 100% mineral fertilizer (**Table 1**). Sutton et al. [37] studied the effects of waste residue rates from ruminant animals and did not find differences in corn productivity among the rates or between mineral and organic fertilizers in 5 years of use. Despite the lack of significant difference, crop yields were always higher in organically fertilized plots.

| | | **Treatments** | | | | | |
|---|---|---|---|---|---|---|---|
| **Crops** | **Crop season** | **T$_1$** | **T$_2$** | **T$_3$** | **T$_4$** | **T$_5$** | **T$_6$** |
| | | **Mg ha$^{-1}$** | | | | | |
| Beans | 2009/2010 | 2.76$^{ns}$ | 2.85$^{ns}$ | 3.25$^{ns}$ | 3.21$^{ns}$ | 3.39$^{ns}$ | 3.16$^{ns}$ |
| Wheat | 2010 | 0.67$^{b}$ | 1.18$^{a}$ | 1.11$^{a}$ | 1.35$^{a}$ | 1.06$^{a}$ | 1.20$^{a}$ |
| Soybean | 2010/2011 | 2.11$^{ns}$ | 2.51$^{ns}$ | 2.34$^{ns}$ | 2.38$^{ns}$ | 2.50$^{ns}$ | 2.28$^{ns}$ |
| Oats | 2011 | 1.71$^{b}$ | 4.49$^{a}$ | 3.88$^{a}$ | 3.50$^{ab}$ | 4.43$^{a}$ | 3.20$^{ab}$ |
| Corn | 2011/2012 | 7.00$^{c}$ | 12.22$^{a}$ | 9.93$^{b}$ | 11.56$^{ab}$ | 10.17$^{ab}$ | 9.59$^{b}$ |
| Wheat | 2012 | 3.36$^{b}$ | 3.57$^{ab}$ | 3.61$^{ab}$ | 3.47$^{ab}$ | 4.06$^{a}$ | 4.02$^{ab}$ |
| Soybean | 2012/2013 | 2.42$^{c}$ | 2.74$^{ab}$ | 2.94$^{a}$ | 2.93$^{a}$ | 2.81$^{ab}$ | 2.99$^{a}$ |
| Oats | 2013 | 1.35$^{c}$ | 2.18$^{ab}$ | 2.48$^{a}$ | 2.06$^{ab}$ | 2.06$^{ab}$ | 2.12$^{ab}$ |
| Corn | 2013/2014 | 7.21$^{c}$ | 11.18$^{a}$ | 10.67$^{ab}$ | 11.64$^{ab}$ | 12.03$^{a}$ | 11.79$^{a}$ |
| Wheat | 2014 | 1.03$^{c}$ | 2.11$^{a}$ | 1.59$^{b}$ | 2.40$^{a}$ | 2.12$^{a}$ | 1.28$^{bc}$ |
| Beans | 2014/2015 | 1.77$^{c}$ | 2.72$^{a}$ | 2.10$^{b}$ | 2.28$^{ab}$ | 2.68$^{a}$ | 2.63$^{a}$ |
| Accumulated | | 31.40$^{c}$ | 47.75$^{a}$ | 43.90$^{ab}$ | 46.79$^{a}$ | 49.82$^{a}$ | 44.26$^{ab}$ |

T$_1$ = Absolute control (without SORs and SMFs); T$_2$ = 100% SMF; T$_3$ = 100% SOR; T$_4$ = 75% SMF + 25% SOR; T$_5$ = 50% SMF + 50% SOR; T$_6$ = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at $P < 0.05$. "ns" indicates not significant by the $F$ test at $P < 0.05$. Source: Romaniw [60].

**Table 1.** Crop yields affected by slaughterhouse organic and mineral residues in no-till system.

Considering the six crop seasons accumulated (**Table 1**), the treatments with the highest productivities were T$_4$ (75% SMF + 25% SOR) and T$_5$ (50% SMF + 50% SOR), representing increases of 49.0% and 58.7% in relation to the control treatment (without SMFs and SORs), respectively.

Many authors [31, 38-40] have reported increases in crop yields due to the use of organic sources in fertilization. However, some of them can only be observed from medium-term to long-term courses due to the slow and gradual soil property change, as observed in Table 1, with changes among the fertilizer treatments observed only after the third crop.

Through cost-benefit analysis (**Table 2**), we could identify that the increase in crop yield and the mineral fertilizer cost reduction, in response to the increase in SOR rates, reflected in increases in net earnings in comparison with SMF fertilization. With the application of the lowest SOR rate (25%), there were savings of 292.57 USD per hectare compared with the mineral fertilizer, but the maximum savings of 1170.27 USD per hectare was achieved with the application of 22 Mg ha$^{-1}$ SOR along the 11 crop seasons.

| | **Treatments** | | | | |
|---|---|---|---|---|---|
| **Crops** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** |
| | | | **U\$ ha$^{-1}$** | | |
| Beans | 280.40 | 125.63 | 241.71 | 203.02 | 164.32 |
| Soybean | 240.86 | 125.63 | 212.06 | 183.25 | 154.44 |
| Corn | 529.65 | 125.63 | 428.64 | 327.64 | 226.63 |
| Oats | 301.51 | 125.63 | 257.54 | 213.57 | 169.60 |
| Wheat | 508.79 | 188.44 | 428.71 | 348.62 | 268.53 |
| Accumulated | 1861.22 | 690.95 | 1568.65 | 1276.09 | 983.52 |

*Base values were obtained from SEAB [41]. $T_1$ = Absolute control (without SORs and SMFs); $T_2$ = 100% SMF; $T_3$ = 100% SOR; $T_4$ = 75% SMF + 25% SOR; $T_5$ = 50% SMF + 50% SOR; $T_6$ = 25% SMF + 75% SOR. *SMF rates were recommended according to cultivated crop and soil analysis, and the SOR rate was fixed at 2 Mg ha$^{-1}$.

**Table 2.** Fertilization costs (SORs and SMFs) along the crop seasons.

### 4.2. Soil organic matter (SOM) pools in crop systems affected by SOR application

As soil organic matter (SOM) is closely linked to C, it is essential to note that it is found in highly variable situations in terms of level of decomposition, chemical composition, size, level of recalcitrance as well as chemical and physical protection. For this reason, fractionation methods were used (chemical or physical) to classify and quantify the effects of SOR application on the SOM pools. In addition, the use of SORs as fertilizers in the medium and long terms can increase TOC content and microbial activity, which results in the recovery of soil quality and increases crops' productive potential.

Analyzing the use of slaughterhouse waste over SOM pools, we could observe an increase in total organic carbon (TOC) stocks at the 0–20 cm layer through the use of a combination of 50% SOR + 50% SMF (**Table 3**). This increase was 33.8% higher than that in the control (without SORs and SMFs) and 28.8% higher than that in $T_2$ (100% SMF). Previous studies developed by Filho et al. [42] and Zhang et al. [43] concluded that fertilization with organic waste in long-term experiments elevated soil carbon (C) and nitrogen (N) levels.

| Soil layer (cm) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| | TOC content, g $kg^{-1}$ | | | | | |
| 0–5 | $18.33^{ns}$ | 18.03 | 20.80 | 19.67 | 21.60 | 18.70 |
| 5–10 | $13.97^{ns}$ | 13.50 | 13.43 | 13.97 | 15.87 | 13.07 |
| 10–20 | $13.00^{ns}$ | 13.37 | 12.53 | 12.70 | 14.27 | 13.17 |
| | TOC stock, Mg $ha^{-1}$ | | | | | |
| 0–5 | $11.45^{ns}$ | 9.15 | 12.52 | 17.13 | 18.59 | 16.78 |
| 5–10 | $8.70^{ns}$ | 10.17 | 9.45 | 9.24 | 10.13 | 10.85 |
| 10–20 | $15.94^{ns}$ | 18.18 | 17.17 | 17.31 | 19.59 | 18.04 |
| 0–20 | $36.09^{b}$ | $37.50^{b}$ | $39.14^{b}$ | $43.67^{b}$ | $48.31^{a}$ | $45.66^{b}$ |

$T_1$ = Absolute control (without SORs and SMFs); $T_2$ = 100% SMF; $T_3$ = 100% SOR; $T_4$ = 75% SMF + 25% SOR; $T_5$ = 50% SMF + 50% SOR; $T_6$ = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at $P < 0.05$. "ns" indicates not significant by the $F$ test at $P < 0.05$. Source: Romaniw et al. [54].

**Table 3.** Total organic carbon (TOC) contents and stocks in response to the use of mineral fertilizers and slaughterhouse organic waste applied in isolated and combined forms in no-till system.

de Andrade et al. [44] observed higher increases in TOC in the second year after the application of sewage sludge biosolids in sugarcane, emphasizing that such effects could be further increased in subsequent years.

The contents and stocks of mineral-associated organic carbon (MAOC) and the contents of particulate organic carbon (POC) presented different responses upon the fertilization treatments (**Table 4**).

| Layer (cm) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| | POC content, g $kg^{-1}$ | | | | | |
| 0–5 | $5.00^{ns}$ | 5.16 | 5.98 | 5.42 | 6.28 | 4.76 |
| 5–10 | $2.96^{ns}$ | 2.66 | 2.79 | 2.67 | 2.83 | 2.71 |
| 10–20 | $2.80^{ns}$ | 2.68 | 2.60 | 2.50 | 3.37 | 2.57 |
| | MAOC content, g $kg^{-1}$ | | | | | |
| 0–5 | $50.00^{ns}$ | 44.73 | 49.70 | 49.27 | 52.53 | 44.73 |
| 5–10 | $38.53^{ns}$ | 37.07 | 35.90 | 39.30 | 40.13 | 37.87 |
| 10–20 | $35.00^{ns}$ | 24.20 | 33.83 | 36.97 | 41.93 | 35.73 |
| | POC stock, Mg $ha^{-1}$ | | | | | |
| 0–5 | $2.03^{ab}$ | $2.18^{a}$ | $2.04^{ab}$ | $2.13^{ab}$ | $1.75^{ab}$ | $1.39^{b}$ |

| **Layer (cm)** | **Treatments** | | | | | |
|---|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** |
| 5–10 | $1.17^{bc}$ | $1.06^{c}$ | $1.28^{bc}$ | $1.19^{bc}$ | $1.98^{a}$ | $1.79^{ab}$ |
| 10–20 | $2.15^{ns}$ | 2.29 | 2.20 | 2.10 | 2.29 | 2.97 |
| 0–20 | $5.35^{ns}$ | 5.53 | 5.52 | 5.42 | 6.02 | 6.15 |
| | MAOC stock, Mg $ha^{-1}$ | | | | | |
| 0–5 | $9.41^{bc}$ | $6.97^{c}$ | $10.48^{abc}$ | $15.00^{ab}$ | $16.84^{a}$ | $15.39^{ab}$ |
| 5–10 | $7.54^{ns}$ | 9.11 | 8.17 | 8.05 | 8.14 | 9.06 |
| 10–20 | $13.79^{b}$ | $15.89^{ab}$ | $14.97^{ab}$ | $15.21^{ab}$ | $17.31^{a}$ | $15.07^{ab}$ |
| 0–20 | $30.74^{c}$ | $31.97^{bc}$ | $33.62^{bc}$ | $38.25^{abc}$ | $42.29^{a}$ | $39.51^{ab}$ |

$T_1$ = Absolute control (without SORs and SMFs); $T_2$ = 100% SMF; $T_3$ = 100% SOR; $T_4$ = 75% SMF + 25% SOR; $T_5$ = 50% SMF + 50% SOR; $T_6$ = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at $P < 0.05$. "ns" indicates not significant by the *F* test at $P < 0.05$. Source: Romaniw et al. [54].

**Table 4.** Contents and stocks of particulate organic carbon (POC) and mineral-associated organic C (MAOC) in response to the application of mineral fertilizers and slaughterhouse waste isolated and combined in no-till system.

| **Layer (cm)** | **Treatments** | | | | | |
|---|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** |
| | C-OXP content, g $kg^{-1}$ | | | | | |
| 0–5 | $1.29^{a}$ | $2.34^{b}$ | $2.74^{b}$ | $2.49^{b}$ | $2.72^{b}$ | $2.86^{b}$ |
| 5–10 | $0.89^{a}$ | $1.71^{bc}$ | $1.74^{bc}$ | $1.54^{b}$ | $1.89^{c}$ | $1.80^{bc}$ |
| 10–20 | $0.67^{a}$ | $1.61^{b}$ | $1.43^{b}$ | $1.28^{b}$ | $1.66^{b}$ | $1.68^{b}$ |
| | C-HW content, g $kg^{-1}$ | | | | | |
| 0–5 | $0.42^{a}$ | $0.67^{a}$ | $1.29^{b}$ | $2.07^{c}$ | 2. | $2.33^{c}$ |
| 5–10 | $0.50^{a}$ | $0.69^{a}$ | $0.69^{a}$ | $1.93^{b}$ | $2.02^{b}$ | $1.95^{b}$ |
| 10–20 | $0.51^{a}$ | $0.67^{a}$ | $0.57^{a}$ | $1.54^{b}$ | $1.95^{b}$ | $2.03^{b}$ |
| | C-OXP stock, Mg $ha^{-1}$ | | | | | |
| 0–5 | $0.81^{a}$ | $1.48^{b}$ | $1.70^{b}$ | $1.57^{b}$ | $1.71^{b}$ | $1.80^{b}$ |
| 5–10 | $0.62^{a}$ | $1.20^{b}$ | $1.22^{b}$ | $1.08^{b}$ | $1.33^{b}$ | $1.26^{b}$ |
| 10–20 | $0.91^{a}$ | $2.21^{b}$ | $1.95^{b}$ | $1.75^{b}$ | $2.28^{b}$ | $2.30^{b}$ |
| 0–20 | $2.35^{a}$ | $4.88^{b}$ | $4.90^{b}$ | $4.40^{b}$ | $5.32^{b}$ | $5.36^{b}$ |
| | C-HW stock, Mg $ha^{-1}$ | | | | | |
| 0–5 | $0.26^{a}$ | $0.42^{a}$ | $0.81^{b}$ | $1.31^{c}$ | $1.30^{c}$ | $1.47^{c}$ |
| 5–10 | $0.35^{a}$ | $0.48^{a}$ | $0.48^{a}$ | $1.35^{b}$ | $1.41^{b}$ | $1.37^{b}$ |
| 10–20 | $0.70^{a}$ | $0.91^{a}$ | $0.79^{a}$ | $2.10^{b}$ | $2.67^{b}$ | $2.78^{b}$ |

| Layer (cm) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| 0–20 | 1.31$^a$ | 1.82$^a$ | 2.08$^a$ | 4.76$^b$ | 5.38$^b$ | 5.61$^b$ |

$T_1$ = Absolute control (without SORs and SMFs); $T_2$ = 100% SMF; $T_3$ = 100% SOR; $T_4$ = 75% SMF + 25% SOR; $T_5$ = 50% SMF + 50% SOR; $T_6$ = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at $P < 0.05$. "ns" indicates not significant by the $F$ test at $P < 0.05$. Source: Romaniw et al. [54].

**Table 5.** Contents and stocks of C oxidizable by potassium permanganate (C-OXP) and hot water (C-HW) in response to the use of mineral fertilizers and organic residues from slaughterhouses applied alone or in combination under a no-till system.

For POC at the 0–20 cm layer, the treatments that provided the highest increases were $T_5$ (50% SMF + 50% SOR) and $T_6$ (25% SMF + 75% SOR), with increments of 12.5% and 14.9%, respectively, in comparison with the control (**Table 4**). For MAOC at the same depth, the highest increases were also provided by $T_5$ and $T_6$, with increments of 37.6% and 28.5%, respectively, in comparison with the control. The increase and maintenance of labile SOM pool stocks are essential for the amelioration of soil quality and for the sustainability of crop systems, since they are essential for soil microbial activity [45].

The C-HW content decreased with soil depth, suggesting a stratification profile in the soil (**Table 5**). This fact is already well reported in no-till systems [46-48], and due to the addition of SORs, this response was even more pronounced, leading to higher biomass-C input from crop residues over the soil surface.

In addition, the SOR application in combination with SMFs increased the C-OXP and C-HW stocks regardless of the SMF combination. Considering the C-OXP pool at the 0–20 cm layer, the treatments that provided the highest increases were $T_5$ (50% SOR + 50% SMF) and $T_6$ (75% SOR + 25% SMF), representing increases of 126.4% and 128.0%, respectively, in comparison with the control. For C-HW at the same depth, the $T_5$ and $T_6$ treatments were also the ones that provided the highest increases, with increments of 310.7% and 328.2%, respectively, in comparison with the control.

This impact of the combinations of SORs and SMFs, in the short term, could be attributed to the increase of labile SOM pools, promoting higher soil biological activity [48]. The input of organic residues also plays an important role in soil aggregation [49] and higher C protection [50]. Similar results were also found by Kanchikerimath and Singh [51] and Rudrappa et al. [52] in the medium term (more than 5 years) in India.

Thus, fertilization with SORs favors soil microbial activity and stimulates soil organic matter mineralization [53]. Therefore, combinations of SORs and SMFs can lead to higher C inputs, and depending on the soil layer, they can even surpass the increments provided by isolated SMFs.

### 4.3. SOR rates affecting C-$CO_2$ emission and soil organic matter pools in incubated soil

The mean C-$CO_2$ flux rate of incubated soils with SOR applications varied from 0.30 to 2.79 Mg ha$^{-1}$ at the lowest rate (0 Mg ha$^{-1}$) at the beginning of the incubation and at the highest SOR rate (16 Mg ha$^{-1}$) at the end of the incubation process (**Figure 1**). At the highest SOR rate, there was an increase in the C-$CO_2$ flux equivalent to seven times compared with the beginning of the process (0.45 Mg ha$^{-1}$ C-$CO_2$).

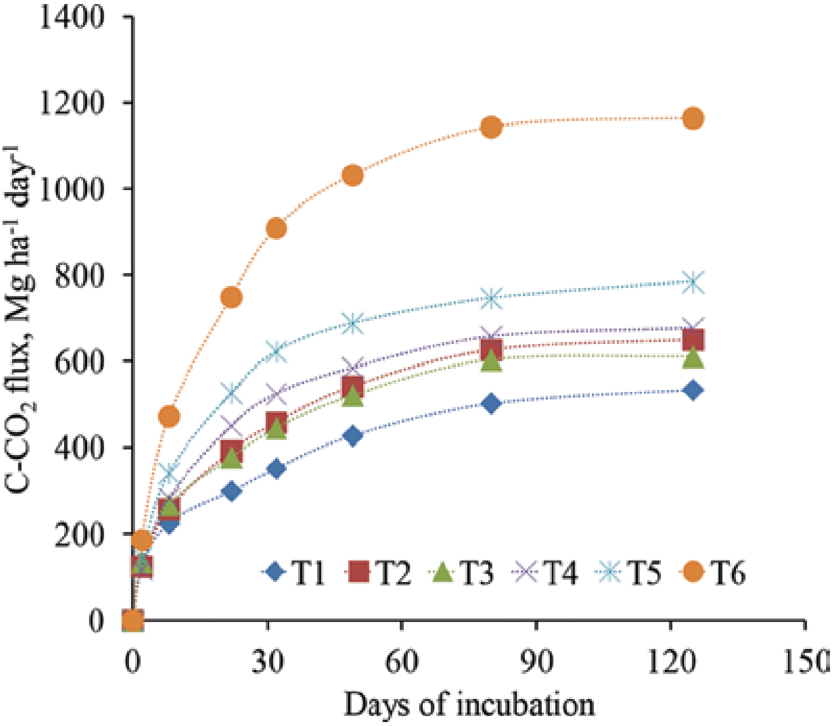

**Figure 1.** C-$CO_2$ flux in incubated soils with different rates of SOR. $T_1$ = Control (0 Mg ha$^{-1}$ SOR); $T_2$ = 1 Mg ha$^{-1}$ SOR; $T_3$ = 2 Mg ha$^{-1}$; $T_4$ = 4 Mg ha$^{-1}$; $T_5$ = 8 Mg ha$^{-1}$; $T_6$ = 16 Mg ha$^{-1}$. Source: Romaniw [60].

The mean C-$CO_2$ fluxes observed due to the increasing SOR applications of 0, 1, 2, 4, 8 and 16 Mg ha$^{-1}$ were 10.3, 12.5, 12.6, 13.0, 15.0 and 22.4 kg ha$^{-1}$, respectively. Therefore, only the highest SOR rate of 16 Mg ha$^{-1}$ is out of the ideal range of 9.8–19.5 kg ha$^{-1}$ as evaluated by the Soil Quality Kit test in long-term experiments [55].

With the increase in SOR rates in incubated soils, we could observe a more pronounced effect 60 days after applications (**Figure 1**), which indicates that the $CO_2$ emission rates of microbial biomass decrease as C starts being fixed in the soil.

After the initial increase on day 45, in general, $CO_2$ emissions among the treatments tended to be similar to the control soils. After 60 days of incubation, all treatments started emitting a similar amount of $CO_2$. Such evolution was also observed by Sánchez-Monedero et al. [56] in an incubation experiment with composted sewage sludge at different stabilization degrees.

According to **Figure 1**, there is a tendency for stabilization of the C-$CO_2$ emissions after 80 days of incubation. This fact may be related to the availability of substrate for microbial activity as reported by Campbell et al. [57]. The balanced fertilization with SORs as a source of labile carbon supports the microbial activity, resulting in increases in C-$CO_2$ emissions.

The increase in SOR rates resulted in a linear tendency with the C-$CO_2$ flux (**Figure 2**). This tendency is probably related to the SOR C:N ratio and structure, which provides higher surface contact with soil particles. These factors allied to ideal conditions of humidity and temperature increase the microbial activity, leading to higher C-$CO_2$ emission rates [58]. The increase in C-$CO_2$ emission with higher SOR rates at the end of the incubation period is probably related to the fast soil microbiota growth and the decomposition of higher organic material amounts. This fact indicates that such higher SOR rates could cause a higher liberation of organic materials in the soil, which easily decompose due to temperature and humidity conditions.

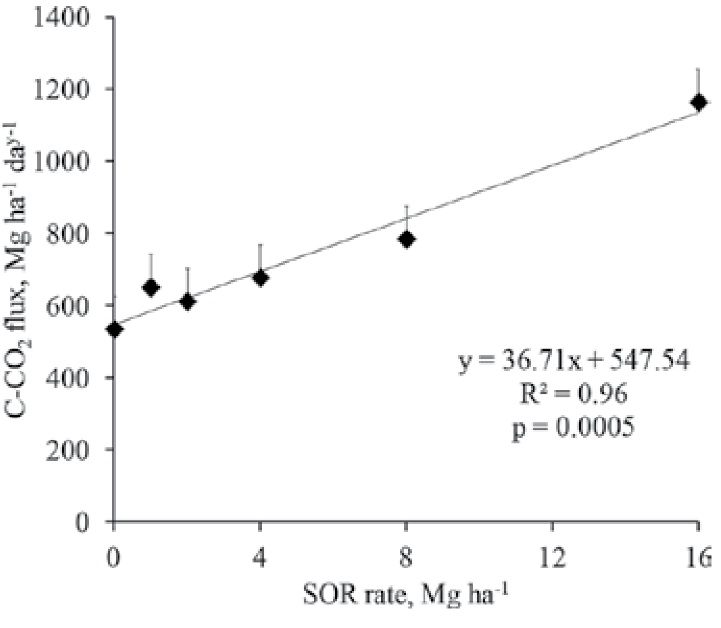

**Figure 2.** Accumulated C-$CO_2$ emissions affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg $ha^{-1}$ after 125 days of incubation. Source: Romaniw [60].

Although the increase in SOR rates resulted in higher C-$CO_2$ emissions, linear increases in the TOC were observed ($P < 0.05$) (**Figure 3**), indicating its influence over soil carbon mineralization. The fast mineralization at the beginning of the incubation process is mainly related to the amount of labile carbon available. As the decomposition process begins, the influence of labile fraction lessens due to its easy degradation [58, 59]. In general, all samples with SOR application presented higher TOC contents compared with the control.

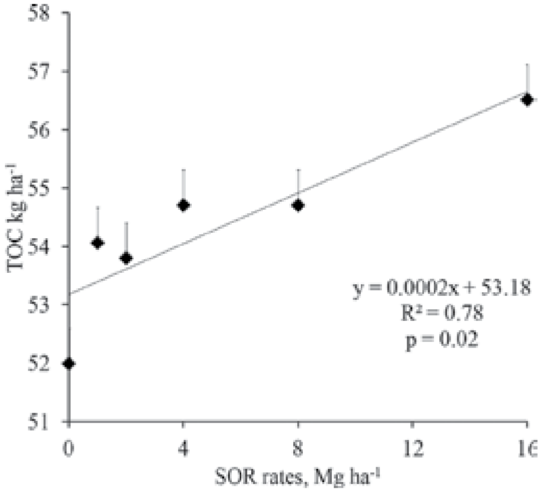

**Figure 3.** TOC content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha$^{-1}$ after 125 days of incubation. Source: Romaniw [60].

The C-HW content decreased at the SOR rate of 16 Mg ha$^{-1}$. The high SOR rate possibly caused a reduction in soil aeration, leading to lower microbial activity and carbon mineralization (C-HW) (**Figure 4**).

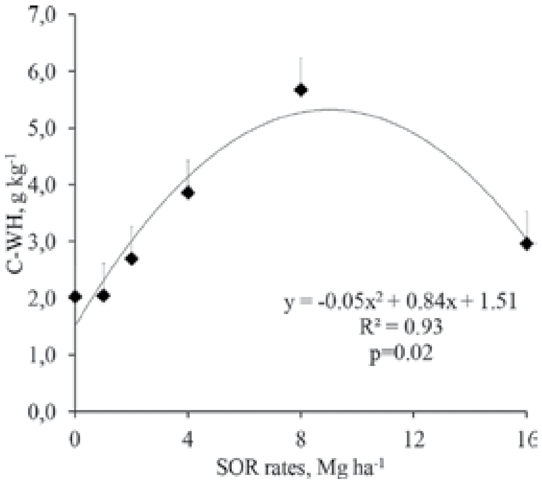

**Figure 4.** C-HW content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha$^{-1}$ after 125 days of incubation. Source: Romaniw [60].

The SOR rate of 8 Mg ha$^{-1}$ provided increases in C-HW and C-OXP (**Figures 4** and **5**), mainly because of the high microbial activity due to the availability of labile carbon. The proportions between labile and recalcitrant fractions differ in the fertilizer that presents a higher concentration of soluble fraction and that with lower fiber contents [61]. The differences in biochemical composition can alter the structure of microbial biomass and affect its efficiency in C use, resulting in differences in C mineralization of different organic sources.

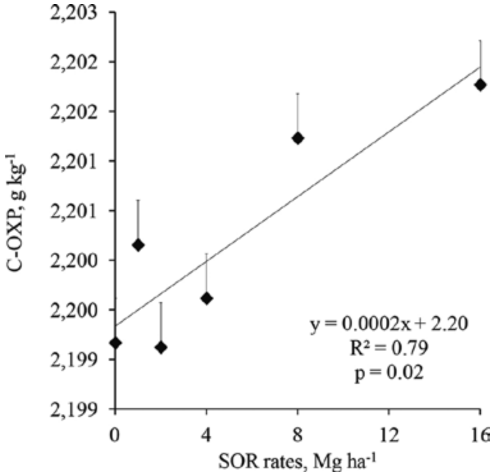

**Figure 5.** POXC content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg $ha^{-1}$ after 125 days of incubation. Source: Romaniw [60].

The C-$CO_2$ flux, when related to the TOC stock, expressed the amount of C-$CO_2$ lost by each Mg $ha^{-1}$ TOC produced according to the SOR rates applied (**Figure 6**). This parameter is a sensitive indicator of the environmental changes that can occur due the increasing SOR applications. It can be used to detect disturbances, reflecting the increase in C-$CO_2$ emission. The TOC stock was greater with the increase in SOR rates, which indicates higher potential for C-$CO_2$ sequestration.

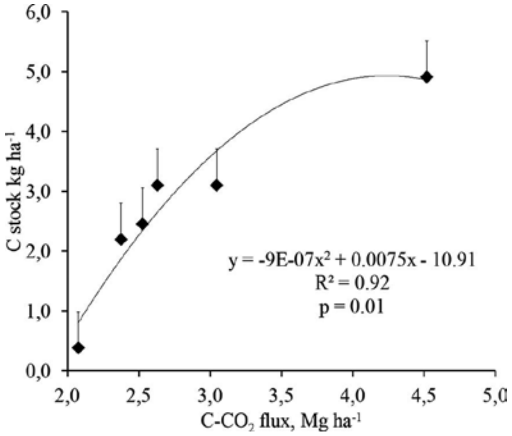

**Figure 6.** Relationship between C-$CO_2$ flux and TOC stock in incubated soils for 125 days with SOR rates of 0, 1, 2, 4, 8 and 16 Mg $ha^{-1}$. Source: Romaniw [60].

The increase in C-$CO_2$ emissions with SOR addition produced an initial increment and variability in TOC (**Figure 6**). This variability suggests disturbance in the microorganisms' activity through the SOR addition. The SOR applications provided accumulated emissions of 1.28, 1.56, 1.58, 1.63, 1.88 and 2.79 Mg $ha^{-1}$ C-$CO_2$ and fixations of 0.24, 1.52, 1.36, 1.92, 1.92 and 3.04 Mg $ha^{-1}$ TOC (reduced values from the initial TOC) in the soil after the 125-day incubation period. These results indicate that, although there is a pronounced flux of C-$CO_2$ with higher SOR applications, the TOC levels also increased. The TOC fixation was higher than the C-$CO_2$ flux for the 4, 8 and 16 Mg $ha^{-1}$ rates.

Therefore, SOR application can be considered a promising strategy in order to provide soil C sequestration, affecting directly the quality and productivity of the system.

## 5. Conclusions

The applications of poultry and pork slaughterhouse waste increased crop productivity, especially in $T_5$ (50% SOR + 50% SMF). The C labile pools (C-HW, C-OXP and POC) were higher in the treatments with elevated SOR applications (50% and 75%), thereby increasing soil quality and sustainability. In addition, fertilization with SORs demonstrated to be an alternative to minimize the costs and use of mineral fertilizers and increase C sequestration.

## 6. Acknowledgements

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## 7. Acronyms

SOR: Slaughterhouse organic residue

SMF: Synthetic mineral fertilizer

N: Nitrogen

C: Carbon

TOC: Total organic carbon

MAOC: Mineral-associated organic carbon

POC: Particulate organic carbon

C-OXP: Permanganate oxidizable organic carbon

http://dx.doi.org/10.5772/63123

C-HW: Hot water extractable organic carbon

GHG: Greenhouse gases

$C\text{-}CO_2$: Carbon emitted as carbon dioxide.

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# Organic Waste as Fertilizer in Semi-Arid Soils and Restoration in Mine Sites

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Additional information is available at the end of the chapter

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## Abstract

The use of organic waste such as tannery sludge which has high organic matter, N and P content, as organic fertilizer is suitable for improving soil fertility in semi-arid soils and for remediation of abandoned mine sites. Retention of heavy metals on fractional processes of organic matter cannot be generalized, it depends on the chemical characterization of organic waste and soil. Addition of tannery sludge containing high concentrations of Cr and carbonates to semi-arid soils resulted in an increase in Cr loss in infiltration and runoff after 6 months of incubation followed by simulated rainfall. Under these characteristics, results suggest that tannery sludge represents a potential ha ls amended with organic compost. Chemical characteristics of organic waste such as nitrogen content, humified organic matter, pH, EC, CEC, ESP (interchangeable sodium percent), and SAR (sodium absorption ratio) are important properties to consider in organic matter amendment to semi-arid soils participating on the complexity and leaching of heavy metals and nutrients in the matrix of soil.

**Keywords:** tannery sludge, simulated rainfall, mobility of heavy metals, mine tailings, heavy metal fractionation

## 1. Introduction

Soils of arid and semiarid regions need the amendment of organic compounds due to being low in organic C content and as consequence unable to improve their physicochemical and biological properties and thus their yield of crops and their natural fertility [1, 2].

Organic amendments, such as farmyard manures, have been used as common organic manure for supplying nutrients to plants. After the second world war, there was a great interest in

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using other organic wastes as organic fertilizer, farmyard manure has been used as a reference when sewage sludge or other organic materials have to be judged [3]. The sewage sludge and sludge compost amendment were ceased when it was found that soluble heavy metals in the soil and the total heavy metals in the crops were increasing when these wastes were applied [4]. However, sewage sludge and compost are the most widely used organic amendments, mainly by their high organic matter, N and P content, which are important nutrients for plant growth, their contribution to soil structure, and resistance to soils erosion [5–8]. On the other hand, application of sewage sludge as agricultural fertilizer is associated to numerous environmental and health problems such as those implicated by toxic metals, organic compounds, and possible other health problems related to pathogens [9–12].

The gradual increase in industrialization and urbanization in the last decades has created an enormous increase in volumes of wastes produced all over the world. Usually, these wastes are discharged to the environment [13], especially in countries which regulatory control is not strict.

The objective of the case study described in this chapter, together with methodology and literature review is to provide different scenarios of the role of organic waste in semi-arid soils poor in organic carbon, the effect of heavy metals on soil biogeochemical processes, their dispersion and mobility in soils, and the availability of heavy metals to plants.

### 1.1. Semi-arid soils and tannery sludge

Tannery sludge derives from a complex combination process where organic and inorganic materials become chemically bound to the protein of the hides and preserve it from deterioration. A significant number of operations within the tannery industry involve large amounts of water, chemicals, and energy leaving as waste large amounts of polluted water. These industrial effluents contain several types of chemicals such as dyes, levelling agents, acids, alkalis, phenols, carbonates, alcohols, cyanide, and heavy metals, among others [14].By-products generated during leather manufacturing are usually rich in proteic matter and organic substances, thus it is a potential resource that can be used as fertilizer in agriculture production. This leather processing waste is an attractive disposal for soil amendment as it has proven to improve the physical properties of soil and supply organic matter and plant nutrients [15–19].

The use of these waste in semi-arid soils as organic fertilizer of plants could be an alternative disposal method and simultaneously it will resolve the environmental risks presented when they are abandoned to open sky.

Under natural environmental conditions, chromium is present in either the trivalent Cr(III) or the hexavalent Cr(VI) [20, 21]. The effect of Cr on health have been widely studied [22]. Cr(VI) is about 300 times more toxic than Cr(III).Health effects of Cr have been reported in lung cancer, and birth defects [23, 24].Cr(III) have relatively low toxicity and are easily precipitated and immobilized; however, Cr(VI) is toxic, water soluble, and highly mobile, and can then be transported into the surrounding surface soil and ground water [25, 26].Tannery sludge contains both trivalent (CrIII) and hexavalent (CrVI) chromium. There is little information about the biogeochemical conditions affecting solubility of heavy metals in arid and semi-arid soils including soil pH, Eh, and dissolved organic carbon contents [27]. The information generated in this study is critically important for assessing the benefits or potential risks of using tannery waste to treat semi-arid soils for re-forestation.

### 1.2. Mine tailings amended with organic wastes

Mine tailing disposal sites from either inactive or abandoned mines are common in arid and semiarid regions throughout the mine region around the world. These tailings have been stored outside and have contaminated local ecosystems and harmed the nearby populations [28]. Today, areas containing mine tailings can be found in urban and agricultural zones and mine tailing storage after the closure of mining operations is becoming increasingly problematic in the arid and semiarid region because of wind erosion. These areas are a source of air pollution giving off particles [29]. Short-term exposure to mine tailing particles can lead to illness, while long-term exposure may lead to premature death in adults and children [30, 31].

## 2. Research methods

### 2.1. Sampling areas

The soil was sampled from three sites: Two around mesquite trees (*P. laevigata*), the dominant vegetation (Dolores Hidalgo, Guanajuato, Mexico), under the canopy and outside the canopy of mesquites (**Figure 1**), and the third one from a site cultivated with maize (*Zea mays*) for 20 years.

**Figure 1.** Under the canopy tree of mesquite (U), Outside the canopy of mesquite (O).

Soil was collected from a 0- to 5-cm layer, where the highest organic contents can be found. The first sampling was taken from under the canopy of four isolated mesquite trees, 1–2 m from the stem in four perpendicular directions randomly selected. The second one at a distance of 6–8 m from the stem, outside the canopy in the same perpendicular directions (**Figure 1**).

The soil was bulked; all the stones, visible roots, and fauna were removed, it was sieved to less than 2 mm and stored at 5°C to use latter.

### 2.2. Tannery sludge

Tannery sludge, produced during leather manufacturing, when processing skin or hide to leather, was sampled from a tannery in Leon (Guanajuato, Mexico). It contained large quantities of hair, fatty fleshings, and soluble proteins, as well as sulphide, lime, chromium-sulphate, salts, dyes, acids, and leather trimmings.

### 2.3. Incubation experiments

A pre-incubation process of soil samples was necessary to allow the soil microbial activity to stabilize after the sampling and sieve management. Soils were pre-incubated for 1 week prior to starting the experiment at conditions similar to the experiment, i.e. at 20°C in the dark, in a temperature and humidity controlled room. Three replicates were destructively harvested at days 0–90 or 120 and stored at −20°C for N mineralization and soil microbial activities analysis.

### 2.4. Soil microbial activities and nitrification

Maintaining soil fertility depends on biomass and activity of soil microorganisms vital in the biological cycles of most major plant nutrients [32]. Microorganisms are also involved in forming soil structure [33]. Several microbiological parameters have been suggested to measure soil environmental quality [34]. For instance, soil respiration and enzyme activities such as dehydrogenase activity and nitrification, can inform about the presence of viable microorganisms, and on the intensity, kind and time length of the effects of pollutants on the metabolic activity of soils.

Sub-samples of 40 g of soils were placed in 110-ml glass bottles, which were then put into 1-l jars containing 10 ml $H_2O$ and a vessel with 20 ml 1 M NaOH solution. The jars were air-tight sealed with plastic lids and incubated at 25 °C for 7 days. After incubation, vessels with 20 ml 1 M NaOH solution were removed, resealed, and stored for future $CO_2$ analysis. At the mentioned dates, soil was removed for analysis of $NO_3^-$–N, $NO_2$–N, and $NH_4^+$–N, done by shaking for 30 min with 100 ml 0.5 M $K_2SO_4$ solution and filtered through Whatman No. 42 paper. Similarly, control fresh samples were extracted. Extractants were stored at −20°C until analysis. Concentration of $NO_3^-$–N and $NO_2^-$–N in the extracts was determined by colorimetric method [35] and $NH_4^+$–N by Indophenol blue [36].

Dehydrogenase activity in soils has been used as measure for overall microbial activity [37]. The method is based on the estimation of triphenyltetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) in soils after incubation at 30°C for 24 h. Soil dehydrogenase activity was measured using a modified form of the method used by Casida [37]. Five grams of fresh soil were incubated at 37°C for 24 h in test tubes containing 1 ml 3% 2,3,5- triphenyl-tetrazolium chloride (TPF), 67 mg $CaCO_3$, and 2.5 ml distilled water. The accumulation of the end-product triphenyl formazan (TPF)was determined in acetone extracts (50 ml) using a PerkinElmer Lamda 3A Spectrophotometer at 520 nm.

### 2.5. Measurement of $CO_2$-evolved

Glass bottles of 110 ml containing the amended substrate and unamended control soils were placed in 1-l, wide-mouthed glass jars, with a glass flask of 30 ml containing 20 ml of 1 N NaOH solution to trap the evolved $CO_2$. Jars were tightly closed and incubated at 20°C for up to 270 days at room temperature. The NaOH solution was exchanged every 7 days during the first 2 months, and monthly thereafter. Jars were aired each time for 2 min when they were opened to exchange the NaOH solution, to avoid anaerobic conditions in amended and unamended soils. Every time that the NaOH trapped were collected, a blank with non-soils in the jars were collected, too. The values of $CO_2$ in the blanks were used to correct the $CO_2$ trapped inside the jars. $CO_2$ trapped in 1 M NaOH solution was measured in a 5-ml aliquot by titrimetric methods with a standard 0.1 M HCl solution using the phenolphthalein indicator method [38].

### 2.6. Chemical analysis

Total organic C in the soil and tannery sludge was measured using dichromate digestion [39], total N was measured using Kjeldahl digestion [40], and total hydrolysable and orthophosphate phosphorus were determined using the stannous chloride method [35]. The particle-size was analyzed using the hydrometer method [41]. To conclude, total Cr in tannery sludge, fleshing waste, and infiltration and runoff solutions was measured using absorption atomic spectrometry with a fitted graphite furnace spectrophotometer (Avanta M System 300, GF 3000 S/N 10288). Tannery sludge and fleshing waste were digested with 4:1 HCl: $HNO_3$ using a digiprep TM digestion system, prior to analysis.

### 2.7. Fractionation of chromium

Tessier et al. scheme [42] is widely used, although application of sequential extraction is still subject controversy. The main problems of sequential extraction procedures are the non-selective use of extracts and the trace elements redistribution among phases during the extraction [43]. In spite of these restrictions, sequential extraction procedures have proved to be useful in the environmental analytical chemistry field [44].

Sequential extraction was utilized for partitioning Cr in soil and sludge amended soils into six operationally defined fractions described by Tessier et al. [42] and modified by Xiong et al. [45]. Six operationally defined fractions, exchangeable, bound to carbonates, bound to Mn oxides, bound to Fe oxides, bound to organic matter and residues according to procedure described by Tessier. Summarizing, 2 g of soil were placed in a 50-ml polycarbonate centrifuge tube and subjected to the following extraction program: Exchangeable fraction (I): soil extracted with 25 ml of 1 M ammonium acetate was shaken for 2 h, then centrifuged. Carbonate bound fraction (II): Fraction I residue extracted with 25 ml of 1 M sodium acetate, adjusted to pH 5 with acetic acid then shaken for 5 h and centrifuged. Mn-oxide-bound fraction (III): Fraction II residue extracted with 25 ml 0.1 M hydroxylamine hydrochloride adjusted to pH 2 with nitric acid then shaken for 12 h and centrifuged. Fe-oxide bound fraction (IV): Fraction III residue extracted with 25 ml of 0.2 M ammonium oxalate, adjusted to pH 3 with oxalic acid, and shaken for 24 h, then centrifuged. Organic and sulphide-bound fraction (V): Fraction IV residue extracted with 5 ml of 30% $H_2O_2$ adjusted to pH 2 with $HNO_3$ then heated in a water bath at 85°C. After cooling, 20 ml of I M ammonium acetate were added, shaken for 2 h, then centrifuged. Residual fraction (VI): Fraction V residue digested with 3:1 HCl: $HNO_3$ in digestion glass tubes. After digestion was completed, 25 ml water were added and then filtered. The levels of Cr in the six fractions (I to VI), plus a fresh sample, were analyzed with atomic absorption spectrometry as described above, at 1, 3, and 6 months of incubation.

Mobility factor percentage was calculated according to the equation:

$$\%mobility\ factor = \frac{\left(I + II + III\right)}{\left(I + II + III + IV + V + VI\right)} \times 100$$

Hexavalent Cr (CrVI) was quantified employing diphenylcarbazide procedure described by Bartlett [46]. One gram of soil was extracted with 3 ml of 10 mM $K_2HPO_4/KH_2PO_4$, pH 7.2. One milliliterazide reagent was added to 8 ml of extract, mixed and stand for 20 min and read the color at 540 nm. Azide reagent was prepared with 120 ml of 85% phosphoric acid, diluted with 280 ml distilled water, to 0.4 g of S-diphenylcarbizide dissolve in 100 ml of 95% ethanol.

### 2.8. Rainfall system experiment

For the experiment of simulated rainstorm, the rainfall system type Morin [47] was used (**Figure 2**).

**Figure 2.** Rainfall system type Morin.

### 2.9. Soil treatments

After adjusted to 50% WHC, sub-samples of 10 kg of soil were placed in 15 kg rough use plastic bags. Two different treatments were applied to the soils: tannery sludge (T) to outside (O) and under the canopy (U) of mesquite tree soils, and tannery sludge plus fleshing waste in outside and under the canopy soils (OTF and UTF, respectively). The amount of tannery sludge was chosen to give less than 300 mg Cr kg$^{-1}$; the critical level of Cr for acceptable use of waste and bio-products in agriculture, as established by the US Environmental Protection Agency [48].

A cotton plug was adjusted to the plastic bags to allow ventilation. After moistening, the treated soils were incubated at 25°C for 1, 3, and 6 months in order to allow mineralization to occur and before they were submitted to a simulated rainfall system. A fresh control was included in order to compare the experimental results with it. The treated soils were air-dried and passed through a 4-mm sieve. They were sampled and then each one was packed to a depth of 4 cm in a 30 × 50-cm box over a 12-cm layer of gravel and 4-cm layer of coarse sand for moisture tension control. Soils were then wetted by capillarity while maintaining the water in a levelled position. Following saturation, the boxes were set at a 3% slope and then allowed to drain for 30 min (**Figure 3**). A total of four runs for each treated group were conducted, each one after an incubation period (0, 1, 3, and 6 months). For each run, the soil treatments were placed on assoil box carrousel and subjected to a simulated rainstorm using the rainfall system type Morin [47] (**Figure 2**). The full description of a simulated rain experiment is in Barajas-Aceves et al. [49].

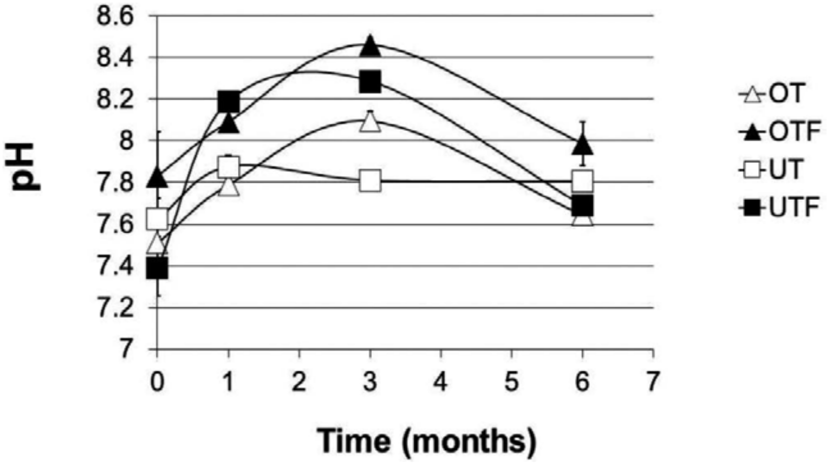

**Figure 3.** pH after a simulated rainfall in semi-arid soils amended with fleshing waste and or tannery sludge. Bars indicate standard deviation. Source: Ref. [49].

Simulated rainfall at intensity of 50 mm h$^{-1}$ and a drop diameter of 3 mm was applied for 60 min. With these specifications, the process simulated the 6 months of a raining period in Dolores Hidalgo, Guanajuato, Mexico. During each run, water percolation through the soil and sediment samples were collected in separated plastic bottles every 5 min for 1 h. Weight of sediment samples and infiltrated water was registered. Double aluminium potassium sulphate (20 ml at 10%) was added to the sediment samples to precipitate suspended solids and separate the water by decantation. The solids in the bottle were dried for 24 h at 105°C and the soil loss was measured by gravimetry, expressed in grams. Once the rainfall was stopped, the soil boxes were left for 24 h to allow the drainage ceased from them, the soil was randomly sampled, allow to dry slowly until it had a moisture equivalent to 40% WHC. Before the rainfall system started, the same soils with the same treatments were sampled and were adjusted to 40% WHC before the soil microbial activities determinations.

The decanted water was transferred to a plastic bottle and weighed. The volume was expressed in $cm^3$ (considering the water density of 1 g $cm^{-3}$). The runoff was calculated by the equation:

$$Runoff(mm) = \frac{V \times 10}{A}$$

where $V$ is the volume of runoff ($cm^3$), $A$ is the area exposed to rainfall (1500 $cm^2$), and10 is the factor to convert cm to mm.

The infiltration was calculated with a similar equation and expressed in mm [50–52].

$$Infiltration(mm) = \frac{V \times 10}{A}$$

The results of all these measurements were the sum of the 12 samples per treatment collected in one run.

## 3. Case study

### 3.1. Introduction

#### *3.1.1. Semi-arid soils and tannery sludge*

Applications of tannery sludge are restricted due to high Cr content, even when Cr(III) content in the sludge is considered to be unavailable. Nevertheless, the use of tannery sludge for reforesting the north of Guanajuato (a natural reserve in Dolores Hidalgo, Guanajuato) is appealing because, it can avoid soil degradation due to the increasing erosion in the region, and at the same time, it can reduce contamination by tannery sludge dumping to open air in Leon, Guanajuato.

The influence of Cr on nitrogen transformation in soils has been studied and reports show somewhat mixed results. James and Bartlett [53] found no inhibition in treatments containing Cr(III) in sewage sludge or tannery effluent but that nitrification was inhibited by Cr(VI) at a concentration of 10 $\mu g\ g^{-1}$ in soil suspensions. Chang and Broadbent [54] observed that nitrogen immobilization, mineralization, and nitrification were inhibited to a great extent by Cr(III) added to a neutral soil, but Cr(VI) was not measured in the extracting solutions used to characterize soil Cr in this study.

Previous studies in semi-arid soils collected in the same natural ecosystem with mesquite amended with tannery sludge to evaluate the biological functioning of soil, show that C and N mineralization increased. Similarly, there was not inhibition in the biological functioning of soil [2].

The aims of this research project were 1) to evaluate the environmental impact of heavy metals from tannery sludge by determining not only the total content in a matrix, but also their bioavailability and their capacity for mobilization and toxicity by chemical fractionation. 2) To indicate the effect of tannery waste type on soil aggregate stability, infiltration, runoff, sediments, nutrients, and Cr loss from semi-arid soils using a simulated rainfall system. This information will be useful for evaluating the ecological risk associated with the use of tannery sludge in semi-arid soils during reforestation projects.

Reducing exposure to contaminants from mining activities is important, especially in the old mining towns like Vetagrande, where large areas have been affected by the presence of mine tailings.

Mine tailings have chemical and physical properties limiting plant growth, like lack of organic matter or macronutrients. Usually, they are acidic, severely toxic, do not have soil structure, have low water retention and slow rates of water infiltration [31]. Organic supplements, such as compost, farmyard manure or biosolids, may be added to overcome these limitations. Moreover, the use of organic compost mixed with tailings could alter several qualities of soil such as potential mineralization of C and N from added organic waste and the mobility and availability of heavy metals in the soil.

The aims of the use of organic amendments on mine tailings were 1) to determine the mobility factor of heavy metals; 2) to evaluate the effect of different organic wastes amended in mine tailings on N and C mineralization potential; 3) to evaluate the availability of Pb and Zn in *Brasica juncea* as indicators of heavy metal availability for pollutants and two shrubs grown in mine tailings and mixed with compost

## 3.2. Methods

### *3.2.1. Sampling area*

The soils were sampled from the natural reserve "El Cortijo", in which the dominant vegetation is mesquite (*Prosopis laeviginata*), located in Dolores Hidalgo, Guanajuato, Mexico. The soils were collected from two sites: under the canopy and outside the canopy of mesquite tree. The average altitude of sites is between 1750 and 2000 m above sea level and the average annual rainfall is between 400 and 600 mm (mainly from June to September).

### *3.2.2. Tannery sludge*

Tannery sludge produced during leather manufacturing was sampled from a tannery in Leon (Guanajuato, Mexico). The sludge contained large quantities of hair, soluble proteins, and fatty fleshings from processing the skin to hide, and sulphide, lime, chromium-sulphate, salts, dyes, acid, and leather trimmings from processing the hide to leather. Two tannery sludge samples were collected in two different tannery industries with different Cr concentrations. A third tannery sludge was sampled from a tannery in Leon where the fleshing sludge was separated from the leather at the beginning of the process and then followed by chemical treatments. The fleshing waste contained small quantities of hair, soluble proteins and fatty fleshing from the processing of skin to hide. The three different sludge samples and fleshing waste were used in different studies with the same semi-arid soils. Chemical characterization is shown in **Table 1**. Tannery sludge was air-dried before used for the experimental aerobic incubation.

| | Tannery sludge 1 | Tannery sludge 2 | Tannery sludge 3 | Fleshing waste (F) |
|---|---|---|---|---|
| Total C (g $kg^{-1}$) | 281 | 257.8 | 7.65 | 5.68 |
| Total N (g $kg^{-1}$) | 53.4 | 18.7 | 0.77 | 1.5 |
| pH | 8.34 | 8.09 | 8.65 | 7.66 |
| Cr (mg $kg^{-1}$) | 6690 | 1663 | 6516 | 136 |
| Na (mg $kg^{-1}$) | — | — | 1174.6 | 119.95 |
| Ca (mg $kg^{-1}$) | — | — | 10.94 | 188.7 |

— = no determined. Data from: Refs. [2, 49, 55].

**Table 1.** Characteristics of tannery waste from Leon Guanajuato, México.

Amounts of tannery sludge 1 and 2 added to soils were in accord with the criteria of the amount of N covering the recommended dose of N for maize crop growth in the region. The amount of tannery sludge 1 which amended soils from outside and under the canopy of mesquite trees was 1.5 g of wettannery sludge to 50 g of soil. The total amount added to under and outside the canopy soils was approximately 1308 mg C $kg^{-1}$, 320 mg total N $kg^{-1}$, 45 mg total P $kg^{-1}$,and 414 mg Cr $kg^{-1}$ soils.

The amount of tannery sludge 2 added was 0.0125 g $g^{-1}$ soils, an amount which covered three times the requirement for the region recommended dose of N for maize crop (i.e. 260 kg N ha $^{-1}$). The following treatments with three replications were applied at a rate of 250 g $g^{-1}$ soil: control (without any amendment),Cr(III), Cr(VI), tannery sludge, Cr(III)+ tannery sludge, Cr(VI) + tannery sludge, Cr(III) ($Cr_2O_3$), and Cr(VI) ($K_2Cr_2O_7$). The criteria for dose selected was near to the value of the maximum upper limits of 300 mg $kg^{-1}$ for acceptable utilization of waste and by-products in agriculture as established by the US Environmental Protection Agency, Part 503 [56].The jars were sealed with air-tight plastic lids and incubated at 25 °C for 180 days. After 0, 30, 60, 120, and 180 days' incubation the $CO_2$ and inorganic N ($NH_4^+$, $NO_2^-$, and $NO_3^-$) were analyzed as described above.

The amount of tannery sludge 3 (**Table 1**) was 0.046 g $g^{-1}$ soils which was equivalent to 22.08 Ton $ha^{-1}$ and fleshing waste was 0.092 g $g^{-1}$ soil and was equivalent to 3.7 Ton $ha^{-1}$. The amount of tannery sludge was applied to give less than 300 mg Cr $kg^{-1}$ that is the critical level of Cr for acceptable utilization of waste and bio-product in agriculture [48]. These amounts of tannery sludge and fleshing waste contained approximately 13.0 and 2.4% less than 170 kg N $ha^{-1}$, which is the maximum dose recommended by the European nitrate directive [57] to reduce water pollution by $NO_3^-$–N from agricultural sources.

### 3.3. Results and discussion

Addition of tannery sludge 1 to outside and under the canopy of mesquite soils had no inhibitory effect on N mineralization and increased $CO_2$ production and inorganic N concentrations, but did not increase available P concentrations. These results suggest that tannery sludge could provide valuable nutrients to mesquite tree, the dominant vegetation in Dolores Hidalgo [2].

#### *3.3.1. N and C mineralization*

The amount of tannery sludge 2 used in another experiment [58]shows that inhibition of nitrification in outside the canopy soils increased when adding tannery sludge plus $Cr_6^+$ from 30 to 180 days. Soils under the canopy, amended with the same treatment, did not show a constant effect on nitrification throughout the incubation time (**Table 2**).

Results from this study showed that nitrification is sensitive to Cr(VI) added alone in outside the canopy soils from 30 to 120 days' incubation (**Table 2**) and to Cr(VI)plus tannery sludge from 30 to 180 days in soils outside the canopy (data not shown).Cr(III)added alone or Cr(III) plus tannery sludge added to the two soils had no specific effect on the microbial activities ($CO_2$ production or dehydrogenase activity) or N-mineralization [58].

| **CO2 production rate** | | |
|---|---|---|
| Treatment | % inhibition (30 days) | % inhibition (120 days) |
| UVI | 21.49 | 25.12 |
| UTVI | 7.57 | 14.71 |
| OVI | 98.48 | — |
| OTVI | 22.11 | — |
| Dehydrogenase activity | | |
| UVI | 15.39 | 15.06 |
| UTVI | 29.93 | 31.48 |
| OVI | 61.26 | 83.63 |

| **CO2 production rate** | | |
|---|---|---|
| OTVI | 49.61 | — |
| NO3-concentration | | |
| UVI | 52.82 | — |
| OVI | 69.64 | 83.81 |
| OTVI | 62.40 | 95.36 |
| OTIII | 43.38 | 27.73 |

All the values are significant at $p \leq 0.05$, — not significant. U = under the canopy, and O = outside the canopy soils, VI = Cr(VI), III = Cr(III), T = tannery sludge 2. Source [55].

**Table 2.** Inhibition of $CO_2$ production rate, dehydrogenase activity, and $NO_3^-$ concentration in under- and outside-the-canopy soils from Dolores Hidalgo, Mexico, incubated at 25°C for 30 and 120 days.

There is a conflicting effect on using mineralization of organic C in metal contaminated soils because of the stimulation and inhibition on respiration [59, 60]. Results of under the canopy soils show that Cr(VI)could have effects on complex soil organic matter and render it less available by reducing Cr(VI) to Cr(III) [61, 62]. Thus, under the canopy soil which had more organic C, there was less inhibition of $CO_2$ production rate than outside canopy soils.

In soils outside the canopy with low organic matter, Cr(VI) may have effects on soil organic matter available which increases with the dying of cells. Thus, the sum of $CO_2$ produced by death and surviving microorganisms will reflect a small or not inhibition of $CO_2$ production by Cr(VI) (**Table 2**). Adding tannery sludge plus Cr(VI)may reduce inhibition of $CO_2$ production rate by complexing the Cr(VI) with organic matter from tannery sludge [62].

### 3.3.2. *Cr fractionation*

Results from Cr fractionation in under and outside the canopy soils amended with Cr(VI), Cr(III), Cr(VI) plus tannery sludge 2, Cr(III)plus tannery sludge 2, or tannery sludge 2 alone was that the level of total Cr increased in the more resistant fraction (fraction VI which is the least soluble form) and increased further over time. The opposite trend occurred with non-residual fraction (sum of fraction I, II, III, IV, and V) which tended to decrease with time in the two soils (**Table 3**).

| **Time incubation** | **30 days** | | **30 days** | |
|---|---|---|---|---|
| Treatments | Residual | Non-residual | Residual | Non-residual |
| Under the canopy | | | | |
| Cr(III) | 5.4f | 4.1g | 41.4c | 0.90 h |
| Cr(VI) | 12.7e | 31.2f | 27.7g | 16.8 f |
| Tannery sludge | 26.0d | 53.6c | 50.1b | 25.7 d |
| Tannery sludge + Cr(III) | 31.2c | 49.1d | 66.4a | 33.0 c |

| Time incubation | 30 days | | 30 days | |
|---|---|---|---|---|
| Tannery sludge + Cr(VI) | 38.4b | 74.4b | 42.2c | 38.0d b |
| Outside the canopy | | | | |
| Cr(III) | 22.0d | 7.0g | 39.7c | 12.0 g |
| Cr(VI) | 25.0d | 44.2e | 26.5h | 24.1 e |
| Tannery sludge | 23.4d | 52.2c | 33.0 d | 32.4 c |
| Tannery sludge + Cr(III) | 45.1a | 39.1e | 29.7f | 26.1 d |
| Tannery sludge + Cr(VI) | 22.0d | 77.6a | 27.6 g | 65.5a |

Residual = fraction VI; Non-residual = sum of fraction I, II, III, IV, and V. Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$, according to the Duncan's test.

**Table 3.** Fractionation of chromium (mg kg$^{-1}$) in semi-arid soils from Dolores Hidalgo, México, amended with Cr(III), Cr(VI) and/or tannery sludge 2, incubated at 25°C for 30 and 120 days.

The greater percentage of Cr in the residual fraction at 120 days of incubation (**Table 3**) (40–65%) probably reflects the greater tendency of Cr to become unavailable once it is in the soil [63]. The residual and non-residual Cr suggest that the metal bioavailability does not only depend on its concentration but is also affected by the characteristics of the tannery sludge and soil components (such as Fe, Mn, oxides, or the quality of organic matter) into which it is sorbed [61].This will have an impact in the interaction between Cr and the biota [64].

Tannery sludge 3 and fleshing waste were added to the same semi-arid soils incubated for 6 months and subsequently subject to simulated rainfall. In this study we evaluated the Cr loss that occurs due to runoff and infiltration, as well as Cr fractionation, Cr speciation, soil pH, and soil microbial activities before and after the simulated rainfall event.

### *3.3.3. pH*

The highest pH was in soils amended with fleshing waste (OTF > UTF) (average 8.49, 8.12, respectively), followed by OT and UT at 3 months' incubation after rainfall (**Figure 3**). Similar results were observed in soil before simulated system [49].

The increment in soil pH also increased the solubility of organic molecules, which is especially important in semi-arid soils(Wolf, 1994).The pH decreases from 3 to 6 months agree with those found in a study conducted by Apple [65] who demonstrated that cycles of drying and rewetting might cause disruption of soil aggregation, resulting in a rapid mineralization by soil micro-organisms due to the release of accessible substrate.

### *3.3.4. Hexavalent chromium*

The concentration of Cr(VI) in soils after application of the simulated rainfall was significantly higher in the soils under the canopy treated with tannery sludge and fleshing waste, or tannery sludge only than in soils outside the canopy with the same treatments (**Figure 4**), even though there were no significant differences between soils treated with tannery sludge or tannery sludge mixed with fleshing waste (data not shown).Values of Cr(VI) remained constant for 1–3 months (1.18–1.74 for outside soils and 2.30–2.80 mg Cr $g^{-1}$ soil for under the canopy soils) under alkaline conditions (pH > 8.0).

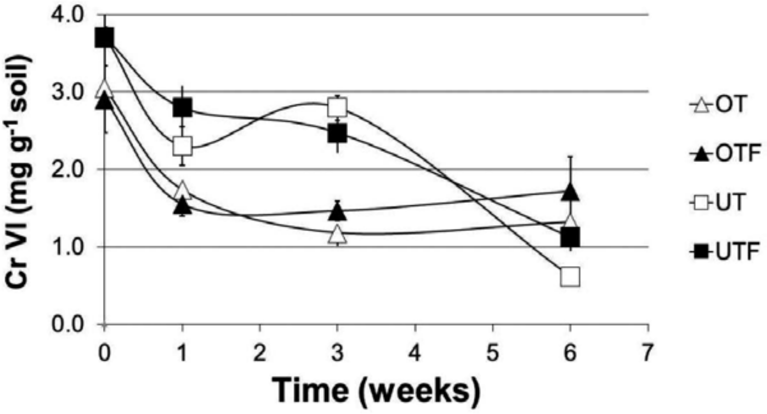

**Figure 4.** Chromium hexavalent after a simulated rainfall in semi-arid soils amended with fleshing waste and or tannery sludge. Bars indicate standard deviation. Source: Ref. [49].

*3.3.5. Total Cr loss by runoff and infiltration*

Total Cr loss in mg was observed in runoff compared to infiltration. The highest values of total Cr released was observed in runoff for UTF treatment, mainly at 1 and 3 months' incubation. The lowest value of total Cr was in infiltration in OTF treatment followed by OT (**Table 4**) [66].

The high concentration of total Cr observed in runoff from 1 to 3 months corresponds with the highest increase in pH (8.01–8.46). Cr measured between these pH values must be all Cr(VI) where anionic Cr(VI) formations are favoured [67]. Thus, the high concentration of Cr loss in runoff from 1 to 3 months suggests that it might be Cr(VI) which was available to the soil solution. Cr values at 1 and 3 months of incubation for under the canopy soils treated with tannery sludge alone or mixed with fleshing waste were higher than the upper limits of Cr (VI) established by the Mexican Norm [68].

| Time (months) | OTF | UTF | UT | OT |
|---|---|---|---|---|
| Infiltration | | | | |
| 0 | 0.42 (0.161) | 0.87 (0.074) | 1.29 (0.123) | 0.50 (0.074) |
| 1 | 0.09 (0.042) | 1.68 (0.134) | 0.97 (0.141) | 0.29 (0.233) |
| 3 | 0.40 (0.124) | 1.02 (0.128) | 2.53 (0.133) | 0.30 (0.132) |
| 6 | 0.34 (0.127) | 1.16 (0.187) | 0.79 (0.147) | 0.50 (0.169) |
| Total | 1.25 | 4.73 | 5.58 | 1.59 |

| Time (months) | OTF | UTF | UT | OT |
|---|---|---|---|---|
| Runoff | | | | |
| 0 | 0.71 (0.156) | 1.24 (0.264) | 1.33 (0.846) | 1.55 (0.285) |
| 1 | 4.84 (0.150) | 11.50 (0.387) | 2.64 (0.548) | 3.84 (0.402) |
| 3 | 1.10 (0.380) | 13.42 (0.402) | 7.89 (0.978) | 6.50 (0.151) |
| 6 | 1.03 (0.191) | 1.10 (0.625) | 1.01 (0.711) | 1.11 (0.205) |
| Total | 7.68 | 27.26 | 12.86 | 13.00 |

U: soil sampled under the canopy of mesquite tree; O: soil sampled outside the canopy of mesquite tree; T: tannery sludge; F: fleshing waste. Values in parentheses indicate standard deviation. Source [49].

**Table 4.** Total Cr (mg) releases in runoff and infiltrations in semiarid soils.

### *3.3.5.1. Chromium fractionation*

Dominant Cr fractionation in semi-arid soils was bound to carbonates (Fraction II) at 0, 3, and 6 months' incubation after rainfall (**Table 5**). Dominant Cr fractionation at 1 month incubation was bound to reducible (Fraction III bound to manganese oxide) followed by Fraction IV (bound to Fe oxides). These results demonstrate that Cr binding was influenced by pH and might also have been influenced by ammonium.

| 0 Months | Cr in fraction (%) | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| OT | 0.91 | 95.30 | 0 | 2.98 | 0.81 | 0.00 |
| OTF | 0.38 | 95.80 | 0 | 2.95 | 0.83 | 0.03 |
| UT | 0.98 | 91.79 | 0 | 4.64 | 2.48 | 0.11 |
| UTF | 0.46 | 92.31 | 0 | 4.64 | 2.47 | 0.12 |
| 1 Month | | | | | | |
| OT | 1.44 | 4.33 | 23.51 | 27.95 | 42.76 | 0.02 |
| OTF | 2.47 | 2.07 | 65.30 | 17.64 | 12.51 | 0.01 |
| UT | 3.76 | 2.20 | 72.89 | 12.08 | 8.82 | 0.25 |
| UTF | 1.64 | 2.43 | 78.43 | 12.22 | 5.26 | 0.02 |
| 3 Months | | | | | | |
| OT | 2.35 | 86.77 | 2.23 | 2.25 | 5.99 | 0.40 |
| OTF | 1.90 | 89.69 | 2.12 | 1.57 | 4.69 | 0.02 |
| UT | 0.47 | 81.25 | 3.69 | 4.01 | 10.54 | 0.05 |
| UTF | 4.47 | 29.58 | 14.20 | 12.73 | 38.81 | 0.22 |
| 6 Months | | | | | | |

| 0 Months | Cr in fraction (%) | | | | | |
|---|---|---|---|---|---|---|
| OT | 2.91 | 84.20 | 5.23 | 4.11 | 3.30 | 0.25 |
| OTF | 2.64 | 84.38 | 5.57 | 4.37 | 2.88 | 0.16 |
| UT | 3.36 | 83.45 | 4.90 | 4.20 | 4.09 | 0.01 |
| UTF | 2.61 | 86.41 | 3.96 | 3.24 | 3.78 | 0.00 |

Source: Ref.[49].
Fractions: I = exchangeable, II = bound to carbonates, III = bound to Mn oxides, IV = bound to Fe oxides, V = bound to organic matter and VI = residues.

**Table 5.** Cr Fractionation (%) in semi-arid soils after rainfall amended with fleshing waste (F) and or tannery sludge 3 (T).

In addition, it has been shown that Cr associated with carbonate becomes susceptible to changes in pH, which results in its becoming soluble [42, 69].

### *3.3.6. Runoff, soil loss, and infiltration*

Water infiltration into soil is one of the most important processes in the hydrological cycle and is crucial in agriculture. Amount of cumulative soil loss and runoff was significantly higher ($P<0.01$) in soils from outside the canopy than those from under the canopy tree. The opposite occurred in infiltration, being higher under the canopy than outside the canopy tree [66]. Amendment of tannery sludge alone to outside the canopy soils reduced the cumulative soil loss and runoff followed by the tannery sludge plus fleshing waste. However, addition of tannery sludge reduced also cumulative soil loss and runoff under the canopy, but the mixture of tannery sludge plus fleshing waste was higher than those with tannery sludge alone. There were no significant differences in soil infiltration outside the canopy soils with or without amendments. However, infiltration under the canopy soils amended with tannery sludge or tannery sludge plus fleshing waste were higher than without amendment. Thus, the addition of tannery sludge plus fleshing waste to under the canopy soil increased the cumulative runoff [66].

Results reported by Barajas-Aceves et al. [66] suggest that high sodium and salt concentrations of tannery sludge plus fleshing waste (1174.68 mg Na $kg^{-1}$ tannery sludge and 119.95 mg Na $kg^{-1}$ fleshing waste and 188.70 mg Ca $kg^{-1}$ fleshing waste) and soil organic C (0.46 g $kg^{-1}$ under the canopy soil) affected soil aggregates of under and outside the canopy soils [70] in different ways, thereby allowing reduction of runoff and loss of solids [66, 71]. While elevated electrolyte concentration may enhance flocculation, sodium has the opposite effect in soils, causing dispersion. The contrary behavior occurred with the application of organic C, thus enhancing water infiltration, delaying runoff, and reducing erosion [72]. Thus, organic matter is known to stabilize soil aggregates. Stronger dispersive conditions (such as higher solidicity, lower salinity, and higher energy or impact of water irrigation) should be needed to disperse a stable aggregate [73].

Results of this study could indicate that the degree of dispersion and flocculation and in the treated soils is not only due to salts and sodium concentrations added in the treatments through tannery sludge and fleshing waste, but also to the clay mineralogy of soil [70, 74]. Thus, differences in CEC values for soils under and outside the canopy (51.3 and 22 meq 100 $g^{-1}$ soil) might reflect the nature of the predominant clay minerals of the soils [75]. Oster et al. [76] studied the flocculation values of montmorillonite and illite suspensions saturated with mixtures of Na and Ca ions in the exchange phase. They suggest that soils with illitic clays are more sensitive to dispersion and clay movement than soils with montmorillonitic clays.

Taken together, these findings suggest that fleshing waste contains sodium at concentrations (data cited above) that adversely impact the infiltration rate of soil collected under the canopy. Previous studies [74, 77] indicate that sodium may cause dispersion and plug soil pores. In this study, repeated wetting and drying of soil could have caused sodium dispersion from sodium coming in the treatments applied to the soil. This, in turn, could have negatively affected the soil structure, reducing infiltration and surface crusting [66, 71, 77].

This would also explain the higher runoff observed in soil collected from under the canopy amended with tannery sludge plus fleshing waste when compared to soil collected from under the canopy and amended with tannery sludge alone. These results suggest that great care must be taken when using waste as an organic fertilizer in semi-arid soil if the wastes contain salts and sodium, to avoid damaging the soil, especially if the goal is to preserve or improve fertile lands. Agassi et al. [74] postulated that the combination of these ESP values and rainwater low salinity caused crust formation and thus high runoff.

#### *3.3.7. Nitrogen loss*

The highest values of $NO_3^-$–N and $NH_4^+$–N concentration losses after simulated rainfall were found in the solution infiltration [66].

Outside the canopy of mesquite soils treated with tannery sludge only or tannery sludge plus fleshing waste showed the highest concentrations of $NO_3^-$–N (341 and 560 mg $l^{-1}$ respectively) and a lower concentration of $NH_4^+$–N (283 and 158 mg $l^{-1}$ respectively) in the infiltration solution together with the highest reduction of soil loss and infiltration [71] (71 and 55%, respectively) [66] suggesting that no changes regarding nitrification took place in those soils. However, the opposite occurred with soils under the canopy treated with tannery sludge plus fleshing waste, a higher concentration of $NH_4^+$–N (452 and 582 mg $l^{-1}$, respectively) and a lower concentration of $NO_3^-$–N (445 and 316 mg $l^{-1}$ respectively) in the infiltration solution were observed. These results, together with no reduction of runoff and low reduction on soil loss (−7 and 43%, respectively) [66], suggest that in areas where treated soil drains poorly, the held water impedes $O_2$ diffusion, creating anoxic areas in the soil at a redox potential appropriate for denitrification. This suggestion is supported by results of high pH (more than 8) in treatments of OTF and UTF at 1 and 3 months of incubation after the simulated rainfall event (**Figure 3**) [49]. Furthermore, the high values of runoff and low infiltration in UTF amendment [66] suggest that clay mineralogy of under-the-canopy soils and Na concentration, plus the tannery waste added disrupted soil aggregates, leaching the $NH_4^+$–N accumulated during the dry intervals prior to the rainfall event [78, 79].

Taken all this information together, it seems that the increasing pH values might have caused competition between chromium oxyanions and OH−, thus decreasing Cr(VI) sorption [80, 81].

Results of the tree different tannery sludge study suggest that tannery sludge characteristics are so important that they define the Cr fractionation in the soil [82]. Thus, the retaining metal in the solid phase depends on metal concentration and abundance of solid phase [83]. Solubility of the metal is mainly controlled by pH, concentration, state of mineral compounds, and type of ligands. Furthermore, these findings indicate that the incubation system used may influence microbial processes in the soil and that the presence of organic compounds plays an important role in determining which fraction of Cr dominates, enhancing metal adsorption to soil phases.

The relative importance of any solid phase for retaining a metal depends on the identity, concentration of metal, and abundance of the solid phase [83, 84]. Solubility of metal is mainly controlled by pH, concentration, type of ligands, chelating agent, oxidation state of mineral component, and the redox potential of the system [85], with TOC enhancing metal adsorption to sediment phases.

The use of tannery sludge with high concentration of carbonates and Cr as organic fertilizer in semi-arid soils could be potentially harmful due to chemical forms related to solubility and carbonate forms favouring heavy metal uptake by plants, and leaching.

### 3.4. Mine tailings amended with organic wastes

There was a reduction in the mobility of Pb when bokashi and compost of vermicompost were added to agricultural and rangeland soils mixed with mine tailings at 0 or 169 days of incubation. However, mobility of Zn was reduced only in both soils mixed with vermicompost and mine tailings. Differences in Pb and Zn mobility between bokashi and compost treatments might have occurred because the effects of organic materials added to soils on soil properties depend on degradation of such materials, which could have affected heavy metals solubility [86, 87]. The high levels of humified organic matter in vermicompost probably influenced the mobility of Pb and Zn [86]. Some published results suggest that availability of Pb and Zn to soil microflora is also influenced by the high humus content in organic matter [62, 88].

Treatments in agricultural and rangeland soils containing mine tailings plus compost showed the greatest inhibition of cumulative C mineralization followed by bokashi [88]. The highest inhibition of N mineralization in agricultural soils was in treatment amended with vermicompost and in rangeland soils in treatments with compost and bokashi plus mine tailings (20.30 and 18.74% inhibition, respectively). The highest inhibition of dehydrogenase activity was observed in both soil amendments with tannery sludge (48–80% respectively) and the lowest in agricultural soil plus bokashi plus mine tailings and rangeland soils plus vermicompost plus tannery sludge (27 and 39% respectively [88]. These results suggest that the quality of the organic material together with the chemical characteristics of the soils could be important factors influencing decomposition of organic materials. Indeed, contents of nitrogen, cellulose, hemicellulose and lignin, the C/N ratio and the lignin/nitrogen ratio [89] have been reported to be some of the most important factors controlling decomposition processes of organic substrates.

Experimental values of N and C mineralization for the two soils in each treatment [88] were used by fitting to four commonly used kinetic models: Zero order, linearized power function, first order, and first order E [90–95].

Nitrogen mineralization in all treatments was best fitted to the linearized power function [88].

$$N_t = K_t^m a$$

The different values of *K* and *m* among treatments and soils did not follow a defined trend. K values in agricultural soils treated with mine tailing alone or with bokashi and vermicompost plus mine tailings increased to 32.1, 26.5, and 31.8% respectively. Similarly, the value of rate constant *K* in rangeland soils plus mine tailing alone increased to 76.7 % compared to the value of K in soil alone. However, in rangeland soils treated with vermicompost plus mine tailing K decreased to 39%. These results suggest that bokashi and vermicompost in agricultural soils provide a similar pool of mineralizable N and the addition of mine tailings modified the decrease of these pools. Behavior of N mineralization in rangeland soils was different as well as the decrease of N mineralization with the three organic wastes [90–92].

The best-fit model for C mineralization was first-order E model for both soils in all treatments [88].

$$C_t = C_0 \left(1 - \exp^{-kt}\right) + \mathrm{C}_1\,\mathrm{b}$$

Potential C mineralization (C0) showed the highest values for treatments with low k values [88]. Murwira et al [93] reported that Co*k parameter can be better estimated than only one parameter. Both single parameters are interdependent.

The combination of those two parameters showed high values of Co∗k in treatments with compost, bokashi, or vermicompost alone in agricultural soils, followed by rangeland soils(11.5–18.2 and 8.1–7.6 respectively). These values are in the range of those reported in the literature for different organic materials (40.6–1.3) [95]. Differences in the values of Co∗k in all treatments in both soils might suggest that the amount of lignin-humus present in the organic compost, the amount of nitrogen content, and the organic waste quality and the type of soil might be important in the process and rate of decomposition [96, 97]. The lowest values of Co∗k were in the treatments with mine tailings alone (3.96 in both soils plus mine tailings) compared with soils alone (56 and 65% less in agricultural and rangeland soils, respectively) or the amendment of organic waste alone. These results suggest that the chemical composition of organic waste (nitrogen content, lignin, and polysaccharides) together with the chemical characteristics of the soils could be important factors influencing decomposition of organic materials and even in the presence of heavy metals [98, 99].

*Brassica juncea* accumulates more Pb and Zn in roots than in shoots [100] growth in soil plus mine tailings. Concentration of Na and Mg measured in mine tailings was 5207 and 102,917 μg $g^{-1}$ soil respectively [100]. These results demonstrated that *Brassica juncea* had the ability to survive and tolerate several metals simultaneously, in the presence of high levels of Na and Mg (SAR between 2.5 and 3.7% and ESP between 18.3 to 20.7%) [100]. All metals measured from mine tailings were accumulated in the root and there was very low translocation to the shoots.

According to the literature, the typical behavior of an accumulator species such as *Brassica* is that there is higher accumulation of heavy metals in the leaves [101], which is opposite to the results of these study.

Results suggest that high levels of Na and salt in mine tailings and the physicochemical characteristic of mine tailings might influence translocation of heavy metals from roots to shoots; metals such as Pb and Zn which are the main metals extracted by *Brassica juncea*. This suggestion is according to reports showing that salt acts antagonistically, thus when plants grow in media with a high Pb concentration and high salt concentration, the amount of Pb accumulated by *Brassica juncea* decreases [102].

The mine tailings amended with 10% compost to growth two shrubs *Acacia retinodes* and *Nicotiana glauca* were able to survive at high concentrations of heavy metals in mine tailings (**Table 1**) when 10% compost was added. The dry biomass of both shrubs increase from 62 to 79% growth in mine tailings plus compost compared to mine tailing alone. *Echinochloa polystachya* was not able to grow on mine tailings, even when it was amended with compost, as was shown by the percentage inhibition data for its root and leaf biomasses. Pb and Zn concentrations in the three plants were higher in roots compared to leaves for all treatments (Pb from 514 to 861 in roots and from 14.6 to 90 (μg $g^{-1}$) in leaves and Zn from 682 to 766 in roots and 541.4 to 254 (μg $g^{-1}$) in leaves) [70]. The elevated contents of Pb and Zn in roots along with the low translocation factors [70] indicate that the two shrub species used in this study are appropriate for Pb and Zn phytostabilization.

## 4. Conclusions

The drastic decrease in soil organic matter in semi-arid soils due to deforestation or in some areas used as deposits for mine tailings are attractive sites for soil restoration. These areas need application of organic residues to avoid subsequent soil erosion by losing soil structure and minimize high risk of pollution in adjacent areas. Application of organic wastes may provide nutrients to pioneering vegetation increasing organic content and improving soil physico-chemical and biological properties and thus their natural fertility.

This work provides a clear demonstration of the role of organic waste to increase or release heavy metals according to the quality of organic matter amendment. Recycling valuable components such as C and N available in semi-arid soils was supported by the potential C and N mineralization, dehydrogenase activity, and plant growth. Retention of heavy metals on the fractionation of organic matter cannot be generalized, it will depend on the chemical characterizations of organic waste and soil. Chemical characteristics of the organic waste such as the nitrogen and humified organic matter content, pH, EC, CEC, ESP (exchangeable sodium percent), and SAR (sodium absorption ratio) are important properties to consider in organic matter amendment to semi-arid soils participating on the complexity and leaching of heavy metals and nutrients in the matrix of soil. Measurements such as heavy metal fractionation, percent of heavy metal mobility, soil biochemical processes, and heavy metals accumulation in roots or translocation to plants give a global picture of the complexity of semi-arid soils amendment with organic waste, either in mine soils with heavy metal content, or when used for organic fertilization to semi-arid soils. Behavior of organic composts or organic waste as organic fertilizer in semi-arid soils will find two directions, one: as the organic matter increases heavy metal immobilization in soil and provides nutrients to plants; two: heavy metals present in organic waste or in semi-arid soils from mine sites will interact with soil microbial activities, plant growth, and heavy metal fractionation. Measurement of infiltration factor in both ecosystems containing high heavy metals and salts concentration under simulated rainfall deserve close attention. Monitoring constantly these types of ecosystems to look for the risk of heavy metal mobility after the season changes, and the potential C and N mineralization and heavy metal stabilization in plants should be a priority in semi-arid soils in process of remediation or reforestation.

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Chapter 12

# Use of Pasteurised and N-Organic-Enriched Sewage Sludge (Biosolid) as Organic Fertiliser for Maize Crops: Grain Production and Soil Modification Evaluation

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Additional information is available at the end of the chapter

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## Abstract

Trough plot-field essay, the effects of two pasteurised-N-enriched sludge loading (3000, 7000 kg ha$^{-1}$) on *Zea mays* L. crop were studied for grain production and soil modifications evaluation. The results of pasteurised sewage sludge application (Plateau-ASP–Active Sludge Pasteurization-ActiSolids©) showed a more grain production by the two biosolid doses in comparison with mineral fertilization (NPK: 15:15:15, 1270 kg ha$^{-1}$). The organic fertilization produced 11 tons ha$^{-1}$ (grain dry matter) by 9 tons ha$^{-1}$ (grain dry matter) for mineral application. No relationships were found between N and P application and grain production. The bisolid application (just for the large dose) derived in a low pH [with a low-aluminium saturation (%)], and low C: N, C: P and N: P soil ratios too, with a P soil content increment. By other hand, the heavy metal soil contents (Cd, Cr, Cu, Pb, Zn, Hi, Hg) are below Galiza-Spanish legislation levels (DOG 107/2012).

**Keywords:** pasteurized sewage sludge, *Zea mays* L. crop yield, soil modifications, heavy metals, grain production

## 1. Introduction

The adequate management of sludge from urban wastewater treatment plants is a critical global environmental concern due to population growth in urban centres, which increases the production of industrial and household waste and the amount of wastewater that must be processed by treatment plants. This issue is especially relevant, as more than 50% of the global population lived in urban centres in 2008 for the first time in the history of humankind [1]. The treatment of wastewater generates a semi-solid residue; the final disposal of this residue requires permanent technical and science-based solutions to prevent contamination of the natural environment. The most economical options that are available for the elimination of sludge entail its use as a fertiliser in agriculture or its disposal in landfills. The National Registry of Sewage Sludge in Spain [2] indicates that 1200 × $10^3$ Mkg m.s. of sludge was generated in 2013, which represents an increase of 62% since 1997. The use of sludge in agricultural soils has increased in recent years: it was applied to 65% of the agricultural surface area in 2006 and to 80% in 2013. In the latter year, the landfill sludge disposal decreased by 8%, whereas its incineration increased by 4%; however, these rates do not account for the use of sludge in non-agricultural soils.

The incentive to apply sludge in agricultural areas is significantly given the ability of this waste product to serve as a fertiliser and their soil positively influence. Conversely, the continual use of mineral fertilisers causes a loss of soil organic matter and other negative impacts (e.g. a decrease in soil fauna or contamination of water bodies) and reduces the capacity of soil to crop production. Considering its different components, the organic matter content in sludge improves the density, porosity and water retention capacity of soil and promotes the activation of microorganisms and soil enzymes. The biosolids application (sanitised, stabilised and dry) favours germination and plant growth, increases the production of various crops and improves the quantity of plant protein and dry matter. The application of this waste product can produce a seasonal shift that causes the early flowering/fruiting of crops (e.g. flax and cotton) and reduce the growing period. Different risks are associated with the chemical composition of sludge, which are primarily related to its heavy metal content. The use of sludge affects the final concentration, solubility (the relative presence of fulvic and humic acid may aid the removal of heavy metals) and final bioavailability of heavy metals in the soil. Similarly, an excessive accumulation of metals negatively influences the development of mycorrhizae. An uncontrolled biosolids application can provoke a soil nutrient excess (N, P) and potentially contaminate aquifers. Deficits in the levels of K and Mn in plants have also been detected, which signals the need for chemical fertilisers to maintain sustained medium-term and long-term production. The sludge use also favours the persistence of pesticides that are applied during crop cultivation [3]. Another consideration is the high variability in sludge composition, which is dependent on the inputs of wastewater treatment plants, season and type of waste post-treatment. Therefore, the sludge chemical composition must be determined prior to its application in crop soils, and field assays should be performed to determine the fertilisation capacity of sludge and its environmental implications.

## 2. Material and methods

### 2.1. Process of obtaining biosolids

According to the legislative norms established by the Autonomous Community of Galiza (NW Spain), all sludge that originates from the purification process of urban wastewater must receive treatment prior to its use in order to sanitise and stabilise it, as well as reduce its volume. Thus, this waste product receives added value due to a chemical treatment process that sanitises and pasteurises sludge, which improves its use as a fertiliser. Both urban and industrial wastewater may be employed as an input. **Figure 1** shows a general outline of the entire purification process, which occurs in a timeframe of less than 1 h. Several chemical reagents are sequentially administered as a function of the type of treatment that is necessary to achieve sanitation. The system of reactors and ducts is closed and completely automatic, which minimises noise pollution and dust. Sludge is treated by an acidification process (with the addition of nitric, phosphoric and/or sulphuric acid) to sanitise and stabilise the organic residue, followed by its neutralisation with anhydrous ammonia. The product is submitted to a thermal process of drying and granulation. The entirety of the process is denominated as Verdiberia-Active Sludge Pasteurisation (Plateau-ASP-ActiSolids©), which includes an enhancement of fertilising capacity (via enrichment with organic N).

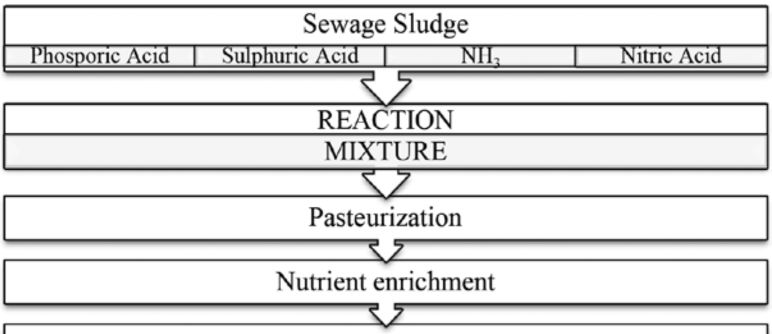

**Figure 1.** General process to obtain Verdiberia-ASP biosolid.

### 2.2. Design of field assay and elemental analysis of biosolid and soils

The field assay was conducted on a private agricultural farm in the municipality of Cospeito (Lugo province, Galiza, NW Spain; 43° 15' 13.15'' N, 7° 26' 31.76'' W). This municipality is characterised by an elevated intensity of agricultural-livestock use. **Table 1** lists the temperature and rainfall conditions that correspond to the study period (June–October 2013), which are characterised by mild average temperatures and moderate precipitation [4].

| 2013 | June | July | August | September | October |
|---|---|---|---|---|---|
| Temperature | 16.1 | 18.2 | 18.5 | 16.4 | 12.9 |
| Rainfall | 52 | 34 | 36 | 68 | 137 |

**Table 1.** Average temperature (°C) and total rainfall (mL) during the study period (corn sawing–corn harvesting).

At the assay site, four parcels of 3 × 4 $m^2$ were randomly and completely distributed for each of the following treatments:

- Control (CT): no application of fertiliser
- Biosolid 1 (BS1): 3000 kg $ha^{-1}$ VerdiberiaASP NP + 450 kg $ha^{-1}$ of potassium sulphate.
- Biosolid 2 (BS2): 7000 kg $ha^{-1}$ VerdiberiaASP NP + 450 kg $ha^{-1}$ of potassium sulphate.
- Mineral fertiliser (MF): 1270 kg $ha^{-1}$ de 15-15-15 + 140 kg $ha^{-1}$ of potassium sulphate.

The soil had an acidity level within the tolerable range for maize crops (pH of 5.5 in water and 10.7% saturation of Al, as an indicator of the cation-exchange capacity); thus, the parameters were not adjusted. To characterise the fertilisation treatments, the P levels of the soil were considered to be normal; however, the K levels were below the desired range. Based on these results and following the indications by [5], 190 kg $ha^{-1}$ of N, 120 kg $ha^{-1}$ of $P_2O_5$ and 260 kg ha $^{-1}$ of $K_2O$ were defined as the base soil conditions for the maize crops in this study (**Table 2**).

| | mg kg$^{-1}$ | | cmol(+) kg$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| **pH** | **K** | **P** | **Ca** | **Mg** | **Na** | **K** | **Al** | **Sat Al** |
| 5.52 | 179.66 | 41.05 | 3.50 | 0.70 | 0.11 | 0.46 | 0.57 | 10.74 |

**Table 2.** Initial main soil parameters (available K and P).

The site was prepared with a mouldboard and rotary plough. The fertilisers were completely incorporated in the soil in the study area according to common practice and uniformly distributed throughout the parcel 1-day prior to maize crop sowing as the final step in the process, which was performed on June 5, 2013. The Automat (Advanta) maize variety (*Zea mays* L.) was employed with a planting density of 70,000 plants $ha^{-1}$. Potatoes had been cultivated the previous year before sowing, by this, the terrain was ploughed and the following phytosanitary treatments were applied as follows: 31.3% S-metolachlor herbicide + 18.7% terbuthylazine herbicide (4 L/ha; SIPCAM Inagra) and 48% liquid chlorpyrifos insectide (1 L/ha).

Soil samples were collected before the maize sowing (control plots without application of fertiliser), 30 days after sowing and at the end of harvest on October 10, 2013.

The pH was measured in a 1:2.5 suspension of soil:water. The C and N contents were determined using a Leco CNS 2000 Autoanalyser, and assimilable P was quantified by colorimetry following the Olsen method [6]. Several elements (Ca, Mg, Na, K and Al) were extracted with a 1 N solution of $NH_4Cl$ [7] and were quantified by spectrophotometry using atomic absorption/emission techniques. Heavy metal soil content was measure through ICP before nitric acid digestion. The parameters that were measured for sludge are summarised in **Tables 3a** and **3b**.

To estimate production, cobs were harvested from ten central plants in each parcel. The fresh weight and dry weight were measured after dried in an oven at 60°C. The grains were subsequently separated to quantify the total production (kg $ha^{-1}$) and the output (Harvest Index = dry weight grain/dry weight cob). In addition, the following weighted production indices were estimated as follows:

Sustainable Yield Index (SYI) [8]

$$SYI = \left(Y - \sigma_{n-1}\right)Ym^{-1}, \text{ with}$$

Y = fertilization treatment yield, σ = standard deviation, Ym = maximum yield (among all treatments) and in the same way the Relative Yield Index (RYI) was calculated, to establish a reference for maximum production with respect to the control:

$$RYI = \left(Y - Yc\right)Ym^{-1}, \text{ with}$$

Yc = Control treatment yield,

and the Agronomic Efficiency (AE) Index, to estimate the nitrogen use efficiency of the crop [9],

$$AE = \left(Y_F - Y_0\right)Nap^{-1}, \text{ with}$$

Nap = N applied in kg $ha^{-1}$

### 2.3. Statistical analysis

The resulting means were compared by a two-factor analysis of variance (two-way ANOVA) with the goal of estimating the influence of the crop grow time period (30 days after sowing–harvest time, which varied according to the specific influence of the crop). A simple ANOVA was performed to determine the significance of the differences in the analysed parameters among treatments in terms of changes in soil characteristics and crop production. The normality of the data was verified (Kolmogorov–Smirnov test), and the homogeneity of variance was verified (Levene test). For cases in which the distribution of the variance was not homogeneous, the Games–Howell test was applied. Assuming homogenous variance, the least significant difference (LSD) method was employed. To compare with the control plot, a bilateral Dunnett's test was performed.

Bilateral Spearman's correlations were calculated for the different production indices, and the regressions between these previously uncorrelated indices and the dose of N and P that was administered by the distinct treatments were also established.

All statistical analyses were performed using SPSS [10].

## 3. Results

### 3.1. Contribution of sludge to soil heavy metal content

Considering the maximum concentration limits of heavy metals in the biosolid and its maximum final contribution to soils as a function of the utilised volume, neither surpassed the limits that were established by normative legislation for the heavy metals that were considered [11]. As listed in **Table 3a**, their levels fell below legal limits for all cases. For example, the Cr content and the Pb content were sixfold and 115-fold lower than the established kg ha$^{-1}$ limits for the application of biosolids.

The biosolid obtained through the Active Sludge Pasteurisation processes shows a lower humidity percentage, for example [12] reported about 16.6% of dry matter, a low OM content [13] or more available P [14].

| | Cr | Cu | Pb | Zn | Ni | Cd | Hg |
|---|---|---|---|---|---|---|---|
| Biosolid | 69.5 | 168.3 | 20.5 | 450.2 | 27.95 | 3.0 | 2.23 |
| Legal limit | 1000 | 1000 | 750 | 2500 | 300 | 20 | 16 |
| kg ha$^{-1}$ (BS2) | 0.4 | 1.1 | 0.13 | 2.9 | 0.18 | 0.02 | 0.01 |
| Legal limit | 3 | 12 | 15 | 30 | 3 | 0.15 | 0.10 |

d.m. = dry matter, BS2 = Biosolid dose applied by 7000 kg ha$^{-1}$.

**Table 3a.** Biosolid heavy metal content and legal limits (mg kg$^{-1}$ d.m.).

| **pH** | **7.98** |
|---|---|
| EC (dS m$^{-1}$) | 48.8 |
| Humidity (%) | 9.56 |
| OM (%d.m.) | 24.16 |
| C/N | 1.39 |
| N—total (%d.m.) | 10.09 |
| N—nitric (%d.m.) | 1.02 |
| N—ammonia (%d.m.) | 2.49 |
| N—urein (%d.m.) | 0.24 |
| N—organic (%d.m.) | 6.34 |
| $P_2O_5$—total (%d.m.) | 8.72 |

| **pH** | **7.98** |
|---|---|
| $P_2O_5$—ammonia citrate-water soluble (%d.m.) | 6.37 |
| $K_2O$—total (%d.m.) | 1.74 |
| $K_2O$—water soluble (%d.m.) | 0.42 |

d.m. = dry matter, EC: electrical conductivity, OM: organic matter.

**Table 3b.** Biosolid chemical characterization.

## 3.2. Evaluation of sludge incorporation rate into soil

The differences in the soil parameters were evaluated between the control parcels at the beginning of the experiment and the parcels that correspond to various treatments during the month of July, 30 days after the initial application of the different fertilisers (**Table 4**). These findings demonstrated a decrease in the pH levels of the treatments compared with CT and in the C/N and C/P ratios for the BS2 treatment, as well as a higher P availability in the same treatment, caused an increase in the relative concentrations of N and P; these elements are specifically provided by pasteurised and minerally enriched sludge. Given that sludge presents very low C/N, N/P and C/P ratios, as 62.8% of N is organic and 73.1% of P is present is its available form, and the soil pH showed a significant decrease after the initial application of sludge (considering the initial pH of the biosolid = 7.98), we can conclude that the fertilisers are rapidly incorporated in the soil. By other hand, during the month of June, a rainfall level of nearly 50 mLm$^2$ and an average temperature of 16.1°C fostered the biological activity of the soil, which also favours the mineralisation process (**Table 1**).

| | **pH** | **C/N** | **N/P** | **C/P** | **$P_a$** |
|---|---|---|---|---|---|
| CT | 5.6 | 13.5 | 62.5 | 844.9 | 50.9 |
| | 0.2 | 0.2 | 17.3 | 231.1 | 8.3 |
| | **a** | **a** | **a** | **a** | **a** |
| MF | 5.0 | 13.1 | 57.8 | 756.5 | 53.4 |
| | 0.2 | 0.4 | 15.9 | 199.6 | 9.8 |
| | **b** | **ab** | **ab** | **ab** | **ab** |
| BS1 | 4.7 | 12.8 | 45.2 | 572.5 | 69.8 |
| | 0.3 | 0.5 | 9.3 | 101.9 | 9.4 |
| | **b** | **ab** | **ab** | **ab** | **ab** |
| BS2 | 4.6 | 12.3 | 31.7 | 390.3 | 103.8 |
| | 0.1 | 0.4 | 3.4 | 34.2 | 9.8 |
| | **b** | **b** | **b** | **b** | **b** |

Only significance differences are showed (different letter means statistical significance difference at $\alpha < 0.05$). $P_a$ = available P.

**Table 4.** Differences between fertiliser treatments and control 1 month after sawing time (Dunnett test). Mean and standard deviation values.

### 3.3. Analysis of temporal changes in soil nutrient content

The independent comparison of the variables over the course of the evaluated time period in terms of their variation enable us to determine whether time serves a role in the evolution of soil characteristics (**Table 5**). After the first time period (30 days after fertilisation and sowing), a significant decrease in soil pH occurred in the BS2 treatment compared with CT and MF. At the end of the harvest, we also observed a decrease in pH for all treatments compared with CT. The average value of the decrease shifted from 0.9 to 0.5 pH units; however, this difference is not statistically significant ($\alpha = 0.25$).

During the first period, only the BS2 treatment had a lower C/N ratio than the CT and MF treatments. In the second period, which corresponded to the end of the harvest, this ratio in BS2 was significantly lower than the CT, MF and BS1. This finding signifies that the relative quantity of N at the end of the experiment increased compared with the CT content for BS2 application (**Table 6**).

Differences in the N/P ratio were detected in the first period (BS2 < CT,MF), whereas BS2 treatment presented lower values for the second period compared with the CT and MF treatments, and BS1 lower than CT and MF.

Only the BS2 treatment demonstrated a lower C/P ratio over the course of the first period compared with the CT and MF. Over the course of the second period, BS1 and BS2 treatments presented lower values for this ratio compared with the CT and MF treatments, and the comparison of BS1 with BS2.

For the concentration of available P, the same behaviour was observed for the two time periods. Both BS1 and BS2 presented higher P values than that of the CT and MF, with differences between BS1 and BS2.

| | **pH** | $\alpha$ | **C/N** | $\alpha$ | **N/P** | $\alpha$ | **C/P** | $\alpha$ | **$P_a$** | $\alpha$ |
|---|---|---|---|---|---|---|---|---|---|---|
| J | BS2 < CT | 0.01 | BS1 < CT | 0.04 | BS2 < CT | 0.01 | BS2 < CT | 0.01 | BS1 > CT | 0.02 |
| | BS2 < MF | 0.02 | BS2 < MF | 0.03 | BS2 < MF | 0.03 | BS2 < MF | 0.02 | BS1 > MF | 0.04 |
| | | | BS2 < BS1 | 0.03 | | | | | BS2 > CT | 0.02 |
| | | | | | | | | | BS2 > MF | 0.01 |
| | | | | | | | | | BS2 > BS1 | 0.01 |
| O | MF < CT | 0.05 | BS2 < CT | 0.01 | BS1 < CT | 0.01 | MF < CT | 0.03 | BS1 > CT | 0.01 |
| | BS1 < CT | 0.02 | BS2 < MF | 0.01 | BS1 > MF | 0.01 | BS1 < CT | 0.01 | BS1 > MF | 0.02 |
| | BS2 < CT | 0.01 | BS2 < BS1 | 0.01 | BS2 < CT | 0.01 | BS1 < MF | 0.01 | BS2 > CT | 0.01 |
| | | | | | BS2 < MF | 0.01 | BS2 < CT | 0.01 | BS2 > MF | 0.01 |
| | | | | | BS2 < BS1 | 0.01 | BS2 < MF | 0.01 | BS2 > BS1 | 0.01 |
| | | | | | | | BS2 < BS1 | 0.01 | | |

(J: 1 month after sawing time, O: harvest time).
Only soil characteristics with significance differences are showed. $\alpha$ = significance level.

**Table 5.** Differences between treatments for each period of soil sample.

| | **CT** | | **MF** | | **BS1** | | **BS2** | |
|---|---|---|---|---|---|---|---|---|
| | **J** | **O** | **J** | **O** | **J** | **O** | **J** | **O** |
| pH | 5.6 | 5.4 | 5.0 | 5.1 | 4.7 | 4.9 | 4.6 | 4.8 |
| | 0.2 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 |
| OM | 7.1 | 7.7 | 6.7 | 7.3 | 6.7 | 7.0 | 7.0 | 7.1 |
| | 0.8 | 0.5 | 0.6 | 0.3 | 0.6 | 0.4 | 0.5 | 0.8 |
| C | 4.1 | 4.5 | 3.9 | 4.2 | 3.9 | 4.1 | 4.0 | 4.1 |
| | 0.4 | 0.3 | 0.3 | 0.2 | 0.4 | 0.2 | 0.3 | 0.4 |
| N | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 |
| | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| C/N | 13.5 | 13.4 | 13.1 | 13.1 | 12.8 | 13.1 | 12.3 | 11.7 |
| | 0.2 | 0.3 | 0.4 | 0.5 | 0.5 | 0.4 | 0.3 | 0.4 |
| C/P | 844.8 | 1192.8 | 756.0 | 1013.5 | 572.3 | 651.8 | 390.3 | 381.5 |
| | 231.2 | 104.1 | 199.4 | 95.0 | 101.8 | 91.9 | 34.2 | 54.0 |
| N/P | 62.5 | 89.0 | 57.8 | 77.2 | 45.2 | 50.2 | 31.7 | 32.7 |
| | 17.3 | 7.8 | 15.9 | 5.5 | 9.3 | 8.7 | 3.4 | 4.9 |
| $K_a$ | 313.6 | 300.8 | 409.3 | 343.1 | 408.7 | 274.2 | 471.2 | 384.3 |
| | 82.6 | 123.5 | 121.9 | 142.4 | 37.8 | 65.9 | 199.1 | 107.0 |
| | | | | | **a** | **b** | | |
| $P_a$ | 50.8 | 37.7 | 53.4 | 42.2 | 69.8 | 63.2 | 103.8 | 108.9 |
| | 8.2 | 4.9 | 9.8 | 5.7 | 9.4 | 5.7 | 7.8 | 16.8 |
| Ca | 6.0 | 4.8 | 5.8 | 4.9 | 4.7 | 5.2 | 5.7 | 5.1 |
| | 0.5 | 0.2 | 1.2 | 0.9 | 1.1 | 1.9 | 1.3 | 0.2 |
| | **a** | **b** | | | | | | |
| Mg | 1.4 | 1.3 | 1.4 | 1.3 | 1.1 | 1.4 | 1.7 | 1.8 |
| | 0.3 | 0.1 | 0.5 | 0.4 | 0.3 | 0.5 | 0.2 | 0.1 |
| Na | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| | 0.01 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 |
| K | 0.8 | 0.8 | 1.1 | 0.9 | 1.1 | 0.7 | 1.2 | 1.0 |
| | 0.2 | 0.3 | 0.3 | 0.4 | 0.1 | 0.2 | 0.5 | 0.3 |
| Al | 0.2 | 0.4 | 0.5 | 0.7 | 1.0 | 0.7 | 0.6 | 0.6 |
| | 0.1 | 0.1 | 0.2 | 0.2 | 0.5 | 0.4 | 0.3 | 0.01 |
| $Al_{sat}$ | 2.8 | 5.7 | 5.7 | 9.0 | 13.0 | 9.6 | 7.3 | 6.8 |
| | 1.7 | 1.7 | 4.3 | 4.8 | 7.5 | 6.3 | 4.8 | 0.7 |

(J = sawing time, O = harvest time) OM, C, N, $Al_{sat}$ = Al saturation (%), $P_a$ = available P (mg $kg^{-1}$), Ka = available K, Mg, Na, Al ($cmol^{+}kg^{-1}$).
Different letters show statistical differences ($\alpha < 0.05$) between time periods for the same treatment.

**Table 6.** Mean and standard deviation values for soil parameters.

Comparing the two periods (July–October), significant differences are detected for the decreasing Ca and the available K content in soil for the CT and BS1 treatments, respectively. No other significant difference was detected. Based on these results, we propose that the applied fertiliser was sufficient for achieving adequate crop production, whereas the extraction of these elements and nutrients over the course of the study did not appear to serve an important role in the variation of their content in the soil.

### 3.4. Total differences in the soil nutrient content as a function of cultivation time and applied treatments

From a general perspective, differences between treatments were observed. The two-way ANOVA (time vs. treatment) only showed significant differences among the distinct treatments ($\alpha = 0.01$); both time and the interaction of time with treatment type were not significant ($\alpha = 0.10$, $\alpha = 0.90$). These results confirm the results of the temporal evolution of soil characteristics as described in the previous section, which indicates that the factor of crop growth does not appear to serve a fundamental role in the modification of the soil parameters. However, the results indicate that the different treatments are responsible for the variations. Significant differences in pH values are detected, which significantly decreased over the course of the experiment relative to the control after the treatments with distinct doses of biosolid (not statistically significant between each treatment) and mineral fertilisation. However, differences in the percentage of Al saturation, for which a decrease in pH does not represent a limitation of the availability of nutritional elements in the soil, were not observed.

| | **pH** | **C/N** | **N/P** | **C/P** | **$P_a$** |
|---|---|---|---|---|---|
| CT | 5.5 | 13.4 | 89.4 | 1202.0 | 37.7 |
| | 0.2 | 0.4 | 13.6 | 202.4 | 5.7 |
| | **a** | **a** | **a** | **a** | **a** |
| MF | 5.1 | 13.1 | 77.7 | 1018.4 | 42.2 |
| | 0.1 | 0.5 | 11.4 | 150.6 | 6.6 |
| | **b** | **a** | **a** | **a** | **a** |
| BS1 | 5.0 | 13.1 | 49.8 | 649.3 | 63.2 |
| | 0.1 | 0.5 | 6.5 | 76.9 | 6.6 |
| | **b** | **a** | **b** | **b** | **b** |
| BS2 | 4.8 | 11.7 | 39.0 | 451.7 | 108.9 |
| | 0.2 | 0.4 | 11.6 | 120.3 | 19.4 |
| | **b** | **b** | **c** | **c** | **c** |

**Table 7.** Soil parameters differences at harvest time (mean and standard deviations values). Only are showed significance differences (different letter mean significance difference at $\alpha < 0.05$. Games–Howell test for pH and P). $P_a$ = available P (mg $kg^{-1}$).

The same tendency is detected in the relationships between nutrients and the C/N ratio, which has a significantly lower value for the BS2 treatment, in addition to the N/P and C/P ratios for

BS1 and BS2. Anyway, the C/P and N/P rates are enough higher to promote a P soil accumulation [15, 16].

With regard to the available P content in the soil, we obtain a greater value in BS2 compared with the other treatments and a greater value in BS1 compared with the MF and CT. In a similar way [17] found more soil P after harvest time (**Table 7**). Therefore, we can assume that the application of biosolid produces a relative enrichment of N and P, which also indicates a potential eutrophication risk due to excess available P as it does not appear to be regulated by crop extraction. [13, 18] report the sewage sludge application as an available P fountain, and by other hand, the C/P and N/P ratios are mainly controlled by P supply [19]. Its content in the soil and the total quantity provided by the biosolid exceed the corresponding maximum limits that were established by legislative norms [11] (48 mg kg$^{-1}$ for P). Based on these established limits, only one application per year of the product tested in this study is permissible. For P [20], recommended a maximum of 150 kg ha$^{-1}$ in order to prevent eutrophication risk.

### 3.5. Production, output and agronomic efficiency of crop

**Table 8** summarises the significant differences for the indices that are related with crop production: grain yield (kg ha$^{-1}$), harvest index, RYI, SYI and AE and their correlations. Corn yield is significantly greater for the BS1 and BS2 treatments (similar for both) than for the CT and MF. In the same sense [21, 22], find largest corn production after sludge applying versus mineral fertilization.

| | **Kg ha$^{-1}$** | **Harvest rate** | **SYI (%)** | **RYI (%)** | **AE** |
|---|---|---|---|---|---|
| CT | 7295.9 (2434.3) **a** | 86.1 (0.05) **a** | 34.5 (17.3) **a** | – | – |
| MF | 9431.3 (2180.3) **b** | 82.6 (0.05) **b** | 51.4 (15.5) **b** | 17.1 (11.3) **a** | 14.4 (10.3) **a** |
| BS1 | 11184.1 (1755.0) **c** | 88.3 (0.03) **ac** | 66.9 (12.4) **c** | 27.9 (11.3) **b** | 16.7 (7.7) **a** |
| BS2 | 10588.5 (1688.0) **c** | 86.8 (0.03) **ac** | 63.1 (12.0 ) **c** | 23.5 (11.7) **b** | 7.3 (4.0) **b** |
| | | Pearson correlations: r$^2$, ($\alpha$) | | | |
| | Kg ha$^{-1}$ | Harvest rate | SYI | RYI | AE |
| Kg ha$^{-1}$ | 1 | 0.24 (0.01) | 1.00 (0.01) | 0.62 (0.01) | 0.71 (0.01) |
| Harvest rate | | 1 | 0.28 (0.01) | 0.17 (0.05) | n.s. |
| SYI | | | 1 | 0.63 (0.01) | 0.67 (0.01) |
| RYI | | | | 1 | 0.61 (0.01) |
| AE | | | | | 1 |

**Table 8.** Differences found for production index and correlation values. Mean and standard deviation values.

For the RYI and SYI, differences follow a similar pattern to production levels. This finding may be attributed to the fact that the difference between production (BS vs. MF) and their low variability are the two factors with more influence in the yield index [yield coefficients of variation (%), CT = 34, MF = 23, BS1 = 16, BS2 = 16]. In the case of AE, the BS1 treatment was significantly greater than the corresponding BS2 treatment, which indicates that a direct relationship does not exist between the contribution of N and grain production. This tendency is confirmed when we analyse the regressions for the distinct production indices and different doses of N, which are not correlated. As observed in **Table 9**, the values of $r^2$ are low, and a statistically significant relationship between the coefficients and the constants was not observed in any case. [23, 24] find that an excessive N fertilization results in low-use efficiency, without any yield benefits and long-term environmental consequences, soil acidification, N-leaching… [25] does not find any relationship between grain yield and N application rates (r = 0.26). Similar pattern was obtained to P addition and yield index, but, for example [26] find a positive relationship between P addition and corn yield response.

| **$Y = N \times 1.27 + 9932.5$** | | |
|---|---|---|
| $r^2 = 0.016$ | $\alpha_1 = 0.16$ | $\alpha_2 = 0.01$ |
| $HR = N \times 4.8 \times 10^{-5} + 0.84$ | | |
| $r^2 = 0.044$ | $\alpha_1 = 0.02$ | $\alpha_2 = 0.01$ |
| $Y = P \times 4.2 + 10464.8$ | | |
| $r^2 = 0.215$ | $\alpha_1 = 0.69$ | $\alpha_2 = 0.13$ |
| $HR = P \times 8.5 \times 10^{-5} + 0.84$ | | |
| $r^2 = 0.239$ | $\alpha_1 = 0.68$ | $\alpha_2 = 0.03$ |
| $\alpha_1$, $\alpha_2$ = Significance value for N, P and constant coefficients. | | |

**Table 9.** Regression values for corn yield (Y) (kg ha$^{-1}$) and harvest rate (HR) in relationship with N and P applied.

## 4. Conclusions

The application of the Verdiberia-ASP-NP (Plateau-ASP-ActiSolids©) biosolid, which originates from the sludge of urban treatment plants, was demonstrated to be suitable for maize production (*Zea mays* L., Automat-Advanta variety). This suitability can significantly increase the yield compared with equivalent mineral fertiliser.

This product does not present a risk of increasing the heavy metal concentration; considering the elemental concentration of the biosolid in total quantities (kg ha$^{-1}$), the resulting values were substantially lower than the established normative limits.

From the analysis of temporal variation, the values measured for the C/N, C/P and N/P ratios during the evaluation time period indicate that sludge rapidly incorporated into the soil with a high rate of mineralisation.

Although the application of this biosolid produced a slight decrease in the pH value at the end of the harvest period, this tendency is not present for the percentage of Al saturation. The availability of nutrients is not negatively affected.

The doses that were employed in this study administered a level of nutrients (NPK) that was adequate for crop development; at the end of the harvest, an excess of available P was detected in the soil. This result may represent a potential problem and the cause of eutrophication of the ground water layers.

Significant relationships between the contribution of nitrogen and phosphorous and the production indicators have not been observed. The rate of mineralisation of the biosolid should be established prior to use in field applications to adequately administer the correct dose depending on the production crop.

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Chapter 13

# On-Farm-Produced Organic Amendments on Maintaining and Enhancing Soil Fertility and Nitrogen Availability in Organic or Low Input Agriculture

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Additional information is available at the end of the chapter

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## Abstract

Maintaining and enhancing soil fertility are key issues for sustainability in an agricultural system with organic or low input methods. On-farm–produced green manure as a source of soil organic matter (SOM) plays a critical role in long-term productivity. But producing green manure requires land and water; thus, increasing biodiversity, such as by intercropping with green manure crops, could be an approach to enhance the efficiency of renewable resources especially in developing countries. This article discusses soil fertility and its maintenance and enhancement with leguminous intercropping from four points of view: soil fertility and organic matter function, leguminous green manure, intercropping principles, and soil conservation. Important contributions of leguminous intercropping include SOM enhancement and fertility building, biological nitrogen (N) and other plant nutrition availability. Under a well-designed and managed system, competition between the target and intercropping crops can be reduced. The plant uptake efficiency of biologically fixed N is estimated to be double that of industrial N fertilizers. After N-rich plant residues are incorporated into soil, the carbon (C):nitrogen ratio of added straw decreases. Another high mitigation potential of legume intercropping lies in soil conservation by preventing soil and water erosion. Many opportunities exist to introduce legumes in short-term rotation, intercropping, living mulch, and cover crops in an organically managed farm system. Worldwide, long-term soil fertility enhancement remains a challenge due to the current world population and agricultural practices. Cropping system including legumes is a step in the right direction to meeting the needs of food security and sustainability.

**Keywords:** soil fertility, nitrogen, living mulch, organic agriculture, leguminous intercropping

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## 1. Introduction

Organic or low input farm is a production system that sustains agricultural productivity by avoiding or restricting synthetic fertilizers and pesticides. It takes soil fertility, which governs the plant productivity of the soil, as a key measure in gaining an optimum yield from a long-term point of view. The establishment and maintenance of soil fertility is a major issue within organic or low input farming systems.

Incorporation of organic residues and manures are key approaches to many integrated soil management strategies [1], including that of nitrogen, one of the key plant nutrients in organically or low input managed farming systems. Green manure, manure, and litter from animal husbandry are considered as soil amendments and major mineral nutrient sources after mineralization. Increased soil organic matter (SOM) is a key issue in maintaining soil fertility and provides plant nutrients. Thus, SOM and N availability are important indices of soil fertility [2]. However, taking economic issues into consideration, industrial N fertilizer is of more benefit than biological N fixation in current agricultural management [3]. In 1987, James indicated that from 1960 to 1977 (during the "green revolution"), legume seed production declined dramatically from 170,000 to 70,000 tons worldwide [4]. Because planting legumes requires land, water, and other resources, the ability to fix N is limited by agricultural conditions.

However, due to the contribution to soil fertility through its effects on the physical, chemical, and biological properties of soils, the role of green manure has been rediscovered and is receiving more attention in soil fertility maintenance and enhancement by farmers, agronomists, and governments around the world. Under current conditions, several opportunities exist for the use of legumes in short-term situations, such as simple rotation, double cropping or intercropping, and cover crops [5].

The method of growing more than one agricultural species mixture together, as intercrops, is generally regarded as one measure to increase the productivity of crop systems. Cover cropping can reduce soil and water erosion, the process by particles detached from the soil mass are transported by running water and wind. Intercropping enhances ecosystem services including crop yield, N use efficiency, pest and weed management, and reduces nitrogen losses to the environment [6]. Thus, the method of intercropping with green manure is of interest in organic or low input farming systems, especially in non-animal husbandry farm systems.

Regarding the question of acceptable long-term productivity with major crop rotation or intercropping with legumes [7], this article discusses soil fertility and the functions of SOM, leguminous green manure as a source of SOM, and its capability to modify the C:N ratio of added organic matter. Increasing N availability and other plant nutrients, the efficiency of intercropping and living mulch, and soil and water conservation are also considered. The objective of the review is to present a way to maintain and enhance soil fertility with green manure intercropping in an organically managed farming system.

## 2. Comprehensive soil fertility and the functions of SOM

### 2.1. Soil fertility aspects

Soil fertility is the crop productivity capacity of the soil due to the supply of plant nutrients and growth media. Long-term productivity can be taken into consideration instead of the yield in one growing season or year. Soil fertility includes sustainable availability and balanced forms of plant nutrients, soil water conservation, and aeration. It covers three aspects: physical, chemical, and biological properties. The physical property aspects mentioned in Table 1 [8] are related to soil texture and structure, which are related to the organization of particles and pores, reflecting effects on root growth, speed of plant growth, and water infiltration. Physical indicators include depth, bulk density, porosity, aggregate stability, texture, and compaction [9]. Loss of soil structure can occur through slaking and dispersion, often linked to intensive cultivation [10], compaction, and vital loss of the pore size distribution needed to maintain soil fertility [1]. Aggregates are the most profitable structural units of soil, offering water, air balance for root development, and the synthesis of complex organic compounds binding soil particles into structural units directly helps to build a loose, open, granular state with medium- to large-sized pores [11].

Chemical aspects include pH, salinity, organic matter content, phosphorus (P) availability, cation exchange capacity, nutrient cycling, and the presence of contaminants, such as heavy metals, organic compounds, and radioactive substances. These indicators determine the presence of soil-plant-related organisms, nutrient availability, water for plants and other organisms, and the mobility of contaminants [9].

| **Physical** | **Chemical** | **Biological** |
|---|---|---|
| Texture,<br>Depth of topsoil,<br>Bulk density,<br>covers soil aeration,<br>water and nutrient holding capacity,<br>water infiltration,<br>crust,<br>temperature,<br>tillage condition. | Organic C,<br>Total N,<br>pH,<br>Electrical conductivity,<br>Extractable N, P, K,<br>Micro- and macronutrients availability,<br>Salinity. | Microbial biomass C and N,<br>Potentially mineralizable N,<br>Soil respiration,<br>Soil born pathogen repression. |

(Modified from Wienhold et al. [8]..)

**Table 1.** Physical, chemical, and biological soil indicators that may be included in a minimum data set for assessing soil quality.

Biological indicators include biomass of micro- and macroorganisms, their activities, and functions. Concentrations or populations of earthworms, nematodes, termites, and ants, as well as microbial biomass, fungi, actinomycetes, or lichens, can be used as indicators [9]. Soil biological properties are based on the soil being a living system; many kinds of organisms are involved in complex biological, chemical, and physical processes. A living soil is regarded as a healthy soil and favorable to plant growth because of the organisms' roles in soil development and conservation, specifically nutrient cycling and determining soil fertility.

### 2.2. The roles of soil organic matter on soil fertility

Organic and low input agriculture are regarded as a procedure to maintain SOM and soil fertility. In Switzerland, a long-term trial biodynamic system was reported to show a stable C content, while a C loss of 15% in 21 years was measured for the conventional system control. In the United States, a field trial showed fivefold higher C sequestration in an organic system (i.e., 1218 kg of C $ha^{-1}$ $year^{-1}$) versus conventional management [12,13]. Lal stated that the rate of organic C sequestration in soil with the adoption of recommended technologies depended on soil texture, soil structure, rainfall, temperature, farming system, and its management [14]. He also found that addition of 1 ton of degraded crop organic matter to the soil may increase crop yield by 20-40 kg $ha^{-1}$ for wheat, 10-20 kg $ha^{-1}$ for maize, and 0.5-1 kg $ha^{-1}$ for cowpeas. Apart from enhancing food security, C sequestration also has the potential to offset fossil fuel emissions by 0.4-12 tons of C $year^{-1}$ or 5-15% of global fossil fuel emissions [14].

Additionally, organic matter has both direct and indirect effects on the availability of nutrients for plant growth. The decay of organic matter liberates these nutrient elements, making them available to the succeeding crop. It is a major source of P and sulfur (S), and essentially the sole source of N through its mineralization by soil microorganisms. Organic matter serves as a source of energy for both macro- and microfaunal organisms. Earthworms and other faunal organisms are strongly affected by the quantity of plant residue material returned to the soil. During the decomposition process of organic tissue, soil particles are attached together as aggregates. SOM enhances the nutrient buffering capacity and the microbial activity, both strengthening soil fertility [15]. Additionally, organic matter contributes 30–70% to the cation exchange capacity, which allows soil particles to hold nutrients, thus preventing them from leaching out. Also, as a buffer, humus exhibits buffering over a wide pH range [16].

Water infiltration and root growth are promoted by lower bulk density, which tends to decrease with organic matter additions [11]. Organic matter has higher water-holding capacity (by 20 times) versus clay. Aggregate stability and water infiltration are increased by organic matter additions. This positive effect on the water-capturing capacity of the soil is likely to increase in importance with climate change, [15] because a higher water-capturing capacity strengthens resilience to droughts and reduces the risk of floods [17]. Thus, the need for irrigation is lowered, which has an additional adaptation and mitigation effect [15,18].

SOM enhances the nutrient buffering capacity and microbial activity, both strengthening soil fertility [15]. The addition of organic matter helps to protect the soil from erosion, acts as a buffer against dramatic changes in acidity, alkalinity, and salinity. The overall effects of organic matter are far greater than the simple analysis of its constituent nutrients; indeed, it is the engine that drives all the biological processes in the soil [19]. Increased SOM and microbial activity in organically farmed soils results from a combination of enhanced C inputs during fertilization and increased grass cover [20].

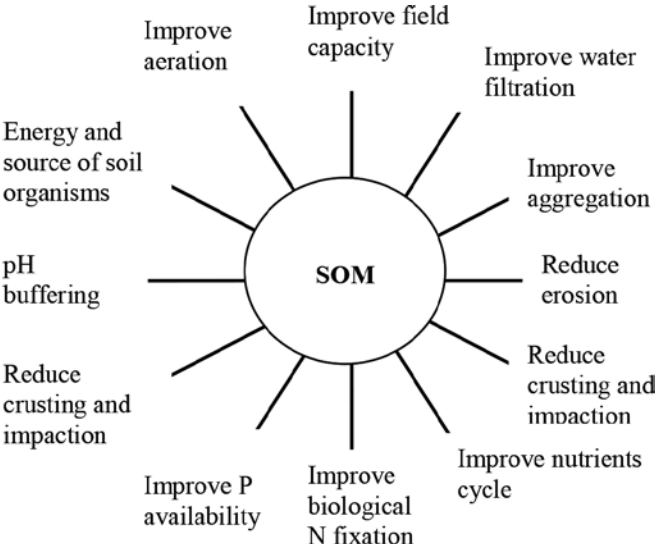

**Figure 1.** Soil organic matter contributes to soil fertility.

## 3. On-farm–produced organic amendments

### 3.1. SOM source and modified C:N ratio of added residues

Leguminous green manure has long been used as a SOM source as a component of cropping system in Africa, Asia, and Latin American. Cover crops and intercropping increase C sequestration in the soil [21]. Another possible benefit of legumes is the result of N fixation by the root nodules, which the amount of biological fixed N is a somewhat contentious issue in some case studies, but most of the organic N can be available to plants after residues have been composted [22]. Generally green manure can produce a dry weight of 5-9 tons or more biomass ha$^{-1}$ year$^{-1}$; about 40% of dry matter is C and 2-4% is N. While the N productivity of several major cultivated legumes has been reported as from 80 kg (berseem clover) to 190 kg (subclover) ha$^{-1}$ [23-25]. The ability of biological N fixation ranges from 40 to 200 kg in aboveground tissues ha$^{-1}$ due to the species of legume, bacterial strain, and agricultural conditions, such as climate and soil (Table 2).

Factors that influence the ability of microorganisms to break down added plant materials includes the C:N ratio of organic matter and components of organic C and N [26]. The C:N ratio of plant tissue reflects the kind and age of a plant from which it was derived. Non-legume plants may have a high C:N ratio, over 60 (cow straw), up to even 250 (wood tissue) [27,28]. Non-leguminous green manure with high a C:N ratio (over 25), will cause microorganisms to tie up available N in the soil. Thus, added materials with C:N ratios above 25:1 can result in N being bound by soil microbes in the breakdown of C-rich crop residues, thus pulling N away from the root zone of crop plants. Legumes usually have a low C:N ratio (about 10-15) and can help to modify the SOM C:N ratio to an adequate level. The optimal C:N ratio for rapid decomposition of organic matter is between 15:1 and 25:1 [29]. The addition of N-rich plant residues, such as legume plants, to aid the decomposition process may be advisable with these high-C residues: the lower the C:N ratio, the more N will be released into the soil for immediate crop use [30].

| **Crop** | **Biomass*** **(tons ha$^{-1}$)** | **Nitrogen (kg ha$^{-1}$)** |
|---|---|---|
| Sweet clover | 4.3 | 130 |
| Berseem clover | 2.7 | 75 |
| Crimson clover | 3.5 | 108 |
| Hair vetch | 4.3 | 118 |
| Subclover | 5.4–10.1 | 184 |

*Dry weight of plant aboveground material (sources: [2325])

**Table 2.** Yearly average biomass yields and nitrogen yields of several legumes.

### 3.2. Intercropping with green manure

Intercropping refers to multiple crops planted at same time on the same land, growing and interacting with each other during the whole or part of the growing season within a crop system [31,32]. Intercrops or mixture crops influence the farming system in ways including the lower density of each species helping to reduce plant pathogen infection opportunities, raising land productivity as a result of reducing the effects of unsuitable conditions, which may be not so unsuitable to other intercrops, positive effects on weed control, border effects, and enhancing plant nutrient and soil humidity use efficiency. Intercropping can be regarded as a method of weed suppression and offering habitats for beneficial organisms. According to space arrangements and temporal practices, intercropping is commonly divided into four subcategories [31]:

1. *Row intercropping*: growing two or more crops at the same time with at least one crop planted in rows.
2. *Stripe intercropping*: growing more than one crop together in stripes wide enough to permit separate crop production using machines but close enough for the crops to interact with each other.
3. *Mixed intercropping*: growing two or more crops with no distinct row arrangement.
4. *Relay cropping*: planting a second crop in a standing crop at a time when the standing crop is at its reproductive stage but before harvest. According to plant density or land features, the crop with high seeding rates is called the major crop, and the other crop is the secondary crop.

Crop mixtures may gather more light and plant nutrients than pure standing crops, as a result of differing root depth and stem height. Generally, the land equivalent ratio (LER) is one of the major measures to judge how complementary crops are under intercropping. The mathematical equation is stated as [33]:

$$LER = (Yij + Yji / Yjj + Yii),$$

where Yij is the grain yield per unit area of species i grown in a mixture with species j, Yii is the grain yield per unit area of species i grown in a pure stand, Yji is the grain yield per unit area of species j grown in mixture with species i, and Yjj is the grain yield per unit area of species j grown in a pure stand.

When the LER is below 1, it indicates that competition exists between different components rather than them being complementary. Generally, under a well-managed intercropping system, the LER is 1.2-1.5 and sometimes even above 1.5. These results are due to the rational use of natural resources for crop growth. As Paolini et al. reported, after 2-year case studies on mixture crops of sunflower and chickpea, total LER figures averaged 1.16 as to aboveground biomass yield and 1.25 as to grain yield [34]. They also pointed out that an unfavorable climate for one species is not so unfavorable to another. The conclusion can be made that when the climate or other agricultural factor becomes the key limiting factor for one crop, the other crop will likely get sufficient plant nutrients and soil moisture water supply; one species will be more tolerant of the unfavorable condition than the other.

Altieri found that in Mexico, 1.73 ha of land had to be planted with maize to produce as much food as 1 ha planted with a mixture of maize, squash, and beans [35]. Additionally, maize + squash + bean polyculture can produce up to 4 tons $ha^{-1}$ of dry matter for plowing into the soil as compared with 2 tons in a maize monoculture. In Brazil, a maize or sorghum mixture with cowpeas or beans can lead to LER values of 1.25-1.58 [35]. Sometimes under intercropping management, a major crop cannot get the maximum yield obtainable in a monoculture due to competition from the other crop or a lower density versus a pure stand. By interplanting, farmers achieve several production and conservation objectives simultaneously [36]. Polycultures produce more combined yield in a given area than could be obtained from monocultures of the component species; sometimes, the LER can be above 1.5, although the yield variability of cereal + legume polycultures are much lower than that for monocultures of the components [37]. The intercrop treatments represented the highest LER was 1.52 for baby corn/pea intercropping system [38]. And other maize/bean intercropping achieved LER values were 1.76 and 1.92 [39]. Cover cropping is also considered as a the practice of growing pure or mixed strands of legumes, cereals, or natural vegetation to protect the soil against erosion, ameliorate soil structure issues, enhance soil fertility, and suppress pests, including weeds, insects, and pathogens [31]. The cover crop approach has been used for thousands years ago and is regarded as a sustainable method of agricultural production.

### 3.3. Living mulch

Living mulch refers to a legume cover-crop, which is undersown with an annual crop. Common living mulches include white clover, hairy vetch, and red clover. A living mulch can improve soil structure and water penetration, prevent soil erosion, modify the microclimate, and reduce weed competition [40]. An ideal crop occupies underused time or space in an existing system. It does not compete with the cash crop for light, water, or nutrients, and attracts beneficial organisms, while keeping harmful pests away. It should be readily established and grow rapidly. It should produce an abundant growth of both shoots and roots in a short time, and its growth habits should encourage ground cover soon after its establishment [41]. Common living mulches can offer soil cover, especially during the seedling period and after harvest of the target crop when the crop plant does not cover most of the soil surface. Subclover planted as a living mulch was able to regenerate and provide the succeeding crop with abundant and N-rich residues [42]. The main benefits of living mulches include enhancement of soil structure, improvement of soil fertility, and positive effects on pest management and environmental quality [43].

Changing from a monoculture to an intercropping system requires several important management practices based on natural laws. Successful management requires investment in experience and research to modify the system into an economically acceptable, ecologically sustainable, and technologically practicable one. In the case of winter wheat, a major crop around the world, legumes, as rotation crops or intercrops, have been tested for the establishment of the cropping systems. According to Caporali and Campiglia, in search of strategies for increasing sustainability in cropping systems, they have been focusing for 10 years on the use of plant resources, such as self-reseeding winter annual legumes (*Trifolium* and *Medicago* species) native to the Mediterranean environment [44]. Although subclover and annual medics are well-known forage crops in cereal-lay farming systems under the Mediterranean climate around the world, their use is practically unknown in more intensive cash-crop sequences, such as the 2-year rotation between a winter cereal (wheat, barley) and a summer crop (rainfed sunflower, irrigated corn), as is common in central Italy. In this rotation, an annual legume is used as a living mulch in winter cereals, and after its self-reseeding, as either a green manure or living mulch for the succeeding summer crop. This alternative cropping system has proved to have the potential to induce a significant shift toward a less energy-intensive and a more environmentally friendly management type, while maintaining the same cash-crop sequence of the conventional one [44].

The foundation of the system began with the screening of self-reseeding legumes species and cultivars and ended with the implementation and performance assessment of an entire alternative cropping system (winter cereal/summer crop rotation). The yield of winter wheat intercropped with subclover was not significantly different from that of a pure wheat stand in a drier crop year, while in the wetter year the grain yield of the intercropping system was significantly higher than the pure stand. However, in both crop years, grain yields were significantly lower that obtained using 130 kg $ha^{-1}$ mineral N fertilizer, by 11% and 23%, respectively. Additionally, a positive correlation between the amount of subclover biomass plowed in and the vegetative and productive characteristic of sunflower was found in dry and wet years. Subclover green manure was so effective that sunflower yield in the alternative system was higher than that of the conventional one fertilized with 130 kg $ha^{-1}$ of inorganic N. Subclover green manure also affected biomass and the composition of the weed community in the sunflower crop. Subclover mulch from a sod strip intercropping system with wheat was also effective in positively influencing the aboveground biomass production of the succeeding crops, the effect being dependent on the amount of dry mulch left by the different subclover species and cultivars [44].

### 3.4. Competition between intercrops and intercropping principles

The struggle for nature's resources is always an issue within any crop system because plant growth needs not only space and time, but also light, mineral nutrients, and water. The competition can be considered to have two main aspects: aboveground competition and root system competition. In rain-fed agriculture, under limited water conditions, a major competition can occur between the target crop and legumes for water resource. The wheat yield under intercropping conditions with legumes reportedly decreases with less water availability versus a pure stand, although legume intercropping with a major crop can enhance the N content of the soil [45]. Mc Gowan and Williams found that subclover depleted soil moisture more than barley. At 19 weeks after sowing, maximal soil moisture was observed when barley was in a high density and a pure stand, 7.5% at 5-15 cm and 9.9% at 15-30 cm depth, while for subclover in a pure stand, it was 6.2% at 5-15 cm and 8% at 15-30 cm depth [46]. Taking into account negative effects between intercrops, how to best balance between competition and companion effects is a task for experimental research.

As Altieri and Rosset pointed out, a production system must be designed to reduce nutrient losses by effectively containing leaching, runoff, and erosion, and improving nutrient recycling mechanisms [40]. Diversity is a natural design, while monoculture is an anthropogenic creation. So intercropping should be organized according to natural laws. The cultivars within a cropping system must be suitable for the local climate and soil conditions. Under the circumstances of intercropping, cooperation between different species is also required; at least, competition should be eliminated as much as possible. As the main competition between two crops is canopy competition and root system competition, the principles of a well-managed intercropping system can be summarized as follows:

Tall and short crops are growing together to minimize struggle for sunlight and reduce air humidity of the microclimate. Crops with deep and strong root systems intercropped with the species with sallow root systems can reduce underground competition. The density of a major crop should be reduced to adjust the growth of itself and leaving optimum space for another intercrop.

Select different maturity dates to minimize competition as much as possible [47].

When crops are planted together according to these principles, competition between different species will be less than would exist within the same species. The success of intercropping systems at low levels of interspecific competition has also been explained in terms of more balanced and efficient use of soil moisture due to temporal complimentarily in water requirements of the two species [48]. In the case of legume intercropping, high companion effects between the two crops are caused by biological N fixation, producing N that benefits the target crop and offering soil cover. Simultaneously, the negative effects of legumes on the major crop should be reduced by a well-managed system.

## 4. Soil N availability and other plant nutrient availability enhancement

Nitrogen plays an important role in yield determination when relatively adequate levels of other agricultural factors exist. Continued use of inorganic fertilizers has not only altered the soil p H, soil structure, and texture, but has also disrupted niches for micro- and mesofauna, which are essential for nutrient recycling [49]. Alternatively, under systems of organic farming management, when industrial N fertilizer is not used, organic matter origin-N, after biological degradation, is converted into mineral N forms, ammonium and nitrate, and becomes a major factor in plant production. However, as the mineral N content in soils increases beyond the capability of plants to take it up, it will cause N leaching and increase other kinds of N losses into the environment. In this case, it is important to understand the N cycle and soil N balance within an agroecosystem.

As common knowledge, the origin of all kinds of N is air $N_2$, 79% by volume of the earth's atmosphere. Soil microorganisms, free-living or associated with legumes, fix atmospheric N. This complex biological process begins with air N and ends with organic N. After organic matter decays, $NH_4^+$, which is ready to be used by higher plants, is released. $NH_4^+$ can be converted into $NO_3^-$ by nitrifying bacteria and generally most $NH_4^+$ is modified into $NO_3^-$ in the soil. Thus, over 90% of soil N is typically $NO_3^-$, not $NH_4^+$, although $NH_4^+$ can be formed from $NO_3^-$ through the process of denitrification in soils [50].

The denitrification process starts with $NO_3^-$ and converts it to $NH_4^+$, N mono-oxides (NOx, greenhouse gases), and $N_2$. Denitrification of nitrate produces about 90% $N_2$ and 10% NOx. However, the natural N balance has been affected by industrial N fixation since the green revolution. Symbiotic N amounts to about 100-175 million tons each year in the 1970s worldwide, with industrial fixation of 3.5 million tons, and lightning may fix 10 million tons of N, a value that has probably not changed over time [51] . In 1989, industrially fixed N increased to 80 million tons in response to the needs for high-yielding crops [22].

By the year 2050, the world population is expected to double from a level of more than 5 billion. It is reasonable to expect that the need for fixed N for crop production will also at least double. If this is supplied by industrial sources, synthetic fertilizer N use will increase to about 160 million tons of N per year [51]. Consequently because of its relatively low plant uptake level, generally around 50% or less, several major environmental reasons exist to seek alternative fixed N fertilizers, including the fact that it affects the balance of the global N cycle, pollutes groundwater, increases the risk of chemical spills, and increases atmospheric nitrous oxide ($N_2O$), a potent greenhouse gas. The global budget for $N_2O$ appears to be out of balance, exceeding sinks by 30-40% and increasing at 0.25% each year [7]. In this case, biological N fixation should receive more attention because about 2 tons of industry-fixed N is needed as fertilizer for crop production to equal the effects of 1 ton of N biologically fixed in a legume crop [51].

In cultivated land, the soil N balance is a complex system covering serial biological processes and physical and chemical processes. It includes plant N uptake, N fixation, organic N mineralization, nitrification and denitrification, nitrate leaching, and other losses, as $N_2$ or

NOx, released into the atmosphere [52]. In the case of organic farms, soil available N is primarily from legumes as green manure and organic fertilizers. SOM reportedly supplies most of the N and S and half of the P uptake by plants within an organic farming system [53,54]. The plant tissues of green manure contain most of the micro or macro plant nutrients, including N, potassium (K), P, and S. Phosphates, K, calcium (Ca), magnesium (Mg), S, and other micro plant nutrients are accumulated by cover crops during the growing season. Hoyt indicated the nutrients content of cover crops; see **Table 3** [55]. These nutrients are maintained in the residues of green manure plants; later, they become available to successive crops after incorporation into the soil.

| Crop | N | K | P | Mg | Ca | Biomass |
|---|---|---|---|---|---|---|
| Hair vetch | 152 | 144 | 19 | 19 | 56 | 3520 |
| Crimson clover | 124 | 154 | 17 | 12 | 67 | 4573 |
| Rye | 96 | 117 | 18 | 9 | 24 | 6057 |
| Austrian winter pea | 156 | 172 | 21 | 14 | 49 | 4443 |

**Table 3.** Green manure biomass productivity and nutrient content (kg ha$^{-1}$) [55].

During the composting process of green manure, some carbonic and other organic acids are formed as by-products of microbial activities. These organic acids react with insoluble mineral rocks and phosphate precipitates, releasing phosphates and exchangeable nutrients [29]. Gardner and Boundy found that wheat intercropped with white lupin (*Lupin albus L.*) has access to a larger pool of P, Mg, and N than wheat grown in monoculture [30]. The former two nutrients were probably mobilized by exudates of organic acids from the lupin root and then taken up by wheat roots.

The nutrients content of different legumes can be estimated by the mathematical formulation described by Peet [56]:

Rye: $N = 0.0194 \times \text{biomass} - 17.4$

Hairy vetch: $N = 0.0409 \times \text{biomass} - 3.1$

Crimson clover: $N = 0.0204 \times \text{biomass} + 13.8$

Austrian winter pea: $N = 0.0402 \times \text{biomass} - 9.2$

Caley peas: $N = 0.0426 \times \text{biomass} - 6.1$

Subclover: $N = 0.0280 \times \text{biomass} + 2.9$,

where "biomass" is the dry weight in kg ha$^{-1}$, and the N content, N, is also in Kg acre$^{-1}$.

For legumes, on average, pounds of K = pounds of N, and pounds of P = 10% of N.

The study of cowpea/maize intercropping shown that cowpea had used atmospheric N for crop growth and also fixed the nutrient into the soil for subsequent crop. The soil residual mineral N was increased by 82% compared with initial soil N. This demonstrated that biological $N_2$ fixation by cowpeas replenished the available N to both crops and also for subsequence crop [57].

## 5. Soil and water conservation by cover cropping

Soil conservation is an important issue in sustainable management, especially on hillsides. Cover crops or living mulch provide important benefits in soil and water conservation. The primary function of alley cropping on sloping lands is erosion control and soil conservation [51,54] . Two forms of soil erosion exist: sheet and rill erosion. Sheet flow is the removal of a relatively uniform thickness of soil and is usually caused by rain-splash, surface runoff, and wind. In rill erosion, water flows with soil particles in small channels [58]. Soil erosion decreases water availability, infiltration rates, water-holding capacity, nutrients, organic matter, and the depth of the soil. Soil erosion not only causes plant nutrient loss but also SOM loss. The latter affects field capacity and soil aggregation structure. Soil erosion has a negative effect on the productivity of soils (Table 4) [59]. The eroded soil typically contains about three times more nutrients than the soil left behind and 1.5–5 times more organic matter. The major costs to a farm associated with soil erosion come from the replacement of the lost nutrients and reduced water-holding ability, accounting for a productivity loss of 50-75% [60].

| **Erosion level** | **Organic matter (%)** | **Phosphorous ( kg ha$^{-1}$)** | **Plant-available water (%)** |
|---|---|---|---|
| Slight | 3.0 | 67 | 7.4 |
| Moderate | 2.5 | 66 | 6.2 |
| Severe | 1.9 | 43 | 3.6 |

**Table 4.** Soil fertility effects of erosion [59].

Soil erosion is connected to water erosion. Water erosion increases the amount of runoff, so that less water can enter the soil matrix and become available to the crop. In severely degraded soils, water infiltration may be reduced by as much as 93%, and so water conservation is linked to soil conservation [61]. Increased SOM content can enhance field capacity and consequently reduce soil erosion. Another effect of reducing soil moisture losses is that the soil cover reduces evaporation in fields. Vegetation acts as a buffer to the soil because rain-splash is an important detaching process in soil erosion. Raindrops striking bare soil have the ability to throw soil particles through the air over distances of several centimeters [62]. A vegetative cover also contributes to slope stability. In Nigeria, in land with a 14% slope and under total rainfall of 1412 mm during a 3-month study period, maize alley cropping with contour hedgerows of *Lucaena leucocephala* and *Gliricidia sepium* established at a 6-m interhedgerow spacing with prunings used as mulch effectively contained erosion by 85% and 73%, respectively [63]. The aboveground components of the plant, such as the leaves and stems, absorb some of the energy of falling raindrops, running water, and wind. The belowground components, the root system, contribute to the strength of the soil, holding soil particles in place. Living mulch can reduce soil and water erosion significantly, as the presence of the canopy slows down raindrops, reducing surface runoff and enhancing water filtration. A well-developed root system holds soil particles together, reducing soil erosion. Moreover, evapotranspiration of plants produces a drier soil environment due to the capable of withstanding a higher intensity and longer duration of rainfall compared with a slope that lacks vegetation [63].

## 6. Conclusions

Agriculture systems have evolved over long periods as a consequence of modifications of climate, agricultural technology, and socioeconomic conditions [64]. In recent decades, cropping systems, both in developed and developing countries, have become increasingly simplified with markedly reduced diversity in vegetation patterns over time and across the landscape. Concomitantly, a large increase has occurred in the use of synthetic fertilizers and pesticides [65]. Consequently, agriculture is suffering stress from environmental issues, such as nitrate leaching to groundwater, nitric oxide release to the air, and $CO_2$ from the fertilizer industry being released into the atmosphere [66]. Thus, the establishment of an adequate crop system that is economically acceptable, environmentally sustainable, and technically practicable is the task of agronomists and farmers.

Organic agriculture is regarded as a sustainable agricultural system, taking into consideration soil fertility conservation, which covers soil physical properties, plant nutrient availability, and erosion control, as its key issues [67]. In organic agricultural practices, biological N fixation has received increased attention from agricultural agronomists and producers. Although the economic value of biological N fixation by legumes varies widely, when the cost of production of the legumes is taken into consideration, opportunities still remain to plant legumes as a short-term rotation crop or an intercrop or living mulch [68]. Seeds of grain legumes can be used as a fast nitrogen available fertilizer in organic production at low temperatures in early spring [26]. Intercropping management has long been practiced and has played a crucial role in sustainable agriculture. One of the main benefits of an intercropping system is the rational utilization of natural resources. The management of intercropping is primarily according to natural laws within an agroecosystem. The regulation that major crops cannot cover the entire soil surface during the whole growing season provides chances for other plants growing as mixture crops, temporarily or spatially. Although competition between intercrops is always observed, a well-managed polyculture system can provide a higher total production than all of the crops planted as pure stands. Crop diversity, in both time and space, appears to be a critical element of sustainable agroecosystems that require few external inputs. Green manure, a major measure for the self-sufficient maintenance of soil fertility, offers organic matter to soil and fixing $N_2$ into organic form in the case of legumes.

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## Chapter 14

# Impact of Organic Fertilizers on Phenolic Profiles and Fatty Acids Composition: A Case Study for *Cichorium intybus* L.

Lovro Sinkovič and Dragan Žnidarčič

Additional information is available at the end of the chapter

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### Abstract

Radicchio (*Cichorium intybus* L.) is an increasingly appreciated leafy vegetable that exhibits great diversity in appearance, including different colored leaves, rosettes, or heads. Varieties of radicchio ('Treviso', 'Verona' 'Anivip', 'Castelfranco', and 'Monivip') commonly produced in Slovenia were investigated for their phenolic and fatty acid profiles. Plants were grown under organic and/or mineral fertilizer managements in greenhouse conditions. High-performance liquid chromatography analysis was used to study phenolic compounds in radicchio leaf samples. Thirty-three phenolic compounds were quantitatively evaluated. Significant differences were found between varieties and across different fertilizer managements. The total phenolic amount (TPA) was found in a wide range from 58 to 403 mg/100 g fresh weight (FW). Between varieties, the highest TPA was observed for var. 'Treviso' (300 mg/100 g FW) and the lowest TPA was observed for var. 'Castelfranco' (125 mg/100 g FW). The main phenolic compounds in radicchio leaves were represented by phenolic acids, chlorogenic acid and cichoric acid, respectively. The fatty acid levels of radicchio leaf samples were determined by the chromatographic analysis of fatty acid methyl esters using gas chromatography with flame ionization detector. The analysis revealed the amounts of C16:0, C18:0, C18:1n9, C18:2n6, C18:3n3, and C20:0 fatty acids. The total fatty acid levels varied from 170 to 500 mg/100 g FW. The highest fatty acid quantity was represented by C18:3n3 (≤63%) followed by C18:2n6 (≤45%) and C16:0 (≤24%). All radicchio samples had a ratio of *n*-6/*n*-3 essential fatty acids below 1 and thus in accordance with the current dietary guidelines. Among different fertilizer managements, the highest total fatty acid levels were found for organic fertilizer (384 mg/100 g FW).

**Keywords:** fatty acids, fertilizers, GC-FID, HPLC-DAD, phenolic compounds, radicchio

## 1. Introduction

Radicchio (*Cichorium intybus* L.; Asteraceae) is a popular salad vegetable in the Mediterranean region, and its usage is increasing in Europe. Other cultivated types of this species are Italian chicory, French endive, witloof, sugarloaf, and succory. It has been known since 1616, when it was first mentioned in Germany. Cultivation began in England in 1886 and later in 1926 also France. There is a discussion about whether radicchio should be classified as a root or a leafy vegetable crop. It can be produced for leaves, rosettes, or heads with a wide range of colors [1]. Radicchio is typically consumed as a raw vegetable in various fresh, mixed, or garnished salads [2,3]. Its popularity among consumers and its nutritional characteristics have great potential for growth in the local markets as well as in the international ones. Most radicchio varieties thrive best during cooler, moist weather and do not tolerate high temperature. Radicchio is a leafy vegetable that can withstand low temperatures, which gives it an advantage for consumption in the winter time of the year when the supply of fresh leafy vegetable in the market is limited [4]. In addition, radicchio represents a plant with several medicinal properties and effects [5].

Vegetable production in many countries depends on high-input systems to maximize yield and product quality, while they try to achieve low production costs, which keep local products competitive in international markets [6]. Conventional high-input farming system is often associated with problems, such as nitrate leaching and ground water pollution, degradation of soil structure, and pesticide contamination [7–10]. The answers to problems associated with conventional practices are alternative cropping systems. Over the past decade, criteria have been developed, which define organic crop production requirements [11]. Now, there exist several national systems of designated requirements to have vegetable products marketed as » organically produced«. In fresh vegetable market, organically grown products of reasonable quality are readily available, but their price is usually much higher compared to those grown by the other than organic manner [12,13].

The polyphenol compositions of vegetable depend on several factors. It is influenced by genetic as well as environmental factors, such as temperature, light, moisture, and the nutritional status of the soil in which the vegetable is grown [14,15]. It is also influenced by the growing manner, phase of maturity, postharvest managements, and storage conditions. Moreover, many vegetables are processed before they are used for consumption. Processing methods, such as cooking and canning, can also influence the polyphenol composition of the vegetable. Regular consumption of vegetables is proven to be associated with lower risks of various types of modern diseases, such as chronic or cardiovascular diseases [7,16].

Polyphenols are organic compounds widely distributed in vegetables. All phenolic compounds have an aromatic ring that contains various attached substituent groups, such as hydroxyl, carboxyl, and methyl groups, and often other nonaromatic ring structures. Phenolics differ from lipids in higher solubility in water and lower solubility in nonpolar organic solvents. These properties greatly aid in the separation of phenolics from one another and from other compounds. Many phenolics arise from the shikimic acid pathway and its subsequent reactions. Among these are cinnamic, *p*-coumaric, caffeic, ferulic, chlorogenic, protocatechuic, and gallic phenolic acids. They are important not because they are abundant in uncombined (free) form but because they are converted into several derivatives besides proteins. These derivatives include phytoalexins, coumarins, lignin, and various flavonoids, such as the anthocyanins [17]. Chlorogenic acid is widely distributed in various parts of many plants and usually occurs in easily detectable quantities. Both chlorogenic and protocatechuic acids have special functions in disease resistance of plants. Gallic acid is important because of its conversion to gallotannins, which are heterogeneous polymers containing numerous gallic acid molecules connected in various ways to one another, to glucose, and to other sugars [18].

Of the various classes of naturally occurring compounds based on the flavonoid skeleton, flavone and flavonols are collectively the most abundant group. The distinction between flavones and flavonols, which are 15 carbon compounds, is an arbitrary one, as flavonols are simply a class of flavone in which the 3-position is substituted by a hydroxyl group [19]. Anthocyanins are present as glycosides, usually containing one or two glucose or galactose units attached to the hydroxyl group in the central ring or to that hydroxyl group at the 5-position of the A ring. When the sugars are removed, the remaining parts of the molecules, which are still colored, are called anthocyanidins. Anthocyanins are soluble and reasonably stable, whereas anthocyanidins produced on acid hydrolysis are insoluble in water, unstable to light, and rapidly destroyed by alkali [20]. Flavones and flavonols are easier to identify than anthocyanins because they are more stable [21]. Several polyphenols, such as derivatives of hydroxycinnamic acids (HCA), flavonoids, and anthocyanins [4,6,22–27], previously determined in radicchio leaves are presented in **Table 1**.

| Phenolic acids | Flavonoids | Anthocyanins |
|---|---|---|
| gallic acid | luteolin 7-O-glucuronide | cyanidin 3-O-glucoside |
| protocatechuic acid | apigenin | cyanidin 3-O-rutinooside |
| caftaric acid (caffeoyl tartaric acid) | apigenin glucuronide | pelargonidin 3-O-glucoside |
| chlorogenic acid | apigenin 7-O-arabinoside | peonidin 3-O-glucoside |
| caffeic acid | quercetin 3-O-glucuronide | malvidin 3-O-glucoside |
| cichoric acid (dicaffeoyl tartaric acid) | quercetin 3-O-galactoside | cyanidin 3-malonylglucoside |
| | quercetin 3-O-rhamnoside | delphinidin 3-O-(6″ malonyl)-glucoside |
| | quercetin malonyl glucoside | cyanidin 3-O-(6″ malonyl)-glucoside |
| | kaempferol 3-O-glucoside | pelargonidin |
| | kaempferol 3-O-glucuronide | peonidin |
| | methyl quercetin glucuronide | malvidin |
| | kaempferol malonyl glucoside | |
| | methyl quercetin glucoside | |
| | isorhamnetin 3-O-glucuronide | |
| | isorhamnetin 7-O-glucuronide | |

**Table 1.** Phenolic compounds reported in radicchio leaves from scientific data.

Lipids are derived from long-chain fatty acids and alcohols or closely related derivatives. They are water-insoluble components of cells that can be extracted by nonpolar solvents. In various parts of the plants, mostly in the cell membranes, are small amounts of lipids (~2%). In higher plants, the predominant fatty acid residues consist of palmitic, oleic, linoleic, and stearic acid.

Fatty acids with <12 and >20 carbon atoms are less common in nature [28]. The most common fatty acids in plants are those containing 16 or 18 carbon atoms. These include saturated palmitic (C16:0) and stearic (C18:0) acids, monounsaturated oleic acid (C18:1n9), polyunsaturated linoleic acid with two double bonds (C18:2n6), and linolenic acid with three double bonds (C18:3n3) [29]. When the carbon atoms in the hydrocarbon chain of a fatty acid hold their full complement of hydrogen, they are described as saturated. Where two adjoining carbon atoms in the hydrocarbon chain of a fatty acid each lack a hydrogen atom, a double bond forms between them. The fatty acid is then said to be unsaturated. The term polyunsaturated fatty acid (PUFA) is accepted as referring to those fatty acids that contain two or more carbon-carbon double bonds within the hydrocarbon chain [30]. Particular PUFAs, which the human system can employ as building blocks while being unable to synthesize them, have been classed as essential fatty acids. The *n*-3 (ω-3, omega-3) PUFAs found in plants refer to a number of health benefits [31]. The most common and most important PUFA is linolenic acid, which is known as a precursor of the long-chain fatty acids (eicosapentaenoic and docosahexaenoic) [32]. Modern agriculture and food industrialization are associated with large changes in the structure of contemporary Western diets. The intake of *n*-6 fatty acids has enlarged during evolution, and the intake of *n*-3 fatty acids has been reduced. Consequently, the *n*-6/*n*-3 ratio increased from 1 to 10 or, in some places, even up to 20 or even 25. These differences in food consumption led to increased risk of numerous modern diseases [33].

Over the past decade, radicchio has become popular for cultivation and consumption in different regions of the world. Scientific literature has revealed that radicchio plants contain important compounds with biological activity and several vitamins and minerals [4,18,34–36]. The effects of fertilizer managements (organic, mineral) on the phenolic and fatty acid profiles in different radicchio varieties (red, red-spotted, green) are poorly discussed in scientific data. This chapter discusses the effect of fertilizers (organic, mineral, and combination) on the total phenolics, the main phenolic classes, and the fatty acids levels of five *C. intybus* varieties. High-performance liquid chromatography (HPLC) was used for the analysis of phenolic compounds and their classes and gas chromatography (GC) was used for the determination of fatty acid levels.

## 2. Materials and analytical methods

### 2.1. Selection of plant material and fertilization experiment

The experiment was carried out in 2012 under the controlled conditions of the central research greenhouse at Biotechnical Faculty (46°04′N, 14°31′W; 320 m a.s.l.). The commercial radicchio varieties were included in our research: red (‘Treviso’, ‘Verona’, and ‘Anivip’), red-spotted

('Castelfranco'), and green ('Monivip'). Photos of individual radicchio variety are shown in **Figures 1** to **3**.

**Figure 1.** Var. 'Anivip' (left) and var. 'Monivip' (right). Photo: D. Žnidarčič.

**Figure 2.** Var. 'Treviso' (left) and var. 'Castelfranco' (right). Photo: D. Žnidarčič.

**Figure 3.** Var. 'Verona.' Photo: D. Žnidarčič.

| Fertilizer treatment | Fertilizer name | N/P/K | Application details | Mark |
|---|---|---|---|---|
| Unfertilized | / | / | Watering | CONT |
| Single basal organic | Plantella Organik | 3/3/2 | 67.5 g/7 L soil | ORG1 |
| Single basal organic | Stallatico Pallettato | 3/3/3 | 45 g/7 L soil | ORG2 |
| Water soluble mineral | Kristalon Blue | 19/6/20 | Irrigation with 9 g/100 L | MIN1 |
| Single basal mineral | Entec perfect | 14/7/17 | 7.9 g/7 L soil | MIN2 |
| Combination of organic and mineral fertilizer | Plantella Organik + Kristalon Blue | 3/3/2 + 19/6/20 | Plantella Organic 3.5 g /7 L soil + after 1 month irrigation with 3.5 g/L Kristalon Blue | ORG1+MIN1 |

**Table 2.** Fertilizer managements used to set up the pot experiment.

The growing experiment in controlled conditions included two mineral fertilizers, two organic fertilizers, a combination of one organic and one mineral fertilizer, and the control (no added fertilizer). In each of the five radicchio varieties, the same six fertilizer managements were applied as presented in **Table 2** in the following design: unfertilized control (CONT), two organic fertilizers (ORG1 and ORG2), two mineral fertilizers (MIN1 and MIN2), and combination of organic and mineral fertilizer (ORG1+MIN1). The experiment consisted of 30 plastic pots filled up with 7 L of soil with application of the selected fertilizers. Sowing was performed on 30 January 2012. Then, the pots were placed in the greenhouse and irrigated appropriately. Water-soluble mineral fertilizer (MIN1) was applied through the irrigation solution containing water and MIN1. The sampling of developed leaves was performed on 10 June 2012. A few leaves from each pot were lyophilized and powdered using a ball mill before analysis. The dry matter content of radicchio leaves varied from 6.8% to 14.8%.

### 2.2. Extraction and identification of phenolic compounds

Radicchio powder was mixed with the solvent 5% formic acid in methanol, which contained flavone as an internal standard. For extraction, an ultrasonic bath at 4°C for 30 min was used. After centrifugation, a 10 μL aliquot of supernatant was injected into the HPLC system. For analysis, reverse-phase HPLC coupled with a diode array detector (DAD) was used. The phenolic compounds were separated on Nucleosil C18 analytical column (250 cm × 4 mm; 3 μm) and eluted using 5% formic acid and HPLC-grade methanol at a constant flow rate. The gradient profile has been flowing to the protocol previously published for the analysis of complex polyphenol mixtures [37].

The DAD was scanning from 250 to 600 nm with four discrete channels. Phenolics were gathered into five classes and monitored at related wavelengths: unknown phenolic compounds (UPCs; 280 nm), HCAs and flavones (320 nm), flavonols (350 nm), and anthocyanins (540 nm). The quantification of each phenolic compound was carried out using the internal standard manner. The phenolic compounds in the radicchio leaves separated by HPLC are presented in **Table 3**. They were classified based on the absorbance spectra [38] and the comparison to representatives [39]. Chlorogenic and caftaric acids were confirmed by previously identified standards [40].

| Compound name/acronym | Peak no. | $R_t$ (min) | UV$\lambda_{max}$(nm) | Phenolic class |
|---|---|---|---|---|
| HCA 1 | 1 | 18.2 | 318, 322 | Monomeric hydroxycinnamic acid |
| Caftaric acid (caffeoyl tartaric acid) | 2 | 19.3 | 330 | Monomeric hydroxycinnamic acid |
| Benzoic acid derivative (protocatechuic acid)? | 3 | 28.3 | 286, 290, 334, 338 | Flavone |
| HCA 2 | 4 | 35.8 | 322 | Monomeric hydroxycinnamic acid |
| HCA 3 | 5 | 41.7 | 330 | Monomeric hydroxycinnamic acid |
| UPC 1 | 6 | 42.6 | 262 | Unknown phenolic compound |
| Chlorogenic acid | 7 | 43.3 | 326 | Monomeric hydroxycinnamic acid |
| HCA 4 | 8 | 53.2 | 326 | Monomeric hydroxycinnamic acid |
| Gallic acid derivative 1 | 9 | 61.4 | 262, 266 | Unknown phenolic compound |
| Gallic acid derivative 2 | 10 | 65.5 | 262 | Unknown phenolic compound |
| HCA 5 | 11 | 66.2 | 310 | Monomeric hydroxycinnamic acid |
| UPC 2 | 12 | 75.7 | 262 | Unknown phenolic compound |

| Compound name/acronym | Peak no. | $R_t$ (min) | UV$\lambda_{max}$(nm) | Phenolic class |
|---|---|---|---|---|
| HCA 5 | 13 | 84.9 | 326 | Monomeric hydroxycinnamic acid |
| Cichoric acid (dicaffeoyl tartaric acid) | 14 | 100.5 | 330 | Oligomeric hydroxycinnamic acid |
| HCA 6 | 15 | 104.2 | 330 | Oligomeric hydroxycinnamic acid |
| HCA 7 | 16 | 112.1 | 330 | Oligomeric hydroxycinnamic acid |
| HCA 8 | 17 | 114.4 | 322 | Oligomeric hydroxycinnamic acid |
| UPC 3 | 18 | 115.1 | 262, 266 | Unknown phenolic compound |
| Gallic acid derivative 3 | 19 | 126.5 | 262 | Unknown phenolic compound |
| HCA 9 | 20 | 131.5 | 326 | Oligomeric hydroxycinnamic acid |
| Kaempferol or quercetin derivative 1 | 21 | 140 | 262, 346, 350 | Flavonol |
| Kaempferol or quercetin derivative 2 | 22 | 141.7 | 262, 346 | Flavonol |
| Kaempferol or quercetin derivative 3 | 23 | 146.3 | 262, 346, 350, 354 | Flavonol |
| ANTHO 1 | 24* | 147.1 | 278, 518, 522 | Anthocyanin |
| HCA 10 | 25 | 149 | 318, 326 | Oligomeric hydroxycinnamic acid |
| Apigenin or luteolin derivative | 26 | 149.5 | 262, 338 | Flavone |
| UPC 4 | 27 | 149.6 | 262, 266 | Unknown phenolic compound |
| UPC 5 | 28 | 155.2 | 262 | Unknown phenolic compound |
| UPC 6 | 29 | 159 | 262, 266 | Unknown phenolic compound |
| FLAVONOL 1 | 30 | 159.5 | 262, 346 | Flavonol |
| FLAVONOL 2 | 31 | 160.3 | 262, 342, 346 | Flavonol |
| Gallic acid derivative 4 | 32 | 161 | 262 | Unknown phenolic compound |
| FLAVONOL 3 | 33 | 164.1 | 262, 266, 342, 346 | Flavonol |

Not detected in var. ‘Anivip’, ‘Castelfranco’, and ‘Monivip’.

**Table 3.** Phenolic compounds in the radicchio leaves separated by HPLC.

### 2.3. Determination of fatty acid levels

Fatty acid levels were analyzed using GC with prior prepared fatty acid methyl esters. In the protocol [41], NaOH and $BF_3$ in methanol were used for transesterification and heptadecanoic acid (C17:0) was used as an internal standard for the quantification of fatty acids. The solution of fatty acid methyl esters was quantified on the GC (Agilent 6890N, USA) with flame ionization detector (FID). At the constant flow rate, the separation was performed on a column for analyses of PUFAs as fatty acid methyl esters. The identification and quantification of fatty acids were carried out using a reference standard mixture of methyl esters of greater fatty acids regularly before the samples. The following fatty acids were detected in the radicchio plants: C16:0, C18:0, C18:1n9, C18:2n6, C18:3n3, and C20:0 (**Table 4**).

## 3. Results and discussion

### 3.1. Phenolic profiles

Thirty-three main phenolic compounds obtained using HPLC detection were selected in all five studied radicchio varieties from six fertilizer managements. Those were grouped according to their absorbance spectra and retention times to UPCs, HCAs, flavonols, flavones, and anthocyanins (**Table 4**). All chromatograms of radicchio samples were similar, but the areas of individual peaks varied considerably. An example of chromatogram for var. 'Castelfranco' is presented in **Figure 4**. Anthocyanins, which are quite unstable, were found in minor quantities in only few radicchio samples.

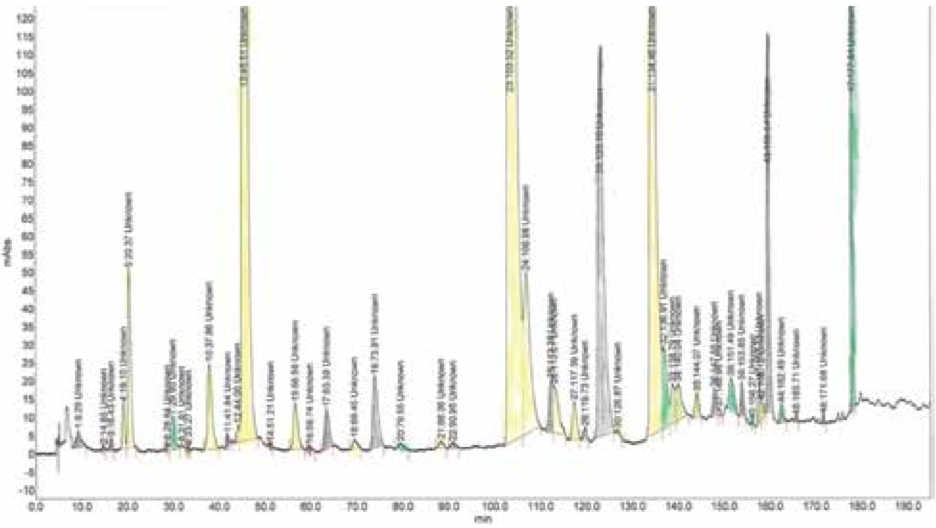

**Figure 4.** Chromatogram of var. 'Castelfranco' from unfertilized management obtained using HPLC analysis.

The phenolic profile data were comparable to former reports, which also found that chlorogenic and cichoric acids are the main phenolic compounds in radicchio leaves [4,6,26,42]. The total phenolic amount (TPA) in the analyzed radicchio leaves under different fertilizer managements varied from 58 to 403 mg/100 fresh weight (FW; **Figure 5**). The results showed large differences between the varieties as well when comparing different fertilizer managements. The average levels over all different fertilizer managements for individual variety showed significantly greater TPA for var. 'Treviso' (300 mg/100 g FW) followed by var. 'Verona' (181 mg/100 g FW), var. 'Monivip' (146 mg/100 g FW), var. 'Anivip' (135 mg/100 g

FW), and var. 'Castelfranco' (125 mg/100 g FW). The red colored var. 'Treviso' showed two times greater TPA in comparison to red-spotted or green radicchio varieties. A high TPA for var. 'Treviso' was reported by D'evoli et al. [34].

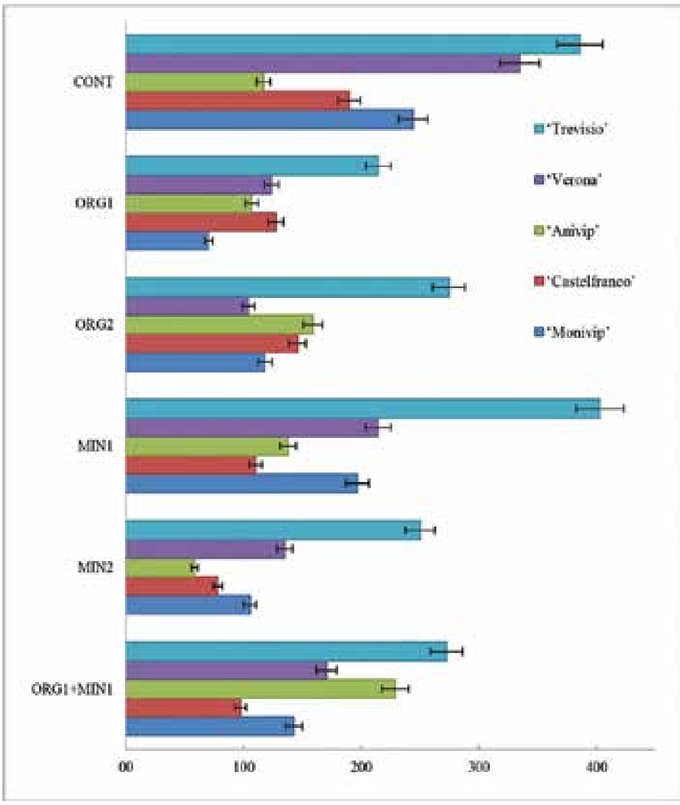

Figure 5. TPA (as mg/100 g FW) in the radicchio leaves among varieties and different fertilizer managements.

Across different managements, the highest TPA was seen for unfertilized (CONT) treatment (254 mg/100 g FW) followed by MIN1 (213 mg/100 g FW), combination of ORG1+MIN1 (183 mg/100 g FW), ORG2 (160 mg/100 g FW), ORG1 (129 mg/100 g FW), and MIN2 (126 mg/100 g FW). Significantly greater TPAs were seen for the radicchio varieties grown under unfertilized management and those with mineral fertilizer (MIN1). Crecente-Campo et al. [43] have reported that the organic or conventional cultivation system did not affect the TPA but only the antioxidant compounds. Vinha et al. [10] found greater TPA for organically grown vegetables, whereas Mitchell et al. [8] obtained only greater amounts for quercetin and kaempferol. According to Oliveira et al. [9], organic manner resulted in greater TPA and vitamin C. Some other studies [44,45] reported that the enzyme phenylalanine ammonia-lyase is involved in the biosynthesis of phenolics and is regulated by nitrogen. In general, the availability of soil nitrogen strongly impacts the synthesis of several phenolic compounds [46]. In relation to nitrogen fertilization, the response of radicchio varieties differs, as high and low nitrogen demanding varieties were previously reported [47].

| **Class** | **Quantity (mg/100 g fresh weight)** | | | | | |
|---|---|---|---|---|---|---|
| | **No fertilizer** | **Organic fertilizer** | | **Mineral fertilizer** | | **Combination** |
| | **CONT** | **ORG1** | **ORG2** | **MIN1** | **MIN2** | **ORG1+MIN1** |
| Hydroxycinnamic acids | | | | | | |
| Treviso | 345.91 ±17.30 aA | 195.67 ±9.78 dA | 257.97 ±12.90 bA | 361.64 ±18.08 aA | 231.91 ±11.60 cA | 232.87 ±11.64 cA |
| Verona | 268.73 ±13.44 aB | 100.08 ±5.00 dB | 82.71 ±4.14 eD | 176.76 ±8.84 bB | 107.98 ±5.40 dB | 131.80 ±6.59 cC |
| Anivip | 100.98 ±5.05 cdE | 94.18 ±4.71 dB | 134.17 ±6.71 bB | 108.03 ±5.40 cC | 46.89 ±2.34 eE | 190.28 ±9.51 aB |
| Castelfranco | 159.81 ±7.99 aD | 104.21 ±5.21 cB | 115.28 ±5.76 bC | 93.91 ±4.70 dC | 60.74 ±3.04 eD | 88.12 ±4.41 dE |
| Monivip | 207.62 ±10.38 aC | 59.02 ±2.95 eC | 86.89 ±4.34 dD | 165.82 ±8.29 bB | 81.74 ±4.09 dC | 109.76 ±5.49 cD |
| Flavonols | | | | | | |
| Treviso | 10.05 ±0.50 cB | 4.38 ±0.22 eB | 10.10 ±0.50 cB | 19.95 ±1.00 aA | 7.88 ±0.39 dA | 11.85 ±0.59 bB |
| Verona | 13.03 ±0.65 aA | 3.14 ±0.16 dC | 3.73 ±0.19 cdD | 11.64 ±0.58 bB | 4.33 ±0.22 cC | 13.74 ±0.69 aA |
| Anivip | 5.19 ±0.26 dE | 2.89 ±0.14 eC | 6.31 ±0.32 cC | 7.89 ±0.39 aC | 5.42 ±0.27 dB | 6.87 ±0.34 bC |
| Castelfranco | 6.53 ±0.33 bD | 4.11 ±0.21 cB | 11.68 ±0.58 aA | 3.94 ±0.20 cD | 4.26 ±0.21 cC | 1.72 ±0.09 dE |
| Monivip | 7.85 ±0.39 aC | 5.23 ±0.26 cA | 2.80 ±0.14 eE | 7.00 ±0.35 bC | 4.37 ±0.22 dC | 5.73 ±0.29 cD |
| Flavones | | | | | | |
| Treviso | 1.33 ±0.07 aB | 0.28 ±0.01 eB | 0.69 ±0.03 dC | 1.22 ±0.06 bA | 1.06 ±0.05 cA | 1.08 ±0.05 cB |
| Verona | 1.89 ±0.09 aA | 0.29 ±0.01 eB | 0.81 ±0.04 cB | 1.20 ±0.06 bA | 0.60 ±0.03 dC | 1.15 ±0.06 bB |
| Anivip | 0.96 ±0.05 bC | 0.15 ±0.01 eC | 0.61 ±0.03 cD | 1.07 ±0.05 aB | 1.06 ±0.05 aA | 0.40 ±0.02 dC |
| Castelfranco | 0.97 ±0.05 bC | 0.32 ±0.02 eB | 1.17 ±0.06 aA | 0.54 ±0.03 cC | 0.50 ±0.02 cD | 0.40 ±0.02 dC |
| Monivip | 1.35 ±0.07 aB | 0.73 ±0.04 bA | 0.44 ±0.02 dE | 0.62 ±0.03 cC | 0.78 ±0.04 bB | 1.36 ±0.07 aA |
| Unknown phenolic compounds | | | | | | |
| Treviso | 28.63 ±1.43 aB | 14.18 ±0.71 dC | 4.14 ±0.21 fD | 19.07 ±0.95 cB | 8.86 ±0.44 eD | 26.67 ±1.33 bB |
| Verona | 48.53 ±2.43 aA | 20.27 ±1.01 cA | 15.64 ±0.78 dC | 23.68 ±1.18 bA | 21.83 ±1.09 bcA | 22.90 ±1.15 bC |
| Anivip | 9.77 ±0.49 dD | 9.75 ±0.49 dD | 17.91 ±0.90 cB | 20.90 ±1.04 bB | 5.00 ±0.25 eE | 31.51 ±1.58 aA |
| Castelfranco | 22.59 ±1.13 aC | 18.81 ±0.94 bB | 17.67 ±0.88 bB | 11.90 ±0.59 cC | 12.76 ±0.64 cC | 7.19 ±0.36 dD |
| Monivip | 27.27 ±1.36 aB | 5.30 ±0.27 dE | 28.13 ±1.41 aA | 23.40 ±1.17 bA | 18.62 ±0.93 cB | 26.03 ±1.30 aB |

The average values ($n$=3) with different lowercase letters in a row are significantly different ($P$<0.001; differences between the fertilizers), and those with different uppercase letters in a column are significantly different ($P$<0.001; differences between the varieties).

**Table 4.** Phenolic classes in the leaves of radicchio varieties derived from different fertilizer managements.

The main classes of phenolic compounds (as mg/100 g FW) among radicchio varieties from different fertilizer managements are presented in **Table 4**. Statistical analysis showed significant differences between both fertilizer managements and the varieties for all of these main classes. HCAs were the greatest represented group of phenolic compounds in radicchios with a range of 60% to 95% followed by unknown phenolics, flavonols, and flavones (**Figure 6**).

Phenolic acids (specifically HCAs) were further on grouped according to their retention times as monomeric (<100 min) and oligomeric (>100 min). HCAs are mostly represented by chlorogenic and cichoric acid in all radicchio samples (**Table 5**). The levels of HCAs varied in a wide range from 47 to 362 mg/100 g FW (**Table 4**). The higher levels of total HCAs were found in var. 'Treviso,' up to two times more than the mean value, whereas var. 'Castelfranco' had the lowest amounts of HCAs. The analysis showed that radicchios contribute a smaller amount of monomeric (27%) in comparison to oligomeric HCAs (56%). Data showed that, across radicchio varieties, var. 'Treviso' had greater total HCA amount compared to other varieties (**Table 4**).

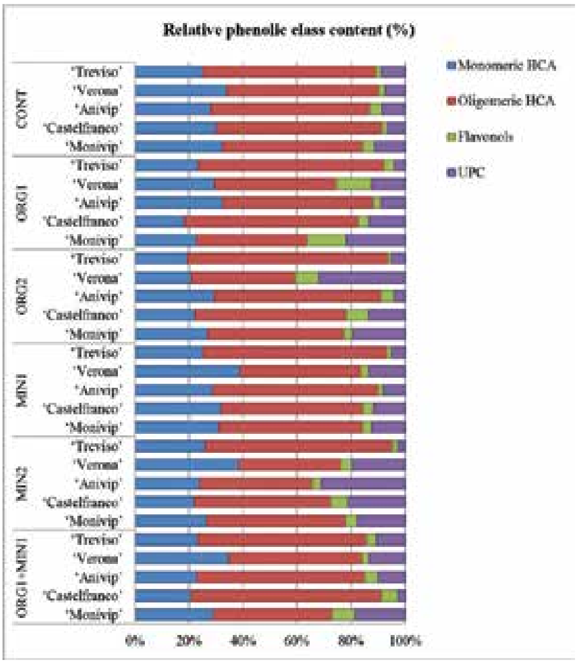

**Figure 6.** Relative phenolic class contents in leaves of radicchio varieties from different fertilizer managements.

The main identified monomeric HCAs were caftaric and chlorogenic acids, whereas the most represented oligomeric was cichoric acid. Cichoric acid was best represented and accounted for 43% of total HCAs, whereas chlorogenic acid with 28% and caftaric acid with 3% were present in lesser quantities (**Table 5**). All three phenolic acids together represent up to 74% of the total HCAs in radicchio samples (**Figure 6**). The HCA quantities were as follow: cichoric acid (16–190 mg/100 g FW), chlorogenic acid (14–89 mg/100 g FW), and caftaric acid (1–14 mg/

100 g FW). Those levels are in accordance with earlier reports, revealing that the caftaric, chlorogenic, and cichoric acids are the most abundant HCAs in radicchio varieties [4,6,22–26].

| **HCA** | **Quantity (mg/100 g fresh weight)** | | | | | |
|---|---|---|---|---|---|---|
| | **No fertilizer** | **Organic fertilizer** | | **Mineral fertilizer** | | **Combination** |
| | **CONT** | **ORG1** | **ORG2** | **MIN1** | **MIN2** | **ORG1+MIN1** |
| Cichoric acid | | | | | | |
| Treviso | 186.37 ±9.32 aA | 120.11 ±6.01 cA | 134.80 ±6.74 bA | 190.12 ±9.51 aA | 128.89 ±6.44 bcA | 133.56 ±6.68 bcA |
| Verona | 123.71 ±6.19 aB | 34.61 ±1.73 cB | 36.79 ±1.84 cB | 65.58 ±3.28 bB | 37.72 ±1.89 cB | 62.75 ±3.14 bB |
| Anivip | 58.46 ±2.92 aE | 15.51 ±0.78 eD | 37.94 ±1.90 bB | 26.87 ±1.34 dD | 26.09 ±1.30 dC | 33.47 ±1.67 cE |
| Castelfranco | 70.99 ±3.55 aD | 24.10 ±1.21 eC | 43.55 ±2.18 cB | 34.27 ±1.71 dD | 27.08 ±1.35 eC | 53.79 ±2.69 bC |
| Monivip | 103.57 ±5.18 aC | 27.41 ±1.37 dC | 41.51 ±2.08 cB | 49.88 ±2.49 bC | 40.36 ±2.02 cB | 42.61 ±2.13 cD |
| Chlorogenic acid | | | | | | |
| Treviso | 85.38 ±4.27 aA | 33.95 ±1.70 cA | 39.15 ±1.96 cA | 80.39 ±4.02 aA | 48.90 ±2.45 bA | 52.83 ±2.64 bA |
| Verona | 89.54 ±4.48 aA | 31.51 ±1.58 dB | 23.80 ±1.19 eC | 77.09 ±3.85 bA | 47.61 ±2.38 cA | 47.84 ±2.39 cB |
| Anivip | 28.79 ±1.44 cD | 23.67 ±1.18 dC | 32.52 ±1.63 bB | 29.07 ±1.45 cC | 14.25 ±0.71 eC | 41.95 ±2.10 aC |
| Castelfranco | 44.41 ±2.22 aC | 17.72 ±0.89 dD | 25.29 ±1.26 cC | 32.25 ±1.61 bC | 15.62 ±0.78 dC | 17.26 ±0.86 dE |
| Monivip | 72.57 ±3.63 aB | 18.02 ±0.90 eD | 24.78 ±1.24 dC | 53.31 ±2.67 bB | 20.23 ±1.01 eB | 36.85 ±1.84 cD |
| Caftaric acid | | | | | | |
| Treviso | 8.52 ±0.43 cB | 11.98 ±0.60 bA | 11.29 ±0.56 bA | 14.29 ±0.71 aA | 11.50 ±0.57 bA | 8.46 ±0.42 cA |
| Verona | 9.18 ±0.46 aA | 5.60 ±0.28 bB | 3.45 ±0.17 dB | 1.86 ±0.09 eCD | 4.37 ±0.22 cB | 4.52 ±0.23 cB |
| Anivip | 1.86 ±0.09 dE | 2.36 ±0.12 cC | 3.45 ±0.17 aB | 2.38 ±0.12 cC | 1.34 ±0.07 eD | 2.76 ±0.14 bD |
| Castelfranco | 3.32 ±0.17 bD | 2.35 ±0.12 cC | 3.92 ±0.20 aB | 1.43 ±0.07 dD | 1.39 ±0.07 dD | 1.56 ±0.08 dE |
| Monivip | 4.88 ±0.24 aC | 1.44 ±0.07 dD | 3.47 ±0.17 cB | 4.00 ±0.20 bB | 3.77 ±0.19 bcC | 3.78 ±0.19 bcC |

The average values (*n*=3) with different lowercase letters in a row are significantly different ($P<0.001$; differences between the fertilizers), and those with different uppercase letters in a column are significantly different ($P<0.001$; differences between the varieties).

**Table 5.** HCA levels in the radicchio leaves.

Both flavonols and flavones are chemosystematic markers found in tribe Cichorieae of the Asteraceae family [27]. Total flavonol amounts of studied radicchio varieties were found in the range of 1.7 to 20 mg/100 g FW (**Table 4**). The flavonols represented below 10% of TPA for most of the radicchio samples, except for var. 'Verona' ORG1 (13%) and var. 'Monivip' MIN1 (14%). Flavones represented only small concentrations ranging up to 2 mg/100 g FW (**Table 4**). Arabbi et al. [48] found similar amounts of flavonoids ranging from 18 to 38 mg/100 g FW.

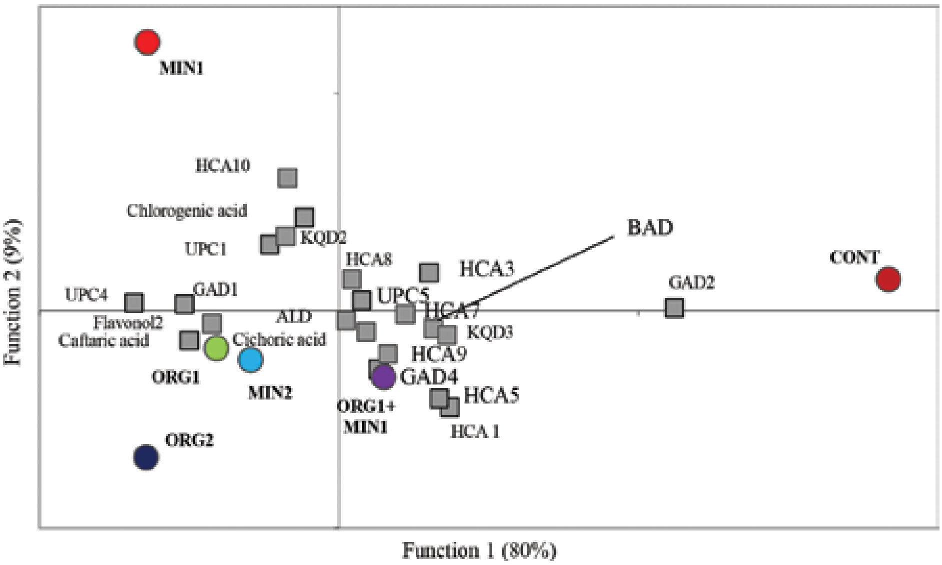

**Figure 7.** LDA plot for 30 radicchio samples according to six fertilizer managements. For legend, see **Table 3**.

Multivariate data analysis by principal component analysis (PCA) and linear discriminant analysis (LDA) was used for plotting the radicchio samples based on their phenolic compounds. All 60 peaks were included in the analysis. Using PCA, 21 phenolic compounds were selected as the most discriminating variables: 10 HCAs, 3 UPCs, 3 gallic acid derivatives, 3 flavonols, 2 flavones, and a protocatechuic acid (**Table 3**). The LDA scores of the data (30 samples, 21 variables) for first two functions are plotted in **Figure 7**. It should be emphasized that ORG1 and MIN2 fertilizer managements are characterized by slow nitrogen release [49].

### 3.2. Composition of fatty acids

The levels of the individual and total fatty acids (mg/100 g FW) of radicchio leaf samples are shown in **Table 6**. Data show significant differences for different varieties and fertilizer managements. Using GC analysis, the following fatty acids were identified and quantified: saturated fatty acids (SFAs) C16:0, C18:0, and C20:0; monounsaturated fatty acid (MUFA); and PUFAs C18:1n9, C18:2n6, and C18:3n3. Linolenic acid (C18:3n3) was represented the most and accounted for 48% to 63% of total fatty acids amount, whereas linoleic acid (C18:2n6) accounted for 16% to 30% and palmitic acid (C16:0) for 14% to 24% (**Table 6**). Stearic (C18:0) and oleic (C18:1n9) fatty acids were less abundant (<5%), and the smallest levels were found for arachidonic acid (C20:0; i.e., <1%). The total fatty acid levels ranged from 173 to 503 mg/100 g FW (**Table 6**). In comparison to other varieties, var. 'Castelfranco' showed greater levels of total fatty acid levels. Between fertilizer managements, there were significantly better total fatty acid levels when the organic fertilizers were used (ORG1 and ORG2). Obtained data are well in accordance to those for forage radicchios [50]. Blanckaert et al. [51] reported almost similar amounts for fatty acid levels of the *Cichorium* '474.'

| Fatty acid | Unfertilised | Combination | Organic | | Mineral | | Sign. |
|---|---|---|---|---|---|---|---|
| | CONT | ORG1+MIN2 | ORG1 | ORG2 | MIN1 | MIN2 | |
| C16:0; saturated | | | | | | | |
| Treviso | 43.55 dC | 42.22 eD | 53.16 aC | 49.94 bC | 46.54 cD | 39.96 fC | *** |
| Verona | 44.04 bC | 31.82 dE | 38.06 cD | 46.36 abD | 47.42 aD | 39.75 cC | *** |
| Anivip | 71.93 aA | 59.59 bcC | 69.74 aB | 66.73 abB | 74.49 aA | 57.24 cB | ** |
| Castelfranco | 60.30 dB | 68.64 cA | 81.50 aA | 72.30 bA | 71.79 bC | 72.19 bA | *** |
| Monivip | 77.12 aA | 64.86 bB | 51.08 dC | 66.28 bB | 65.13 bC | 59.45 cB | *** |
| Sign. | *** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 59.39 ab | 53.43 c | 58.71 b | 60.32 ab | 61.08 a | 53.72 c | *** |
| C18:0; saturated | | | | | | | |
| Treviso | 4.76 bC | 4.24 cD | 5.12 aB | 5.27 aB | 3.86 dE | 3.93 dC | *** |
| Verona | 4.84 abC | 3.00 cE | 4.29 bC | 5.44 aB | 4.87 abD | 4.69 abC | ** |
| Anivip | 4.78 edC | 4.94 dC | 7.87 aA | 5.56 cB | 6.79 bA | 4.29 eC | *** |
| Castelfranco | 6.02 eB | 6.53 cdA | 8.25 aA | 6.74 cA | 6.46 dB | 7.01 bA | *** |
| Monivip | 7.15 aA | 5.72 bB | 4.65 cC | 7.00 aA | 5.71 bC | 5.93 bB | *** |
| Sign. | *** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 5.51 b | 4.89 d | 6.04 a | 6.00 a | 5.54 b | 5.17 c | *** |
| C18:1n9; unsaturated | | | | | | | |
| Treviso | 7.13 cBC | 7.90 bC | 5.49 dD | 10.79 aC | 5.17 eD | 5.46 dC | *** |
| Verona | 7.76 bB | 4.88 cE | 5.26 cD | 9.15 aD | 7.16 bC | 3.50 dD | *** |
| Anivip | 6.57 eC | 11.22 dA | 16.85 aB | 15.10 bA | 14.42 bA | 13.16 cB | *** |
| Castelfranco | 8.56 dA | 9.29 dB | 24.90 aA | 13.81 bB | 11.84 cB | 12.48 cB | *** |
| Monivip | 6.52 dC | 5.70 eD | 7.47 cC | 8.61 bD | 5.42 eD | 15.17 aA | *** |
| Sign. | *** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 7.31 f | 7.80 e | 11.99 a | 11.49 b | 8.80 d | 9.95 c | *** |
| C18:2n6; unsaturated | | | | | | | |
| Treviso | 59.37 bB | 58.07 cD | 49.40 fD | 77.52 aC | 50.17 eD | 55.01 dD | *** |
| Verona | 63.57 bB | 45.29 dE | 54.60 cC | 72.02 aD | 49.84 cdD | 32.09 eE | *** |
| Anivip | 62.57 cB | 100.62 aA | 94.58 abB | 97.68 abB | 90.52 bB | 99.71 abB | *** |
| Castelfranco | 77.89 dA | 75.46 dB | 127.46 aA | 111.23 bA | 98.67 cA | 109.32 bA | *** |
| Monivip | 58.52 cdB | 65.49 bC | 55.42 dC | 77.74 aC | 61.61 cC | 74.43 aC | *** |
| Sign. | ** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 64.38 d | 68.99 c | 76.29 b | 87.24 a | 70.16 c | 74.11 b | |
| C18:3n3; unsaturated | | | | | | | |

| Fatty acid | Unfertilised | Combination | Organic | | Mineral | | Sign. |
|---|---|---|---|---|---|---|---|
| | CONT | ORG1+MIN2 | ORG1 | ORG2 | MIN1 | MIN2 | |
| Treviso | 173.17 cB | 154.52 dC | 197.75 aC | 189.77 bC | 140.90 eE | 127.69 fC | *** |
| Verona | 188.05 aB | 129.16 bD | 147.12 bE | 191.62 aC | 175.60 aD | 91.98 cD | *** |
| Anivip | 250.41 bA | 187.66 deB | 213.95 cdB | 238.56 bcA | 316.64 aA | 162.02 eB | *** |
| Castelfranco | 242.55 cA | 246.32 bcA | 253.40 aA | 233.67 dB | 250.50 abB | 244.30 bcA | *** |
| Monivip | 234.30 abA | 246.70 aA | 161.34 dD | 234.73 abAB | 232.15 bcC | 221.10 cA | *** |
| Sign. | ** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 217.70 a | 192.87 b | 194.71 b | 217.67 a | 223.16 a | 169.42 c | *** |
| C20:0; saturated | | | | | | | |
| Treviso | 0.26 bB | n.d. | 0.94 aB | 0.59 abB | 0.67 abD | 0.62 abB | * |
| Verona | 0.52 bcB | 0.15 cC | 0.60 bcC | 0.83 abAB | 0.79 abcC | 1.25 aA | * |
| Anivip | 1.12 aA | 0.46 cB | 1.16 aA | 0.96 abAB | 0.98 abB | 0.70 bcAB | ** |
| Castelfranco | 0.80 eAB | 1.08 bcA | 1.22 aA | 0.98 cdAB | 1.17 abA | 0.93 dAB | *** |
| Monivip | 1.27 aA | 1.02 bA | 0.86 cB | 1.20 aA | 1.18 aA | 0.73 dAB | *** |
| Sign. | * | *** | *** | Ns | *** | Ns | |
| Average of all cultivars | 0.80 a | 0.54 b | 0.96 a | 0.91 a | 0.96 a | 0.85 a | *** |
| Total fatty acid levels | | | | | | | |
| Treviso | 288.25 cB | 266.96 dC | 311.86 bC | 333.89 aD | 247.31 eE | 232.66 fD | *** |
| Verona | 308.78 abB | 214.31 dD | 249.93 cE | 325.42 aE | 285.68 bD | 173.26 eE | *** |
| Anivip | 397.38 bcA | 364.47 cdB | 404.15 bcB | 424.58 bB | 503.84 aA | 337.11 dC | *** |
| Castelfranco | 396.12 cA | 364.47 cA | 496.73 aA | 438.73 bA | 440.44 bB | 446.24 bA | *** |
| Monivip | 384.88 abA | 364.47 abA | 280.82 cD | 395.57 aC | 371.20 bC | 376.82 abB | *** |
| Sign. | ** | *** | *** | *** | *** | *** | |
| Average of all cultivars | 355.08 c | 328.51 d | 348.70 c | 383.64 a | 369.69 b | 313.22 e | *** |

Sign.: levels of significance, ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; Ns, not significant.
The average values ($n$=3) with different lowercase letters in a row are significantly different ($P<0.001$; differences between the fertilizers), and those with different uppercase letters in a column are significantly different ($P<0.001$; differences between the varieties).

**Table 6.** Fatty acid levels (mg/100 g FW) of radicchio varieties produced with different fertilizer managements.

The nutritional information of radicchio varieties for most optimal fertilizer management (ORG2), which signified the uppermost total fatty acid levels, is presented in **Table 7**. PUFAs represent the range from 79% to 81% of total fatty acid levels, SFAs the range from 16% to 19%, and MUFAs the range <3.6%. The ratio of $n$-6/$n$-3 fatty acids was below 0.48 for all radicchio varieties. Simopoulos [52] reported that past human diets had a ratio of $n$-6/$n$-3 fatty acids near 1, whereas modern Western diets have that ratio much higher (up to 20). The optimal ratio of *n*-6/*n*-3 fatty acids is believed to be from 1 to 4 [33,52]. Schreck et al. [53] found a higher ratio of *n*-6/*n*-3 fatty acids for the lettuce seedlings, whereas some prior readings on wild *Cichorium* leaves showed much lower values [54,55]. All analyzed radicchio varieties had the ratio at values are considered as optimal and fully in agreement with current nutritional recommendations [56].

| Variety | Relative ratio (wt. %) | | | | | PUFA/SFA | *n*-6/*n*-3 |
|---|---|---|---|---|---|---|---|
| | SFA | MUFA | PUFA | *n*-3 | *n*-6 | | |
| Treviso | 16.72 | 3.23 | 80.05 | 56.83 | 23.4 | 4.79 | 0.41 |
| Verona | 16.18 | 2.81 | 81.01 | 58.88 | 22.39 | 5.01 | 0.38 |
| Anivip | 17.25 | 3.56 | 79.2 | 56.19 | 23.23 | 4.59 | 0.41 |
| Castelfranco | 18.24 | 3.15 | 78.61 | 53.26 | 25.57 | 4.31 | 0.48 |
| Monivip | 18.83 | 2.18 | 78.99 | 59.34 | 19.95 | 4.19 | 0.34 |

**Table 7.** Nutritional information of different radicchio varieties derived from organic fertilizer (ORG2) management.

## 4. Conclusions

The phenolic profiles and distribution of classes of the five analyzed radicchio varieties (three red, one red-spotted, and one green) produced by different fertilizer managements under greenhouse conditions are extensively diverse. The analysis of phenolic profiles using HPLC allowed the identification and quantification of prevalent compounds. In the radicchio leaves, the predominant phenolic compounds are cichoric and chlorogenic acids. The phenolic distribution in radicchio leaves is very predisposed by both variety and fertilizer use. The highest TPAs were found for the unfertilized samples followed by the management with the water-soluble mineral fertilizer and the combination of organic and mineral fertilizers. The analysis of fatty acid profiles in radicchio leaves using GC determined six fatty acids. The main fatty acids consist of polyunsaturated linolenic acid (C18:3n3) and linoleic acid (C18:2n6). The main SFA was palmitic (C16:0). Significantly higher fatty acid levels among the fertilizer managements were seen for organic fertilizers. Radicchio seems to have an excellent nutritious balance of essential fatty acids. In summary, the phenolic and fatty acid profiles of radicchio are highly influenced by growing conditions and indicate considerable dietary and nutritional value due to its bioactive phytochemicals.

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Chapter 15

# Productivity and Structures of Marandu Grass Fertilized with Poultry Manure Both with and Without Soil Chiseling

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Additional information is available at the end of the chapter

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## Abstract

With annual increase in production poultry the use of manure can be for the fertilization of pastures. The objective of this study was to evaluate the production of Marandu grass fertilized with poultry manure applied to the soil with and without soil chiseling, betweenSeptember 2012 and September 2013. The study design was a randomized block with four replications in a 5 x 2 factorial arrangement with five doses of manure (0, 1.073, 2.074, 4.148, 6.222 t $ha^{-1}$) both with and without soil chiseling. The cuts were made with light interception of 95% of the canopy with a depth of residue of 0.15 m. With accumulated production during the period there was no interaction (Dose x Management, Cutting Number, Dry Mass (Total and Waste), Leaf Blade (Total and Waste) and Mass Dead of Waste). The application of poultry manure doses caused changes in the stem and the sheath(total and waste) and mass dead total as well asthe production of dry mass, blade blade and stems and sheath. All set to the linear model, and the production of dry mass and blade blade 19.31 and 13.52 Mg $ha^{-1}$ at the highest dose of manure.Poultry manure can be an alternative fertilizer for productive of the leaf blade recovery of Marandu grass.

**Keywords:** fertilization, forage crops, management, forage mass, soil

## 1. Introduction

Globalization has promoted the need foragricultural systems to becomeefficient and highly productive. Addressing theratio of production to the environment and creating links between differentsupply chains, whilst maintaining their sustainability, is a challenge for any agro-industrial system model.

In the Brazilian agricultural production context, in 2012, highlightingpoultry, there were 5.23 billion slaughtered animals and the production of eggs and number of hens was499.85 million [9]. According to [11], broiler breeding produces on average four tonnes of bed per year per1000 birds.It has been calculated that1000 hens produce about 0.12 t day$^{-1}$of manure [14]. Every year 42.85 million tonnes (t) of solid waste is generated in Brazil which could be used both in agriculture and in pastures.

There is a need to research development enabling the use of agricultural waste, so it be comespossible to improve the physical, chemical and biological aspects oftropical soils. Organic fertilizers can provide a greater contribution under these conditions when compared to mineral fertilizers [10]. The use of residue in agricultural soils is favoredbecause of the low cost of manure fromfarms, with the buyer beingresponsible for low-density transport and huge volume.

The use of solid manure is a cost-saving alternative to fertilized pastures [2]; poultry manure has higher levels of organic matter, total nitrogen, total phosphorus and carbon than other types of manure [3]. According to the Ministry of Agriculture Livestock and Supply [6], it is permitted to use poultry manure on pastures and forages with a grace period of 40 days after application and incorporation.

The mechanical soil incorporation of poultry manure on recovering areas can be performed with the use of light tiling with a disc harrow or chisel plow. In this process the disc harrow causes greater movement of soil and forage, due to cutting by the action of the discs, while the chisel plow opens small grooves preserving forage crops on the soil surface.

The *Brachiaria brizantha* cv. Marandu, with 30 years of cultivation in Brazil, is the forage crop used most favorably by producers, who do not practisesoil fertility control and proper pasture management. There areinformation gaps on the structural characteristics of the crop during regrowth under nitrogen and pasture management [1]. It is known that the maximum rates of dry matter forage accumulation are associated with light interception in 95% of incident radiation [19, 23].

Although there is a complex dynamic process between soil, plants, climate and animals it is also important to know the mechanical and fertilization alternatives forthe recovery the forage. As the dry matter production of Marandu grass fertilized with chicken manure and managed with, and without soil chiseling, in light interceptions of 95% at canopy heightsup to 0.15 m, total dry mass of the canopy at ground level and dry mass residue to height of 0.15 m, and their structures as Leaf Blade, Stem + Sheath and dead material. Assessing how the chicken manure applied on grass Marandu changes its production within this system.

## 2. Productivity and structures of Marandu grass

The experiment was conducted in an area of 0.16 hectares cultivated with Marandu grass which had experienced 10 years of grazing, had a slope of 5% in distrophic Red Latosol with the presence, in the 0.0-0.2-m layer, of 87.25%, 1.00% and 11.75% sand, silt and clay, respectively.

Chemical analysis of soil collected at 0.0-0.2 m, showed average levels of pH ($H_2O$) = 6.00; C = 8.23 g $dm^{-3}$; P = 6.28 mg $dm^{-3}$; $H^{+}$+ $Al^{+3}$ = 2.54 $cmol_c$ $dm^{-3}$; $Ca^{+2}$ = 0.87 $cmol_c$ $dm^{-3}$; $Mg^{+2}$ = 0.57 $cmol_c$ $dm^{-3}$; $K^{+}$ = 0.13 $cmol_c$ $dm^{-3}$ and Fe = 180.86 mg $dm^{-3}$; Zn = 4.04 mg $dm^{-3}$; Cu = 3.20 mg $dm^{-3}$; Mn = 144.21 mg $dm^{-3}$; S-SO42- = 3.25 mg $dm^{-3}$.

The poultry manure Hy Line W36 lineage posture with cages, was stored in the shade for 45 days and covered with canvas. Chemical analysis of the manure was found to introduce the following: pH ($H_2O$) = 6.98; organic material = 60%; $N_{total}$ = 6.64%, $P_2O_5$ = 2.41%; $K_2O$ = 3.73%; CaO = 3.53%; MgO = 5.51%; C/N = 4.96:1; Cu = 220.10mg $kg^{-1}$; Mn = 1226.90 mg $kg^{-1}$and Zn = 368.00 mg $kg^{-1}$.

Toraise the saturation of soil bases by 50% manual application of 0.490 t $ha^{-1}$ of dolomitic lime (32% CaO and 15% MgO) was applied to the Marandu grass in August 2012.After 25 days a standardization cut was made using rotary mowers to 0.1 m above the ground.

The application of organic fertilizer rates should be determined based on the need for production and forage cutting in order to avoid the associated risks of soil and water contamination [8]. According to [5], the contamination problem is restricted to some micronutrients and especially to macronutrients such as nitrogen and phosphorus.

Adose of 50 kg $ha^{-1}$ of $P_2O_5$ was based on maintaining species of Group III [13, 16] present in 2.074 t $ha^{-1}$ manure. The experimental design was a randomized block with four replications in a 5 x 2 factorial arrangement, comprising five doses of manure (0, 1.037, 2.074, 4.148, 6.222 t $ha^{-1}$) and two management regimes with and without soil chiseling. A treatment was added of mineral fertilization of Nitrogen (N), phosphorus (P) and potassium (K) contained in 2.074 t $ha^{-1}$ of the poultry manure:138 kg $ha^{-1}$ (N), 50 kg $ha^{-1}$ ($P_2O_5$), 77 kg $ha^{-1}$ ($K_2O$).

Atthe end of September application was made of 0.270 t $ha^{-1}$ of gypsum and a unique manure dose,as well astreatment with mineral fertilizer $P_2O_5$ and 1/3 of the N and $K_2O$, parceled every 60 days. Soilchiselingwas then undertakenin the corresponding portion usinga tractor to plow (SubsoilerTanden IKEDA brand, DPT320M). This featured a cutting disc, positioned at each helical rod to cut and not accumulate spall the Marandu grass and inclined ferrule steel blades to 0.2 m depth.

A productive evaluation of Marandu grass was via alight interception (LI) of 95% by the canopy of Marandu grass [23];measured using anAccuPAR model LP - 80 PAR/LAI Ceptometer with weekly evaluations being made viareadings above the level canopy and below the soil surface in each plot. The residual height was 0.15 m [23] and was lowered[7] by the use of a mechanical trimmer followed by manual removal of all biomass.

Three to nine cuts (95% IL) were performed between the control combination (without manure) and chiseling with the largest poultry manure dose given without chiseling (**Figure 1**). There was an increase in number of cuts (NC) with an increase in poultry manure doses and soil management with scarification over auniform cutting period within treatments. With dose of 6.222 t ha$^{-1}$ of poultry manure and mineral fertilizer (NPK),without scarifying, the first cut occurred at 40 days and without the use of manure the period was longerwith 143 days after the application of manure with and without chiseling. Among the manure doses given accompanied by physical changes to thesoil viachisel plowing there was a longer period for early cutting compared to that without physical soil management.

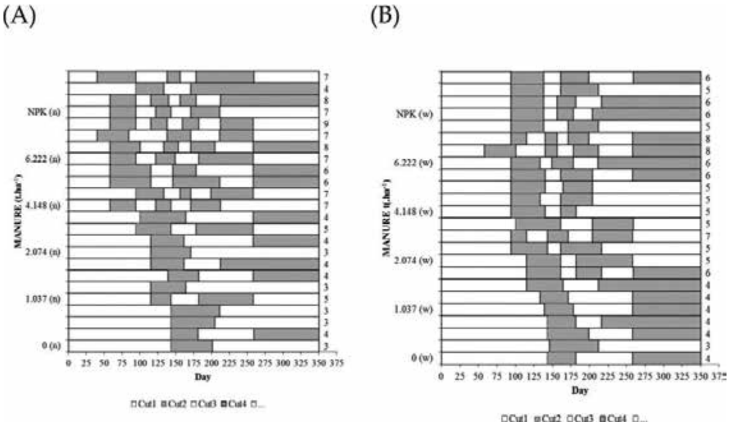

**Figure 1.** Section period of Marandu grass fertilized with poultry manure and soil management: A - without (n) and B –with soil chiseling (w).

With a dose of 6.222 t ha$^{-1}$ of poultry manure and mineral fertilizer (NPK) without scarifying the first cut occurred at 40 days and without the use of manure the period was longerwith 143 days after the application of manure both with and without chiseling. Among the manure doses accompanied by physical changes of soil by chisel plowing the period was longer for early cutting compared to that without the physical soil management.

The highest average interval between cuts was 177 days for lower doses of manure and control. According to [19], under this situation the chance of the plant community in any way replacing the used reserves in the recovery of a new canopy are greater, creating changes to accumulation patterns. The lowest average interval between cuts was 44 days accompanied by the higher dose of manure and chemical fertilizers. According to [7], the greatest Marandu grass efficiency is achieved with more frequent cuts. The results confirm [23]adependence of Marandu grass on thenatural soil fertility and higher nitrogen fertilization fornew structures of the canopy divide the incorporation of nitrogen in organic compounds largely occur in young cells and the growth of roots.

The favorable weather conditions in this region also contributed to the frequency of cuts between 90 and 210 days mainly viahigh temperatures and rainfall. After this period of time, thefrequency wascompromised by low temperatures, around 20 °C, typical to this region, with prevailing climatic characteristics, Cfamesothermal humid subtropical (Köppen classification). Regarding soil management with and without soil chiseling, the possibility of increased use of pasture was identified withtreatments of 6.222 t ha$^{-1}$poultry manure and mineral chemicals (NPK) - both with application on Marandu grass and without the mechanical handling of soil scarification.

With adose of manure of 6.222 t ha$^{-1}$, the results of number of cuts (NC)are similar to those found working with a cutting interval expressed in days, varying according to wet and dry seasons, totaling 6-7 cuts over a year evaluation [18, 22]. Despite the relevance of this work, the cuts made at IL 95% consider the physiological aspects of forage during the highest production levels with greater production of leaf.

The difference in management, and the increased availability of macronutrients and mineralized micronutrients, has contributed to the evolution of the leaf area index (LAI) and light interception (LI), provided by higher tilleringdue to larger spaces LI and structural restoration sward in less time by the appearance of younger leaves. In grass Marandu cuts to 0.15 m high[7] it was observed more stable populations of tillers, confirming [21], with lower grass level in maintained pastures, higher population density of small tillers the greater incidence of light within the sward.

According to [19] the role of the leaves inincreasing the LAIis key, while high growth rates can be achieved in a LAI that causes almost total interception of the incident light. Thus, canopies where cutting or grazing occurs over shorter intervals, keeping an IL close to 95%, will be more efficient in assimilating carbon [20]. However, as the regrowth time is shorterthe result is a lower average rate of forage accumulation.

According to [15], the recovery of the leaf area index (LAI), which can be defined as the land area occupied by leaves, with 95% IL, the removal of part of the biomass produced as a result of cutting or grazing, is variable due to environmental conditions - especially soil nitrogen availability.

In forage mass production, quadrats were used 0.25 m$^2$ (0.50x0.50 m), allowing a total of four samplings per plot, and two samples at ground level IL 95% and two 0.15 m. In each forage sample two aliquots were withdrawn, one for determining the dry mass at IL 95% forstem and sheath total (SST) and the residue of 0.15 m stem and sheath residue (SSR) and the other for separation of the morphological components of forage. A subsample of IL 95% and residue of 0.15 m was carried out via manual separation for structural characterization: green leaf blade (LB) fraction, green stem and sheath (SS) and dead (D) material. There were weighed and placed in a forced air circulation oven at 55 °C for 72 hours for subsequent weighing of the dry fractions. The dry mass amounts of forage and structural features were retained and converted to Mg ha$^{-1}$ to compare the results.

The poultry manure doses caused significant changetobiomass accumulation IL95% SST and dead material total (DT), the residue of 0.15 m for SSR and production of dry mass (DM), LB and SS. There was interaction with the mechanical handling in the number of cuts, total dry mass (DST), leaf blade total (LBT), dry mass residue (DMR), leaf blade residue (LBR) and dead material residue (DR). Dead material (D) was not significant, being assigned to the handling system with IL 95% and reinforcing the conclusions from [19] and [23].

| | **Mean squares** | | | **Re** | **CV (%)** | **Manure Dose (t ha$^{-1}$)** | | | | | **NPK** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Dose (DO)** | **Management (M)** | **DOXM** | | | **0** | **1.037** | **2.074** | **4.148** | **6.222** | |
| (n) NC | 15,13 | 0,08 | 2,33 | 0,96 | 18,54 | 3,25 a | 4,00 a | 4,00 a | 6,50 b | 7,75 b | 6,50 b |
| (w) | | | | | | 3,75 a | 4,50 a | 5,50 ab | 5,25 ab | 6,75 b | 5,75 ab |
| (n) DMT | 814,07 | 23,26 | 120,04 | 42,61 | 16,37 | 23,86 a | 30,08 a | 32,62 a | 49,97 b | 56,62 b | 50,30 b |
| (w) | | | | | | 27,21 a | 33,74 ab | 41,04 ab | 40,99 ab | 45,61 b | 46,51 b |
| (n) LBT | 209,86 | 0,12 | 26,92 | 8,74 | 20,33 | 6,60 a | 9,50 a | 9,97 a | 17,86 b | 22,82 b | 20,17 b |
| (w) | | | | | | 8,21 a | 11,95 ab | 15,50 bc | 15,76 bc | 18,92 c | 17,21 bc |
| SST | 96,61 | 7,08 | 8,55 | 4,32 | 19,07 | 6,24 a | 7,94 ab | 9,76 bc | 12,59 cd | 15,04 d | 13,85 d |
| DT | 21,22 | 6,40 | 14,44 | 7,76 | 19,31 | 11,88 a | 13,23 a | 14,33 a | 16,07 a | 15,20 a | 15,85 a |
| (n) DMR | 331,02 | 41,51 | 75,42 | 22,993 | 20,27 | 14,16 a | 17,07 a | 18,56 a | 31,38 b | 37,15 b | 29,15 b |
| (w) | | | | | | 16,42 a | 19,75 ab | 22,96 ab | 23,76 ab | 26,44 ab | 26,98 b |
| (n) LBR | 27,85 | 4,65 | 9,65 | 2,16 | 29,96 | 2,58 a | 2,57 a | 3,09 a | 7,33 b | 9,08 b | 6,66 b |
| (w) | | | | | | 3,07 a | 3,70 ab | 4,68 ab | 4,29 ab | 5,62 ab | 6,23 b |
| SSR | 52,83 | 5,50 | 4,96 | 3,00 | 27,87 | 3,14 a | 4,10 a | 4,85 ab | 7,38 bc | 9,70 c | 8,13 c |
| (n) DR | 35,44 | 3,80 | 13,43 | 5,51 | 18,76 | 8,32 a | 10,16 ab | 11,88 abc | 15,73 cd | 17,03 d | 13,64 bcd |
| (w) | | | | | | 10,31 a | 11,66 a | 12,67 a | 13,02 a | 12,43 a | 13,31 a |
| DM | 116,05 | 2,62 | 8,60 | 9,31 | 18,81 | 10,24 a | 13,50 ab | 16,07 bc | 17,90 bc | 19,31 c | 20,33c |
| LB | 87,89 | 6,32 | 8,22 | 4,48 | 21,99 | 4,58 a | 7,33 ab | 9,11 bc | 11,00 cd | 13,52 d | 12,24 cd |
| SS | 8,01 | 0,10 | 0,82 | 1,58 | 26,91 | 3,10 a | 3,84 ab | 4,90 ab | 5,20 b | 5,33 b | 5,71 b |
| D | 4,75 | 0,33 | 2,71 | 3,76 | 101,46 | - | - | - | - | - | - |

Notes:
Re = residue; CV = coefficient of variation; NPK =mineral source;
* significant ($p < 0.05$); means followed by the same letters in the row do not differ by Tukey test ($p > 0.05$); n – management no soil chiseling; w – management with soil chiseling.

**Table 1.** Summary of the analysis of variance and test for number of cuts(NC), dry mass cumulative (DM), leaf blade (LB), stem and sheath (SS) and dead material (D) with IL95% (T), residue (R) between the two management systems for Marandu grass fertilized with poultry manure without dose (n) and soil chiseling (w).

With an equivalence of NPK minerals contained in a dose of 2.074 t ha$^{-1}$ of poultry manure, chemical treatment (NPK) was similar to the highest doses of manure to NC values, dry herbage mass and structural characteristics between the management systems, all except DR (**Table 1**). The 60-day periods of application at 1/3 dosage for N and K contributed to the availability of these macronutrients for Marandu grass. The results were in accordance with those reported by [12] in which the chemical fertilizer, with a dose of 100 kg $ha^{-1}$ N, was higher than the corresponding dose of 5 t $ha^{-1}$ of poultry litter.

With IL 95%, increasing the application of poultry manure doses promoted an increase in the number of cutson no soil chiseling (NCn) and with soil chiseling (NCw) with adjustments to linear models (**Figure 2A**). Being assigned mineralization of increasing amounts of macronutrients and micronutrients supplied on the highest doses of manure to Marandu grass.According to [15], the recovery of blade area index is partly dependent on soil nitrogen availability evidenced by higher emission ranging from young leaves intercept light from the sun.

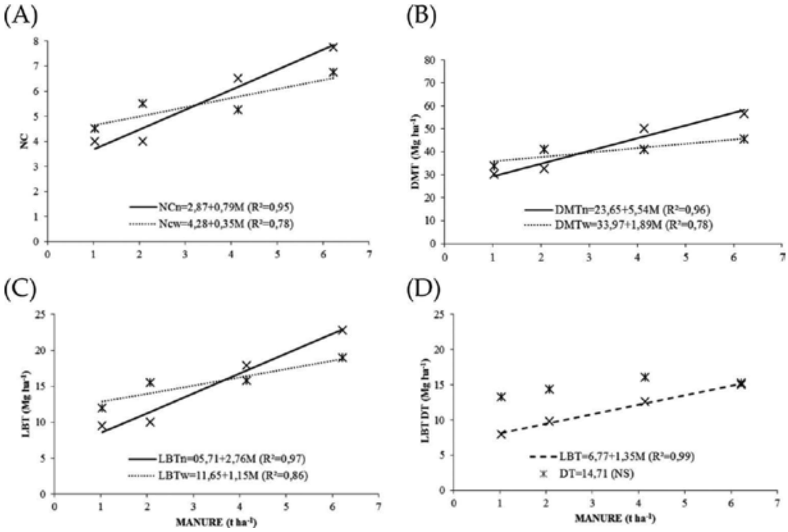

**Figure 2.** Assessment of Marandu grass fertilized with poultry manure with IL 95% for: A. number of sut (NC), B. dry mass total (DMT); C. leaf blade total (LBT) and D. stem and sheath total (SST) and dead material total (DT). Applied manure (M) and management with no soil chiseling (n) and with soil chiseling (w).

The most NCn in relation to the NCw between doses of 1.037 and 6.222 t $ha^{-1}$ is related to the physical changes caused by the plow chisel shank, which can be unbundled from the soy,next to the ground roots. This justifies the reduction in NC even with increasing doses of manure. Withfertilization lower than 3.20 t $ha^{-1}$ NCw is greater than NCn, the increased macroporosity of 0.04 m $m^{-3}$ (layer 0.0-0.20m) provided by greater microorganism action in the decomposition of organic matter in this layer even with a low manure dose applied to the surface soil with grass Marandu. The occurrence of this new recycling of nutrients by physical alteration of the soil is because of the new interaction and development of decomposing microorganisms populations of organic matter in soil [4].

Regardingdry mass without soil chiseling (DMTn) and with soil chiseling (DMTw), linear models demonstrate that the increase of manure and soil management offered favorable production conditions (**Figure 2B**). Regarding the applied dose range of 1.037-6.222 t $ha^{-1}$ of poultry manure, there is an increase of 26.53 Mg $ha^{-1}$DMTn and 11.86 Mg $ha^{-1}$DMTw. According-ing to [1], this increase is probably because of the increase in blade area and better relationship between carbon and nitrogen and new tillers and leaves, especially in higher doses.

The DMw exceeds DMTn when fertilization with manure is less than 2.82 t ha$^{-1}$. Indeed the physical changes in the soil allow the mineralization of organic nutrients for microorganisms and higher conditions for absorption even at low doses of manure. Withhigherfertilization doses than2.82 t ha$^{-1}$DMTn was greater thanground DMTw.

There was an adjustment to linear models for blade production total accumulated without soil chiseling (LBTn) and with soil chiseling (LBTw). With poultry manure doses, there was production of 14.33 Mg ha$^{-1}$ for LBTn and 5.96 Mg ha$^{-1}$ for LBTw, which corresponded to an increase of 140.37% in the management without soil chiseling (**Figure 2C**). The LBTn and LBTw increased according to manure dose increase, with similar valuestomanaging with a dose of 3.68 t ha$^{-1}$.

The increase in poultry manure doses promoted linear increase in the SST production of Marandu grass (**Figure 2D**). The increase of SST among the tested doses was 7.09 Mg ha$^{-1}$, an increase of 89.24%, caused by higher timbering due to the greater availability of nutrients coming from manure and performed by NC. In the case oftotal cumulative dead material (DT), no adjustment to the two proposed designs with an average of 14.71 Mg ha$^{-1}$.

For DMR, without soil chiseling (DMRn) and with soil chiseling (DMRw), linear models increased according to the increasing doses of poultry manure (**Figure 3A**). The DMRn and DMRw are similar forapplications of 2.44 t ha$^{-1}$, and in a dose of 6.222 Mg ha$^{-1}$DMRnand DMRw is greater than 43.55%. The results differ from [17]who found no differences between the forms of application of urea (superficial and incorporated with cultivator) in Marandu grass to the mass of the accumulated residue on the soil surface.

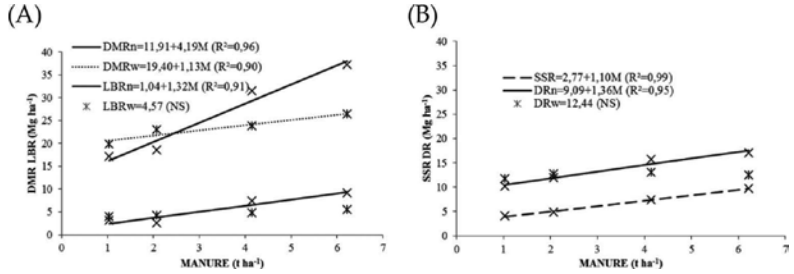

**Figure 3.** Assessment of Marandu grass fertilized with poultry manure for: A. dry residue mass (DMR) and leaf blade residue (LBR), B. stem and sheath residue (SSR) and dead material residue (DR). Applied manure (M) and managementwith no soil chiseling (n) and with soil chiseling (w).

The foliar blade residue without soil chiseling (LBRn) was linear with the increase in poultry manure dose and soil chiseling (LBRw) no adjustment to the proposed models. The greatest-production LBRn with was with 9.08 Mg ha$^{-1}$ poultry manure dose - showing an increase of 283.46% compared withthe lowest dose. Regarding thedependence on the nutrients provided by the manure, the LBRn relates to the ability of an increased light interception and a photosynthetic assimilation of carbon buildup. With frequency and cut intensity, results confirm those of[23] in the management of Marandu grass with 0.15 m waste time, increasing the accumulation of waste sheets in extracts near the soil surface, with higher IL by the leaves left over.

The SSR matter fitted the linear model with a production of 3.92 and 9.67 mg ha$^{-1}$ at a dose of 1.037 and 6.222 t ha$^{-1}$ manure, respectively (**Figure 3B**). The favorable production conditions, with increased mineralization of chicken manure nutrients coupled and with appropriate management with residue tall (0.15 m) were stimulate the generation of new tiller's by increased lighting within the swardthat will form new stems and sheath for issuing its leaves.

The mass of DR without soil chiseling (DRn) showed a linear increase with increase in fertilization with manure - an increase of 7.08 Mg ha$^{-1}$fora dose of manure. The DR is important for reducing erosion, keeping moisture in the soil surface and favoring the action of microorganisms to synthesize various enzymesthe breaking of the material for formation of new organic compounds with release of macro- and micronutrients for own grass Marandu [17].

In the production of dry mass (DM) between IL95% and waste 0.15 m showed alinear growth with an increase of poultry manure dose. Adose of 6.222 t ha$^{-1}$ led to the production of 19.69 Mg ha$^{-1}$MS, or 38.22% greater than the dose of 1.074 t ha$^{-1}$ (**Figure 4A**). For [12], the dry matter yield of Marandu grass increased linearly with the increase of poultry litter doses, with a maximum dose of 20 t ha$^{-1}$ (80 kg $P_2O_5$) and production of 21.3 Mg ha$^{-1}$ DM, which was higher by 126% compared to the dose of 5 t ha$^{-1}$. In extracted, the increased production of grass DM Marandu with application of chicken manure without interaction handling with or without chiseling the soil is very favorable because for the farmer would be a cost with the use of machines incorporating this soil residue.

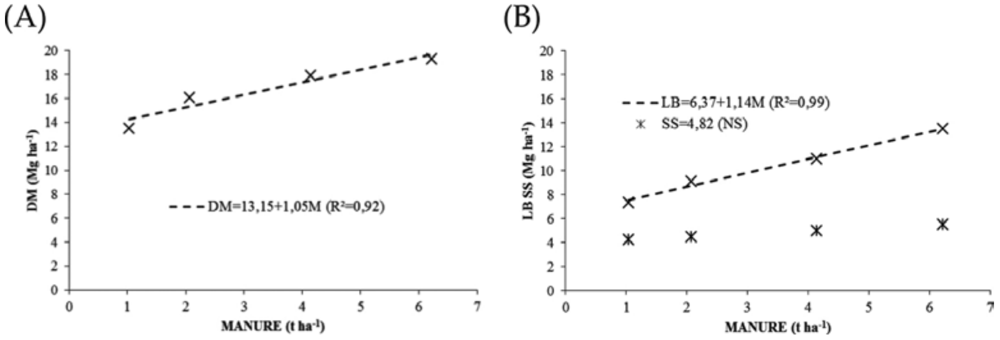

**Figure 4.** Evaluation of Marandu grass production for: A. dry mass (DM), B. leaf blade (LB) and stemsheath (SS). Applied manure (M) and management with no soil chiseling (n) and with soil chiseling (w).

For leaf blade (LF) there was linear adjustment with an increase of 5.94 Mg ha$^{-1}$ on the applied manure doses (**Figure 4B**). The increase of 78.58% representsthe increase of macronutrients and micronutrients in the soil by fertilization with poultry manure, mainly nitrogen mineralized by microorganisms in the forms of ammonium and nitrate. This encourages the increase of production and expansion of sheets in the Marandu grass. For SSthere were no adjustments to the proposed models as a result of the proposed management systems. Between IL 95% and waste 0.15m is the ideal extract where the Animas will feed with the cutting blade leaf younger and more massive, little stem and dead dead leaves of grass Marandu.

## 3. Conclusion

Poultry manure application management without soil chiseling, showed a higher cumulative production of total dry matter and residue of Marandu grass.

Matter yield and accumulated blades were higher with poultry manure application on grass Marandu.

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# Organic Fertilizers: Public Health Intricacies

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**Abstract**

Organic fertilizers are an essential source for plant nutrients and a soil conditioner in agriculture. Due to its sources and the composition of the organic inputs as well as the type, functionality and failures of the applied treatment process, the organic fertilizer may contain various amounts of infectious agents and toxic chemicals, especially the antibiotics that can be introduced to the subsequent food chain. A range of human and animal pathogens of bacterial, viral and parasitic origin have been the cause of food-borne epidemics due to unintended contamination from organic fertilizers. The use of antibiotics by humans and in animal feeds will also end up in the organic fertilizers. These antibiotics and other chemicals, depending on the sources of the organics, will enhance the likelihood of occurrence of resistant and multi-resistant strains of microorganisms in society and have been reported to cause ecotoxicological environmental effects and disruption of the ecological balance. Exposure of microorganisms to sublethal concentration of antibiotics in the organic products induces antibiotic resistance. WHO guidelines for the reuse of excreta and other organic matters identify the risk for the exposed groups to the reuse of the excreta and are applicable in the use of organic fertilizers in agriculture.

**Keywords:** organic fertilizers, food-borne illnesses, pathogens, antibiotics, ecotoxicity, WHO

## 1. Introduction

The potential health intricacies linked with organic fertilizers relate to their origin, their treatment and human exposure within a system perspective from origin to use, including products like crop type. Since organic fertilizers mainly are “faecal material/manure and urine from different animals and/or humans, with the addition of plant materials (organic solid wastes), or in special situations waste materials 1 from food or plant processing industries", the origin of the different fractions and their amounts partly defines the risk. Usually the risk is outbalanced by a wide range of benefits that the use of organic fertilizer exerts in agriculture as nutritional fertilizers and for soil conditioning. It has been further implied as more environmental friendly than the inorganic fertilizers [1] and its effect more tender on biotic components of the ecosystem without much shift in the ecological balance [2]. This is partly reflected by organisms like earthworms which may be negatively affected by inorganic fertilizers but promoted by the use of organic fertilizers and also incorporated as decomposers in aerobic composting processes [3, 4].

As this chapter deals with the public health aspects and risks involved, we define the organic materials utilized by its sources and thus relate to the following:

- Human faecal materials (also sludge from domestic treatment plants and from on-site sanitation, e.g. pit latrine emptying).
- Human urine (if separated).
- Animal manure (some risk differences depending on the species of animals/birds).
- Animal urine (often collected/spread separately, but impacted by the animal faeces).
- Other types of organic solid wastes (plant materials, domestic, industrial from organic food/fodder processing industries).

Additionally, the risk may relate to some storage-specific factors like

- Regrowth of specific bacterial pathogens or opportunistic ones (occurs when the material that, for example should be/are composted, are not well stabilized or broken down. During these circumstances, for example *Escherichia coli*, *Salmonella* sp., *Listeria* sp. and spore formers will regrow in the material if present).
- When the collected/stored/kept organic fractions or mixture thereof (see above) function as a breeding site for flies and mosquitoes that serve as vectors of parasitic diseases.
- Development of spore-forming thermophilic fungi and Actinomycetes in composting processes, where the spores can cause diseases in both immune-competent and immune-compromised individuals upon inhalation. An example of such an organism is *Aspergillus fumigatus*.

Based on source, the risk will vary to a great extent, depending on the health of the animals/humans that primarily defines the microbial concentration and partly occurrence of antibiotics and chemical components in the organic wastes (from domestic or animal sludge fractions) that may be conveyed to the agricultural sites and crops fertilized. Additional components may apply if organic industrial wastes are utilized. An indirect organic fertilization may occur through irrigation using wastewater effluent, where the nutrient load serves as an advantage. This is widely applied in developing countries [5]. However, this may result in additional inputs of antibiotics, toxic organic and inorganic compounds and pathogens. All these concepts are further deliberated in this chapter. The possibilities of recycling food-borne pathogens via agricultural crops to the final end consumers of the crops will additionally be discussed. Food-borne pathogens are especially important for animal faecal-based fertilizers used on fruits and vegetables farms meant to supply salads in restaurants. Other dynamics are residual antibiotics which are sometimes locked in the components of the organic fertilizers with attending public health implications to be further enumerated in this chapter.

## 2. Treatment and risk reduction

The concept of organic fertilization is ever worthwhile, with combined considerations of the public health intricacies that cannot be overemphasized [6]. Several alternative treatment methods can be employed to stabilize the organic fertilizers before use and at the same time reduce the concentrations of potential pathogens, thereby the risks. The efficiency of these will vary based on time, load and different external factors.

| | **Pathogens** | **Treatment option or process** | **Log reduction** | **Duration (months)** | **References** |
|---|---|---|---|---|---|
| 1 | *Escherichia coli* | Settling ponds | 2.2 | 6 | [7, 8] |
| | | Unplanted drying/dewatering beds (for pretreatment) | 4.9–5.5 | 5 | [9] |
| | | Composting (window, thermophilic) | 4 | 4 | [10] |
| | | pH elevation >9 | 2 | 1 | [11] |
| | | Anaerobic (mesophilic) | 2 | 0.67 | [12] |
| 2 | Helminths eggs (WHO, 2006) | Settling ponds | 3 | 4 | [13] |
| | | Planted dewatering drying beds (constructed wetlands) | 1.5 | 12 | [14] |
| | | Unplanted drying/dewatering beds (for pretreatment) | 0.5 | 0.3–0.6 | [15] |
| | | Composting (window, thermophilic) | 1.5–2.0 | 3 | [16] |
| | | pH elevation >9 | 3 | 6 | [17] |
| | | Anaerobic (mesophilic) | 0.5 | 0.5–1.0 | [18, 19] |
| 3 | Viruses | Settling ponds (enteroviruses) | 1.5 | 3.3 | [20] |
| | | Planted dewatering drying beds (constructed wetlands) | 99.9% with aluminium | –- | [21] |
| | | Unplanted drying/dewatering beds (for pretreatment) | 1–3 | –- | [22] |
| | | pH elevation >9 (Porcine circovirus type 2) | 2 | 1 | [23] |
| | | Anaerobic (mesophilic) (Norovirus) | 1.1–1.4 | 0.8 | [24] |

**Table 1.** Efficiency of some pathogens' reduction techniques for low-cost sludge treatment strategies.

Low-cost options for pathogen reduction and nutrient recovery from faecal sludge are of special importance to low-income countries. They include settling ponds, planted dewatering drying beds (constructed wetlands), unplanted drying/dewatering beds (for pretreatment), composting (window, thermophilic), pH elevation > 9, anaerobic (mesophilic) and simple storage. They have varying pathogen reduction efficiencies on bacteria, parasitic protozoa and viruses. **Table 1** summarizes the efficiencies of these pathogen reduction techniques with *E. coli* as an example for the bacterial group, helminth's eggs for parasites and some viral examples as stated. The figures serve as examples. Variations can be large based on prevailing local conditions.

Other methods most commonly used in developed countries to treat the sludge include incineration and pasteurization. The former one ensures a total destruction of all pathogenic organisms while the efficiency of the later one depends on time and applied temperature (normally 70°C for at least 1 h). Irradiation with $\beta$- or $\gamma$-rays is an approved method in the USA, and it reduces the pathogenic content to a high extend but is not widely used.

### 2.1. Organic waste stabilization

Organic wastes can be used as soil amendments or organic fertilizers after an effective stabilization and disinfection. Effective stabilization and disinfection of sewage sludge prior to land application are important not only to protect human health. Currently, some of the most commonly used waste stabilization methods are composting (solid state), aerobic digestion (liquid state), anaerobic digestion, lime stabilization [25, 26] and sludge drying. The aerobic and anaerobic methods of waste stabilization are among the most prominent [27]. Furthermore, there have been growing concerns about the survival of pathogenic microorganisms in sewage treatment processes, resulting in the release of antibiotic resistant microbial species to the environment [28, 29]. These are further considered below.

### 2.2. Composting

Composting is defined as the biological conversion of organic wastes, under controlled conditions, into a hygienic, humus-rich, relatively biostable product that improves land and fertilizes plants [30]. It has the combined effect of pathogen reduction while at the same time stabilizes and converts the organic wastes into product that can be easily handled [31, 32]. The type and concentration of pathogens present in sewage sludge is largely determined by a number of factors including population's state of health, presence of hospitals, abattoirs and factories processing meat [33]. Composting is one of the essential decontamination processes to reduce the load of pathogens in animal wastes. The composting efficiency to ensure inactivation of pathogens depends on allotted time and temperature. Inefficient composting leaves loads of pathogenic bacteria which may be passed on to the end consumers.

Metals such as zinc, copper, cadmium, lead, arsenic, chromium, mercury, vanadium and nickel are usually of great concern [34] when sludge from industrial effluent are used as feed stock for composting both from a health perspective and in the degradation of the productivity of land. Industrial sludge may contain elevated heavy metal concentration which makes them unsafe for garden use. Despite the fact that copper and zinc are important micronutrients, the possibility of bioaccumulation to phytotoxic or deleterious level for human consumption still makes them a concern.

Zoonoses are among the public health concerns associated with improperly sanitized organic fertilizer. Zoonotic diseases and emerging zoonoses that could be associated with organic fertilizer includes salmonellosis, entrohaemorrhagic *E. coli* (EHEC), anthrax and Newcastle diseases just to mention a few [35]. *Thermoactinomyces vulgaris* is another organism of importance. It produces heat-resistant endospores that can survive high temperature during composting. This organism is the causative agent of "farmer's lung" which is an allergic disease of the respiratory system of agricultural workers. The pathogens present in soil amendments are directly related to the organic waste source. The reduction or removal of pathogens in a compost will depend on the composting temperature and the process used [36]. This implies that improperly carried out composting leaves the organic matter poorly sanitized with the compost becoming a source of recontamination with pathogenic or parasitic organisms [37]. *E. coli, Salmonella* sp. and a few others possess advantage for regrowth in compost [38, 39]. Also, due to rich nutrient composition, contaminating *E. coli* grows very rapidly in pre-sanitized organic fertilizers [40–43] that is not properly composted or stabilized. *Salmonella* spp. equally grow in composted sewage sludge if the carbon/nitrogen ratio is >15 and the manure content 0.2 index.

| Organisms | Lethal temperature and necessary time |
|---|---|
| *Salmonella* spp. | 15–20 at 60°C; 1 h at 55°C |
| *Escherichia coli* | 15–20 at 60°C; 1 h at 55°C |
| *Entamoeba histolytica* | 68°C; time not given |
| *Taenia saginata* | 5 min at 71°C |
| *Necator americanus* | 50 min at 50°C |
| *Shigella* spp. | 1 h at 55°C |
| *Mycobacterium tuberculosis* | 20 min at 70°C |
| *Corynebacterium diphtheria* | 45 min at 55°C; 4 min at70°C |
| *Ascaris lumbricoides* eggs | 60 min at 50°C; 7 min 55°C |
| Viruses | 25 min at 70°C |

**Table 2.** Temperature-time relationship required for killing specific pathogens [35, 36, 49].

There is therefore need to ensure that the mature compost does not contain plant and human pathogens. In composting, the thermophilic temperature is the effective determinant of destroying the pathogen and the efficiency further related to the exposure time. The required time at a given temperature for efficient pathogen inactivation, according to USEPA [44] can be estimated using a time-temperature formula:

$$D = 131700000 / 10^{0.1400t}$$

where D is time in days and t is temperature (°C).

| **Organisms** | **US** | | **New Zealand** | **UK** | **New South Wales** | **EU** |
|---|---|---|---|---|---|---|
| | **Class A** | **Class B** | **Class A** | | **Class A** | |
| *Escherichia coli* | N/A | | <100 MPN/g | 1000 CFU/g | N/A | 0/50 g |
| Faecal coliforms | <1000 MPN/g | <2,000,000 MPN/g | N/A | | <1000 MPN/g | |
| *Salmonella* spp. | <3 MPN/4 g total solids | | <1/25 g | Nil | 0/50 g | $<10^3$ MPN/ g |
| Enteric viruses | <1 PFU/4 g | | <1 PFU/4 g | | <1 PFU/4 g | |
| Helminth ova | <1/4 g | | 1/4 g | | <1/4 g | |

MPCN, most probable cytophatic number; MPN, most probable number; PFU, plaque-forming unit.

**Table 3.** Standards for maximum concentrations of pathogens in biosolids and composts used as organic fertilizers [49, 52, 53].

In a properly ventilated composting pile, the temperature usually reaches between 55 and 68°C. This temperature level can last for a few days to months depending on the size of the system and the composition of the ingredients [45–47] and is the determinant for the sanitization effectiveness. The average time required for killing specific pathogen is exemplified below (**Table 2**). *Salmonella* spp. and *E. coli* have been known as pathogen indicator bacteria in organic fertilizer, supplemented with soil-transmitted helminths [48] and enteric viruses when a broader spectrum of organisms needs to be assessed. Several national and international standards/guidelines have been established to ensure public health safety when using these organic fertilizers (**Table 3**). Due to high heat resistance of some bacteriophages, they have been suggested as an indicator of properly sterilized compost [35].

### 2.3. Aerobic digestion

This occurs in engineered ecosystems where biomass consisting of a mixed microbial community and other solids are constantly maintained in a suspension in an aerobic basin supported by mixing [50]. This is usually used in stabilizing sewage and wastewater, producing high-quality treated effluent through the metabolic reactions of the microbial community [51]. The sanitation efficiency of this system depends largely on time, temperature and loading rates [28, 50]. This process still yields poorly stabilized organic matter with a fluid product, having little or no volume reduction and pathogen reduction efficiency is usually low [28].

Moreover, using them as organic fertilizers in an inefficiently sanitized stage can further result in direct microbial contamination of surface water or via runoff from lands amended with such organic waste [28] in addition to their direct exposure effects and effects through crops. Most aerobic sewage sludge treatment plants operate at mesophilic temperatures (30–35°C). Within this temperature range, the stabilization processes are inefficient in the removal of viruses, bacteria and Parasite's eggs [28].

### 2.4. Anaerobic digestion

Anaerobic digestion involves the breakdown of complex organic material into simple monomerics or fraction and production of biogas (bioenergy) in closed system through the activity of anaerobic microorganisms [54]. Anaerobic digestion can be carried out either at mesophilic (30–38°C) or at thermophilic (50–55°C) temperatures. Compared to composting, there is lesser heat generation during anaerobic decomposition, which reduces the sanitizing effect of the process on organic waste [37]. Digesting organics at high temperatures reduces the time required for bacterial inactivation, which eventually results in faster bacterial kill during thermophilic digestion compared to mesophilic [55]. Bacterial spores including *Bacillus cereus* and *Clostridium perfringens* are normally resistant to temperature inactivation at both mesophilic and thermophilic ranges [55–57]. Chauret et al. [58] also noted the resistance of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts to anaerobic sludge digestion. This finding is of importance since *Cryptosporidium* sp. oocysts can persist in soil amended with sludge for at least 30 days [59].

### 2.5. Lime (alkaline) stabilization

Lime stabilization is a preferred alternative compared to anaerobic and aerobic stabilization processes due to its cost efficiency and enhanced sanitizing effect [25, 60]. It effectively reduces the concentration of pathogens in sludge (**Table 4**), heavy metal availability and enhances its agricultural uses [25]. Free calcium ions resulting from the lime solution form complexes with odorous sulphur species and organic mercaptans; moreover, the high pH precipitates metals from the sludge thereby reducing their solubility and availability. Alkaline stabilization involves the addition of lime slurry in the form of $Ca(OH)_2$ or CaO to the liquid sludge in order to raise its pH to about 12 or higher [60]. Apart from the high pH, the addition of quicklime to the liquid sludge can result in thermophilic temperature (up till 70°C) which inactivates the viruses, bacteria and other microorganisms [61,62]. In a study by Farzadkia and Bazrafshan [25], addition of lime slurry to sewage sludge resulted in a reduction of faecal coliforms with more than 99.99% in stabilized sludge. Arthurson [26] noted that there is a need for further investigation on the potential of alkaline stabilization methods since this process is an effective sewage sludge sanitization method but some contradictory results exist.

| Type of treatment | Viruses | Bacteria | Parasite egg |
|---|---|---|---|
| **Pasteurization** (heat, 30 min at 70°C) | Good | Good | Good |
| **Irradiation** (ionizing radiation, 300 rad) | Poor | Good | Good |
| **Lime treatment** | | | |
| Slacked lime (high pH) | Good | Good | Good |
| Quick lime (High pH; 80°C) | Good | Good | Good |

| Type of treatment | Viruses | Bacteria | Parasite egg |
|---|---|---|---|
| **Anaerobic digestion** | | | |
| Mesophilic (30–35°C) | Poor | Poor | Poor |
| Thermophilic (50–55°C) | Good | Good | Good |
| **Aerobic digestion** | | | |
| Mesophilic (up to 20°C) | Poor | Poor | Poor |
| Thermophilic (50–55°C) | Good | Good | Good |
| Compost (50–60°C) | – | Good | Good |

**Table 4.** Pathogen-reduction performance of the different treatments of sludge [28, 63, 64].

## 3. Potential human pathogens in organic fertilizers from faecal materials and implications

Pathogen-free organic fertilizer can be developed and microbiological safety assured for the reuse of sludge and manure. Various factors affecting the survival of pathogens in composting include time, temperature, pH, aerobic/anaerobic, biological activity, UV or irradiation, moisture, combination and chemical effects (e.g. ammonia). These factors are considered with regard to some pathogens discussed in Sections 3.1 and 3.2.

The inherent pathogens in an organic fertilizer depend on the animal source of the faecal materials used. When considering heat-dependent anoxic degradation of product for manure from dairy cattle, studies have shown the rate of kill of *E. coli* O157:H7 at 55°C to be 3 logs per 30 min and 4 logs per 100 min [70]. **Table 5** show the heat-based inactivation of some pathogens with values of decimal reduction time (*D*) at test temperature (*T*) Thermal death times for *Salmonella* to achieve reduction of 9 log has been reported to be 40 min at 55°C. Some pathogens can survive for longer periods of time in compost especially when they are located on the surface part of the compost pile where the heat effects may be inefficient. They also survive better in mesophilic composting (<45°C) than at elevated temperature. Moisture availability in biowaste compost (denoted by water activity, $a_w$) is also an important determinant for the survival time of many pathogens.

| | Pathogens | $T$ (°C) | $D_T$ (*s*) | References |
|---|---|---|---|---|
| **Protozoan parasites** | Soil-transmitted helminths (STHs) | 20–30°C | Several | [69] |
| **Bacteria** | *Campylobacter* sp. | 55.4–61.2 | 89–10.3 | [70] |
| | *Escherichia coli* | 55–70 | 1281.6–1.86 | [71] |
| | *Escherichia coli* O157:H7 | 55 | 1500 | [72] |
| | *Listeria* sp. | 55–70 | 3370.14–7.56 | [73] |
| | *Salmonella* sp. | 55–70 | 3370.14–7.56 | [74] |

| | Pathogens | *T* (°C) | $D_T$ (*s*) | References |
|---|---|---|---|---|
| | | 55 | 720 | [75] |
| **Virus** | Hepatitis A | 80 | 73.2–733.2 | [76] |
| | | 90 | 13.2–180 | |
| | Rotavirus | ~20°C | 20–100 | |
| | Coliphage | 51 | 1860 | [75] |
| **Fungi** | *Botrytis cinerea* | 40–48 | 1800–36 | [77] |
| | *Monilinia fructigena* | 39–45 | 1302–150 | [77] |
| | *Monascus ruber* | 70 | 2238–4379 | [78] |

**Table 5.** Heat inactivation: values of decimal reduction time (*D*) at test temperature (*T*) (Adapted from Romdhana [68]).

### 3.1. Soil-transmitted helminths

Both human and animal waste and wastewater may contain different soil-transmitted helminths (STHs). These are among the most resistant microorganisms and will develop in soils or poorly treated biosolids from the non-invasive stages that are excreted to an infective one. Thus, when poorly handled, soil and crops get contaminated with eggs or larvae of STHs, which in turn will be transmitted orally through crops or due to accidental ingestion (e.g. *Ascaris* sp.) or penetrate bare skin (hookworms). Due to their resistance to environmental stress, helminth parasite eggs are widely used as hygiene indicators. STHs are resistant to sublethal composting temperatures and they require longer time at alkaline pH (months at pH 9–10, but much more rapid at pH 11–12) to effect appreciable die-off. A report by Jensen and Vrsle [65] showed that it would take a period of 117 days to achieve 99% die-off of an *Ascaris suum* eggs when placed on human excreta with pH levels between 9.4 and 11.6. When temperatures of above 50°C are reached, a rapid die-off occurs. Thus, a properly composted night soil with crop residues can destroy the parasitic infective stages efficiently.

When assessing the effectiveness of composting, *A. suum* eggs from pigs may be utilized as a model for the survival of human parasitic roundworm, *A. lumbricoides* [66,67].

### 3.2. Zoonotic organisms in waste dung as components of organic fertilizer

Chicken litters and pig dungs are rich in nutrients and are valuable animal wastes as organic fertilizer. However, chicken litter exemplify one organic fertilizer that may contain important human pathogens like *Salmonella* sp., *Campylobacter jejuni* and *Listeria monocytogenes*. If not properly sanitized these pathogens can easily get deposited on crop/plants, with transmission to consumers with, for example, fruits and vegetables [79,80]. Several human pathogens have been reported in organic fertilizer and may be conveyed to human, while other may function as animal or plant pathogens [81]. *L. monocytogenes* is a typical example of a pathogen easily conveyed via food crop.

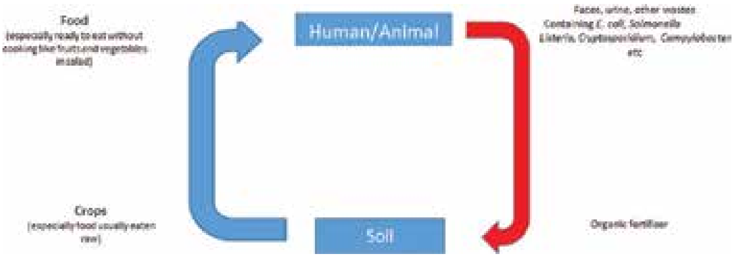

**Figure 1.** The cycling of potential pathogens in faecal materials for making organic fertilizer.

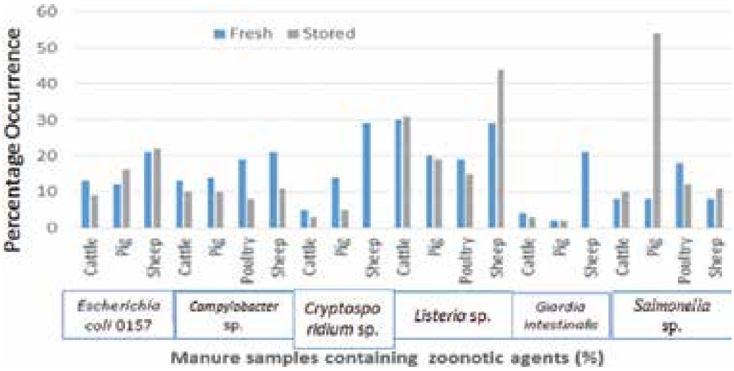

**Figure 2.** Prevalence and concentration of zoonotic pathogens observed in British livestock manure (modified based on Ref. [84]).

Boulter et al. [82] reported that *Salmonella* sp. was observed among several other Gram negative bacterial potential pathogens in green compost for organic fertilizer. Even some Gram positive bacteria may occur, for example *Bacillus cereus* which is associated mainly with food poisoning and as a cause of serious and potentially fatal non-gastrointestinal-tract infections [83]. This resembles contamination of the farmland through poorly formulated organic fertilizers that circulates egested pathogens from human or animal back to them or another is pictorial as a cycle (**Figure 1**). Pathogens from wastes like *E. coli, Salmonella* sp., *Listeria* sp., *Cryptosporidium* sp. and *Campylobacter* sp. among others are usually conveyed to the farmland through poorly composted organic fertilizers or through contaminated irrigation water. **Figure 2** illustrates the occurrence of a number of different zoonotic pathogens found in manure [84].

Treated wastewater effluents contain nutrients (nitrogen, phosphorus and potassium), inorganic matter (dissolved minerals) and other chemicals which can complement the enrichment of the farmland in enhancing plants' growth. Enhanced concentrations of different excreted pathogens may also occur in wastewater being used for irrigation. Most of these pathogens are of known aetiologies of various infection (exemplified in **Table 6**). This is likely more prevalent in developing countries where wastewater for irrigation is not pretreated and disease prevalence may be higher. Intestinal nematodes released with the irrigated water are of special concern. The risk becomes higher in a farmland in which organic fertilizer is already in use, as it enriches the environment for the pathogens to thrive.

**Table 6** gives the summary of potential human pathogens found in wastewater effluent and sewage sludge as components that are used for organic fertilizers. Some of these pathogens have been reported in zoonotic infection as discussed hereafter.

| **Pathogens** | | **Potential disease (s) /Symptoms** |
|---|---|---|
| Gram positive bacteria | *Staphylococcus* sp. | Osteomyelitis, furuncles, carbuncles, impetigo, wound infections, food poisoning |
| | *Streptococcus* sp. | Skin infection, otitis media, respiratory infection |
| | *Clostridium perfringens* | Gas gangrene, gastroenteritis (food poisoning) |
| | *Clostridium botulinum* | Botulism |
| | *Bacillus anthracis* | Anthrax |
| Z-N positive bacteria | *Mycobacterium* spp. | Leprosy, tuberculosis |
| Gram negative bacteria | *Salmonella* spp. | Gastroenteritis, typhoid fever |
| | *Shigella* spp. | Bacillary dysentery |
| | *Escherichia coli* (enteropathogenic strains) | Gastroenteritis |
| | *Pseudomonas aeruginosa* | Otitis externa, skin and wound infections (opportunistic pathogen) |
| | *Yersinia enterocolitica* | Gastroenteritis |
| | *Campylobacter jejuni* | Campylobacteriosis: diarrhoea, fever, nausea, vomiting, abdominal pain, headache |
| | *Listeria monocytogenes* | Listeriosis |
| | *Vibrio cholera* | Cholera |
| | *V. parahaemolyticus* | Acute gastroenteritis |
| Viruses | *Coronavirus HKU1, Klassevirus* and *Cosavirus* | Severe acute respiratory, gastrointestinal and hepatic illnesses |
| | *Norovirus* | Gastroenteritis |
| | Enteric viruses including *Adenovirus (AdV), hepatitis A virus (HAV) and Rotavirus (RV)* | Enteric infection |
| | *Polioviruses* | Poliomyelitis |
| Parasites | Soil-transmitted helminths (*Ascaris* | Helminthiases |

| Pathogens | | Potential disease (s) /Symptoms |
|---|---|---|
| | *lumbricoides*, whipworm and hookworm) | |
| | *Giardia* sp. | Giardiasis |
| | *Cyclospora* sp. | Cyclosporiasis |
| | *Cryptosporidium* sp. | Cryptosporidiosis |

**Table 6.** Potential human pathogens identified in municipal wastewater and sewage sludge being used for fertilizing farmland [26].

### 3.3. Pathogens from organic fertilizers into crops and vegetables

Pathogen can be passed on to crop plants through direct contact, through deposition on the surface or in splash contamination. Human pathogens may also get internalized in plants from fertilizers in the soil, where the probability for internalization may be increased by mechanical damage. The pathogens may migrate within the plants' tissues. **Figure 3** gives a pictorial illustration of the process of internalization of pathogens from organic fertilizers into crops and vegetables. Pathogens in the organic fertilizers are deposited on the surface of the crops and/or vegetables. The pathogens in subsurface parts of the crops are difficult to be removed or disinfected. The potential for enteric pathogens to be absorbed by roots has been considered [85].

Enteric pathogens may further enter plant tissues through both natural apertures (stomata, lateral junctions of roots) and damaged (wounds, cut surfaces) tissue (**Figure 3**). Researchers [86–88] have demonstrated the internalization of *E. coli* from soil into hypocotyl of spinach, lettuce and cabbage using different bioluminescent labels.

Regrowth contribute to high concentration of pathogens. Either *E. coli* O157:H7 or *Salmonella* may Multiply, get internalized into the tissues of raddish [89–91] and mung bean [87]. Surface

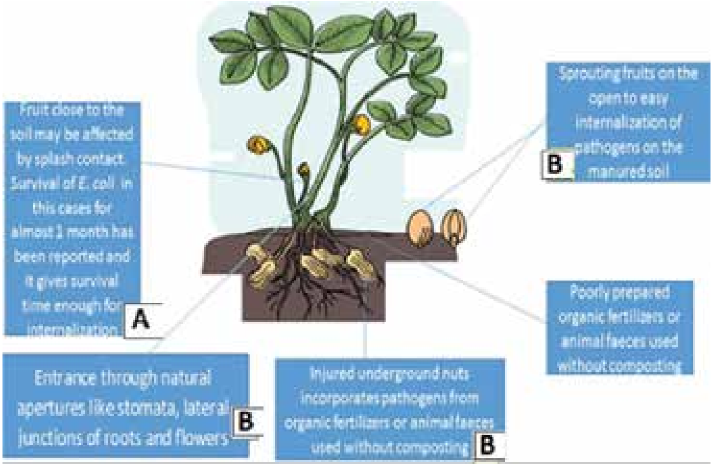

**Figure 3.** Pathogens' deposition (A) and internalization (B) in crop on an organically fertilized farm.

sterilization will then have little effect as the pathogens are already within the tissues. Similar experiment involving *Salmonella* and alfalfa seeds was demonstrated by Gandhi et al. [92] in which the bacterium penetrated into the hypocotyls.

### 3.4. Fate of pathogens in consumers of the plants products

Pathogens associated with plant products can be conveyed to consumers through crops mainly eaten raw from contaminated organic fertilizers. Surface washing will reduce surface-associated pathogens [48, 93]. Fruit- and vegetable-related outbreaks have been reported globally, affecting from a few infected person to causing major epidemics [94–96]. One recent outbreak was in 2011, affecting several countries in Europe involving ingestion of *E. coli* O157 from fruits and vegetables [94]. About 46 million food-related cases with 400,000 hospitalization and 3000 deaths were summarized by Scallan et al. [95,96]. The increasing numbers of immunocompromised individuals globally will enhance the effects of pathogens from contaminated fruits and vegetables. The risk to public health exists, and it is imperative for each country to remodel the agriculture extension to address this challenge.

The original source before food contamination differs. Some pathogens like norovirus and *Salmonella* sp. serotype *Typhi* are sustained in human reservoirs, but several others are sustained in animal reservoir. Surface contamination and/or internalization of pathogens in fruits and vegetable may not be the major pathway for contamination of food supply. However, the outbreaks via this channel may have high public health significance [97, 98]. An estimated, 131 produce-related food-borne outbreaks were reported in the USA between 1996 and 2010. A large *E. coli* O157:H7 outbreak of food-related illness involving vegetable occurred in 1996 in Japan in which >11,000 individuals were reported severely ill. Several deaths occurred among young school children [98].

In England, 60 outbreaks of food-related illnesses from fruit- and vegetable-related infections were reported during 7 years, beginning from 1992. Contamination with human pathogens on farms can be attributed not only to faeces from human, and manure from farm and wild animal but also to poor environmental waste handling [99]. A report of an *E. coli* outbreak confirmed the contributions of water, manure from cattle dung and wild pig faeces, etc. towards contamination on spinach [100]. The same strains of pathogens found in spinach fields have also been found internalized in the spinach. This informed the initiation of safety plan against *E. coli* O157 infection through pathogens in vegetables [101].

When fruits harbour internalized pathogens, they pose an enhanced risk especially when used for sprouted seeds and unpasteurized fruit juices [102–104]. This is more important for internalized fruits as surface contaminant are usually steam washed away in processing companies.

### 3.5. Exposure pathway and health risks when reusing contaminated organic materials as agricultural fertilizers

Consumption of crops, including fodder crops, serves as the most common transmission pathway to chemical and pathogens from biosolids used as fertilizer. Investigations have also been performed related to contamination of crops used for medicinal products and supplements [105]. The direct exposure of agricultural workers is also significant and relates to different transmission routes, as well as the frequency and duration of exposure. Farmworker exposure has been examined [106, 107], including the impact on family members [108]. Direct exposure relates to the level of manual work and mechanization. The risk further relates to the type of fertilizer, from human and animal urine to untreated or treated wastewater, manure or human excreta. A special situation is when stored organic fractions or mixture thereof function as breeding site for fly/mosquito vectors of parasitic disease or attract vermin's that can act as carriers of pathogens. This is for example considered in the USEPA guidelines [109].

In addition to microbiological contaminants, organic fertilizers may, especially when sludge constitute parts of the input material, contain metals and other chemicals that may affect the receiving soils as well as be of relevance for occupational exposures. To appropriately assess human risk from chemicals found in biosolids, the form of the chemicals, and their fate, transport and bioavailability needs to be known, for example, arsenic, lead, mercury, antibiotics.

Jerkins et al. [110] reported two studies that were suggestive that compost workers were affected by fungi. One cross-sectional study in Germany reported a significant increase in symptoms from lungs and airways as well as dermal effects and related these to increased exposure to fungi and Actinomycetes. The other was a prospective study in multiple US cities where significant increases in eye and skin irritation occurred and fungal colonization was documented but no serological evidence of other infections was reported. Indirect evidences were presented by Harrison and Oakes [111] that reported 39 incidence of illness among neighbours to biosolids application sites. The evidences were however not appropriately backed up.

The infection risks have been estimated using quantitative microbial risk assessment (QMRA) when urine or human faeces are used for garden fertilization [112]. A study in South Africa reported enhanced infection risks of *Salmonella* sp. and *Ascaris* sp. associated with spinach or carrots fertilized with human excreta [113]. An assessment of the health risk associated with daily consumption of vegetables (lettuce, 11.5 g) fertilized with compost was done by Watanabe et al. [114]. If the concentration of pathogenic virus in compost, for example is $10^{-1}$–$10^{2}$ PFU/g of lettuce, the risk would still be higher than the WHO tolerable annual infection risks.

## 4. Residual antibiotics (AB) and antibiotic-resistance genes (ARGs) in organic fertilizers

Veterinary drugs are introduced into the environment through a number of routes like direct applications as in aquaculture, application of manure and/or slurry to agricultural fields and through disposal of wastes during the production processes. An investigation also indicate a link between the proximity of swine farms exposed to these antibiotics through contact with animal feed and development of antibiotic resistance in bacteria among small wild animal accessing into barns and feed storage areas [115]. The presence of drugs and their metabolites in the environment have frequently been reported. For instance, low levels (<1 μg/L) of antibiotic residues have been detected in surface water samples in both Germany and the USA collected from sites considered susceptible to contamination [116]. The residual antibiotics in organic fertilizers using animal manure from large-scale livestock farms (mainly including slurry and dung from pigs, cows and chicken) have been investigated with their presence confirmed [117].

The residual antibiotics found in organic fertilizers may emanate from administration to humans either as prophylaxis or for therapeutic purposes. They are also being used as components of animal feeds to promote growth, to treat or prevent diseases of farm animals and sometimes against diseases in plants [118–126]. Hence, tetracycline concentration, for example, in liquid organic fertilizer could be as high as 20 mg $kg^{-1}$ [127]. So, the use of manures as organic fertilizers on farmland containing antibiotics is fast becoming a serious environmental issue of concern [128]. Up to 200,000 tons of antibiotics are both used per annum by humans and administered to farm animals [129]. About 70% are consumed as growth promoters [131, 131], irrespective of the 1998 EU embargo [132, 133]. Massive utilization of antibiotics in veterinary practices remains in China, Russia, Europe and the USA [134, 135] where the largest producer and user of antibiotic is China. Tens of thousands tons of penicillin and tetracycline derivatives were produced in early 2000s [136]. The prescription in China is/was equally over double the amount in the Americas [137].

Therefore, antibiotics that end up in manure or fertilizers might have come from any of the following:

**a.** Feed additives (especially in fish farming)

**b.** Human and veterinary drugs

**c.** Effluents from pharmaceutical industries [138, 139]

Pharmaceuticals are excreted to the environment through the excreta (either mainly through the urine or through the faeces) from humans or animals in a semi-digested active form or as derivatives and end up in wastewater or biosolid. Some of them may be retained in the final organic fertilizers (biosolids), as well as in wastewater and reach surface water and sediments [127, 140, 141]. All kinds of manures, wastewater sludge and excreta from human are vehicles for carrying residual antibiotics in the environment [142–146]. Zhang et al. [146, 147] reported that residual antibiotics were highest in pig manure, followed by chicken manure and cow manure in that order, but this is mainly a reflection of the local situation. The concentration is, as expected, higher from large-scale agriculture farm than from subsistence farm. Rainfall will naturally add to the run-off of these from agricultural land to surface and groundwater. It also enhances the potential for distribution to other biomes with ecotoxic effects. They also get lodged in the soil organisms like earthworms, soil arthropods, fungi and bacteria.

Like internalized pathogens, the potential high uptake of antibiotics by vegetables fertilized with biosolids globally is of enhanced public health concern [148, 149].

Future attention is needed in the issue of bioaccumulation in vegetable with residual antibiotic because

**a.** Vegetable rapidly take up harmful substance(s) during short growth cycle. The acute and long-term cytotoxicity effects on the consumers are unclear.

**b.** Vegetables, either leafy or root, are often consumed raw. Thermal effect in cooking may affect advantageous sublimation of some harmful compounds, but this is not guaranteed and

**c.** They are stored for short-term and consumed fresh, bringing about timely delivery of residual bioaccumulated antibiotics and other pollutants.

Therefore, they bring about any of the following environmental impacts:

**a.** Emergence of bacterial resistance through long-time exposure to sublethal concentration of the residual antibiotics, genetic variation resulting from innate adaptative drives of the bacteria and also provide a pseudo-biofilm environment for exchange of antibiotic-resistant genes (ARGs) [150–152]. It is an established fact that exposure to low-level or sublethal or sub-minimum inhibitory concentration (sub-MIC) of antibiotic drug has effects on the bacterial physiology and its genetic or phenotypic variability, and the potentials of antibiotics to function as signalling molecules. All these factors contribute to prompt emergence and spread of antibiotic-resistant bacteria among humans and animals.

Laboratory-based methods have been developed to determine the effect of exposing bacteria to sublethal concentrations (sub-MIC) of antibiotics. This has affirmed the implication of the antibiotics in environment, including those in organic fertilizers, on the emergence of antibiotic resistance. These kinds of research also encompass the *in vitro* pharmaco dynamic models, concentration and exposure time of susceptible bacteria to selected conventional antibiotics before the emergence of resistance. The concentration variations to be employed for such studies will be informed by the concentration of the extracted antibiotics in the organic fertilizers.

**b.** As it is a generally accepted fact that all drugs, including antibiotics have their side effect. It is only advantageous if taken to remove a more serious infection. Continuous exposure of farmers to residual antibiotics in dust [127] from soil fertilized with organic fertilizer exposes them to risk associated with accumulative effect of the gradual exposure.

**c.** Ecotoxic effects on other biotic components of the environment.

## 5. Guidelines for reuse of human and animal waste products as organic fertilizers

The WHO operational monitoring guidelines for the reuse of wastewater, excreta and greywater to fertilize crop strictly advocate certain validation requirements, operation monitoring parameter and technical measures, and verification monitoring are as stated in **Table 7** for safe reuse of waste. WHO guidelines [48] exemplify the die-off efficiency with a temperature of 50°C for at least 1 week before compost or ecohumus is considered safe for reuse. If this temperature is not achieved, a longer composting/storage time has been advocated by WHO. One to two years of storage is recommended for systems that generate ecohumus for proper removal of bacterial pathogens and appreciable reduction of viral and parasitic protozoa. WHO [48] identified the risk on the exposed groups to the reuse of the excreta and wastewater, and recommended health protective measures. The guidelines also include standards for chemical in fish and vegetables. According to the guideline, ≤1 helminth's eggs (arithmetic mean number) per litre or per gram total solid applies for excreta to be used on edible products and organic fertilizers to which agriculture workers would be exposed. The guidelines also contain threshold values for bacterial pathogens (based on $\leq 10^4$–$\leq 10^5$ CFU *E. coli* per 100 mL or g total solid) and for trematode eggs (absent) in aquaculture.

| **Control measures** | **Validation requirements** | **Operation monitoring parameter and technical measures** | **Verification monitoring** |
|---|---|---|---|
| Fertilizer handling | Reduce direct contact with insufficiently treated material and environmental contamination | Wearing gloves<br>Washing of hands and equipment used | Informed farmers using excreta<br>special equipment available |
| Fertilized field | Time needed for pathogen die-off under different climatic conditions and withholding time between waste application and crop harvest to ensure minimal contamination | Working excreta into the ground, information and signs avoiding overfertilization | Analyse plants' contamination |
| Fertilized crop-produce restriction | Survey of product consumers to identify species always eaten after thorough cooking | Harvesting and transport practices | Testing of excreta/greywater to ensure that it meets WHO microbial reduction targets |
| | Analysis of marketability of different species/crops Economic viability of growing products not for human consumption. Harvesting, transport and trade consumption | Withholding time between fertilization and harvest Types of crops grown in excreta use areas crops cooked before eating | Proper preparation and cooking of food products Domestic and food hygiene |
| | Contamination of hands, kitchen utensils, food | | Hand washing |

**Table 7.** Validation requirements, operation mornitoring parameter and technical measures, and verification monitoring for reuse in fertilization (adapted from WHO [48]).

## 6. Conclusions and research gaps

The WHO guidelines Vol 2 (Wastewater Use in Agriculture) and Vol 4 (Excreta and Greywater Use in Agriculture) [48, 153] form an evidence base and referral point for risk management strategies and risk mitigation. As such they are applicable for the planning and implementation of health aspects, especially related to pathogens, of use schemes for organic fertilizers, whether defined as biosolids, faecal sludge, manure, urine or different mixtures of these and with plant materials. The guidelines are building on microbial risk assessment (MRA) with identification and characterization of hazards, exposure assessment and risk characterization and management that can be applied with different levels of sophistication. This can be part of a scenario or model approach or built into a management approach. With modifications but with its different components it formed the base for "Human Health Risk Assessments of Pathogens in Land-applied Biosolids" [154] in the USA, with a model and scenario-based approach. It further forms a base for the simplified risk management approaches within the WHO sanitation safety plans (SSPs) [155].

For organic fertilizers in agriculture, the major differences in the hazard identification and characterization are locally specific, partly driven by the sources of the organic fertilizers used and partly reflecting the regional and socio-economic situations. In this context, the risk may partly be regarded higher in transient and developing global economies. It further relates to the treatment and application barriers, where regulations and enforcement against most often will be more stringent in developed regions and economies [156–158].

The WHO guidelines are further framed around a risk-reduction strategy accounting for a multiple risk barrier approach, which embrace both technical and handling barriers. This is applied to ensure a reduced exposure risk, which in relation to the application of biosolid, faecal sludge or manure etc. should reduce the risks in relation to both the crop and soil, to agricultural workers, communities or due to secondary run-off and impact. The technical reduction barriers here naturally play a fundamental role where different treatment methods have different efficiency. In the USA, a pathogen equivalency committee [159] should be able to assess new methods to ensure a high level of safety. Safety is also ensured in the way that the application is made in the agricultural fields, the crop selection and the impact of environmental factors (e.g. sunlight, temperature etc) on pathogen die-off. Again, large differences occur locally, seasonally and between different economic regions and social strata.

Even if the different risks and the level of risk can be identified, the epidemiological evidences are still poor for different types of organic fertilizers and especially if we should value this transmission route in relation to others. This further relates to different global regions and socio-economic conditions. The study outcomes from specified investigations in the USA, in EU or in Australia, for example, cannot be directly transferred to the conditions and situations on other continents and vice versa.

Low-cost treatment and handling approaches applicable for developing regions need further attention, where seasonal variations also need to be further accounted for.

The evidence base related to microbial die-off under different field conditions need to be substantially broadened and performed studies so far systematized in relation to effect.

The relationship between animal waste, water and environmental quality and human health have been addressed from a zoonotic livestock perspective, including management practices, exposure interventions and risk analysis but need much further attention related to organic fertilizers [160].

Crop contamination is documented but the relative impact between pre-harvest contamination by organic fertilizers and irrigation water on the one hand and post-harvest handling and storage contamination on the other needs to be further addressed. The specific situation with the potential impact of internalization and uptake of pathogens as compared to deposition on outer surfaces need much more attention and documentation, before long-term handling and management practices can be issued and related to modes of application.

Also, the specific situation, partly addressed in this chapter with uptake of antibiotics (and other organic contaminants) as well as the impact of use of these in livestock and among humans and the further fate in agricultural fields need to be addressed. Linked to this is also the large problem complex with the occurrence, transmission and impact of antibiotic-resistant bacteria especially, but also including other antimicrobial drugs.

At the current stage, the authors believe and conclude that the benefits with human- and animal-based organic fertilizers in the field far outmaster the potential negative impacts. However, we also firmly believe that a broadened evidence base and application of this in a risk-management perspective and framework will further enhance the positive benefits and counteract negative impact.

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This book, Organic Fertilizers - From Basic Concepts to Applied Outcomes, is intended to provide an overview of emerging researchable issues related to the use of organic fertilizers that highlight recent research activities in applied organic fertilizers toward a sustainable agriculture and environment. We aimed to compile information from a diversity of sources into a single volume to give some real examples extending the concepts in organic fertilizers that may stimulate new research ideas and trends in the relevant fields.

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