Earthworms
The Ecological Engineers of Soil

Edited by Sajal Ray

EARTHWORMS - THE ECOLOGICAL ENGINEERS OF SOIL

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Meet the editor

Sajal Ray received his MSc and MPhil degrees from the Calcutta University in Zoology and Environmental Science, respectively, and his PhD degree from the Jadavpur University. His thesis reports immunotoxicity of pesticide in an economically important snail of India. As an awardee of Fogarty Visiting Fellowship, Dr. Ray carried out his postdoctoral research in Cardiac Pathology at the National Institutes of Health, USA. His research interest is studying immunological responses of mollusc, sponge, crab and earthworms exposed to pollutants. His team is engaged in understanding the evolutionary mechanism of immunity in phylogeny. He presented his research in conferences including World Congress of Malacology, Washington, DC. He is a Professor of Zoology at the Calcutta University and teaches Zoology for nearly 30 years at the postgraduate level.

Contents

Preface

Earthworms are an important biotic component of agricultural soil. They bear an immense significance in its agricultural and ecological processes. Their relative abundance and general physiological status serve as indicators of the health of various types of soils. These oligochaete annelids are effectively utilized in vermicomposting technology throughout the world. They greatly influence the fertility, porosity and other characteristics of agricultural soil. Their maneuvering potential of soil characters earned their name and fame as "ecological engineers". These moist-bodied tubular organisms greatly influence the characteristics of soil by effective composting and controlling its microbial and physicochemical features. Currently, the alarming level of contamination of soil by chemical toxins has been indicated as a serious ecotoxicological threat for both soil and its inhabitants. Intimate association of earthworms with contaminated soil renders these species to be the victims of acute physiological stress and early mortality. Pesticides, heavy metals, nanotoxins, pharmaceutical wastes and other emerging toxins of soil affect these worms both at organismal and molecular levels. Toxin-induced shift of genetic and immunological makeup of earthworm has been identified as another biological risk which demands a special scientific attention. Toxin-induced alteration of physicochemical profile of soil might affect the microbial environment—which, according to some workers, may act as an influencing factor of the general immunological status of earthworms.

However, these engineers of soil not only influence the soil properties, they also affect the chance of resource availability to other organisms. According to the engineering activity, they are broadly classified as extended phenotype and accidental engineers. Both of these types generate "ecological hot spots", a unique microbial environment characteristic to a special soil type. Extended phenotype engineers exhibit a high potential to generate ecological heterogeneity due to their site-specific activity. On the other hand, the mobile accidental engineers are involved in uniform distribution of soil resource within a broad range of soil. These two nonsimilar strategies of ecosystem engineering help the ecologists and system biologists to understand the role of individual species in ecosystem functioning in a better way. Physiological response of earthworm toward environmental stressors and toxins has been of interest to many scientific workers. Coelomocyte-mediated immunological reactivity has been established as a marker of soil contamination. Thus, they are often considered as an effective tool to monitor the physicochemical quality of soil. For the last two decades, a paradigm shift in earthworm research has been observed in the areas of ecological engineering, molecular biology and immunology. Scientists had reported immunological and proteome-based monitoring methods of various contaminants of soil ecosystem using earthworm as sentinel species. This important soil invertebrate, in recent years gained a special focus for its importance in agroecology, biomonitoring and biotechnology. Vermicomposting has

been advocated as a major alternative for sustainable and biosafe agriculture across the globe. Interestingly, not all species of earthworms are equally efficient in composting wastes and thus are subjected to microbial and genetic screening in a controlled environment. Exploration and inventorization of the diversity of earthworm have been identified as challenging fields of research. Newer sets of taxonomical, ecological and physiological information have been continuously enriching one existing knowledge base on this group. Scientists identified the scope of genetic improvisation of earthworm for a better composting potential in selected species. With the introduction of various species of nanotoxins and chemical compounds into the global environment, the earthworm and other infaunas of soil ecosystem have been encountering the threat of extinction. This problem is presumed to be aggravated in the future under the backdrop of climate change.

This edited volume comprises research and review articles representing the current trend of research in biology and ecology of earthworm and vermicomposting. General health and abundance of earthworm are often correlated with the level of soil fertility.

The first section "Introduction" contains the Introductory chapter which describes history between humans and earthworm.

In section entitled "Ecology and Diversity", the ecological and geographical uniqueness of distribution of earthworms was reported in the landscapes of Siberia. A long-term observation was used to construct schematic maps in exhibiting quantitative variations of earthworm in landscape and regional ranges. The effect of climate aridity was reported to be an influencing factor of species number. Abundance and diversity of earthworm in managed and nonmanaged fallow lands were reported in Calakmul Reserve of Mexico. Calakmul reserve is a well-preserved rain forest where fallow land management was introduced. Earthworm's density and richness were recorded to be correlated with the age of the fallow land. The type of fallow land was assumed to affect the biomass of earthworm in this reserve. Functional interaction of earthworm with soil nematodes was reviewed under the backdrop of ecological variation. Phytonematodes often cause substantial damage of the crops in various ecological conditions. According to the authors, earthworm may be considered as a biological agent to reduce the extent of plant damage caused by selected species of nematodes. A novel approach of biodiversity inventorization of earthworm was discussed from taxonomical point of view. Development of online database may supplement the traditional method of morphotaxonomy of earthworm. This new approach of identification and conservation is expected to fill up some important lacunae of our present-day understanding of the diversity of oligochaetes. In the section entitled "Vermicomposting", the authors discussed the principle of vermicomposting. They pointed out the academic confusion and controversy which centre on the identity and biology of potential composting species. Importance of "pure culture" and the method of cultivation of earthworm of high composting efficiency were highlighted. Temperature, moisture content and physicochemical properties of feed mixtures influence the growth rate, maturity, reproduction rate and population size of earthworm during vermicomposting. In this section, the authors reported the influence of several physicochemical parameters on the general vermicomposting process. They also reported the differential responses of many species of earthworms toward selected parameters of soil. This book reports the basic and applied aspects of ecology and biology of earthworms with an emphasis on vermicomposting. The paradigm shift that occurred in the field of research during the last two decades has been reflected in this book. This title is expected

to provide new directions of research to the scientific workers investigating on different disciplines of earthworm biology.

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Introduction

Introductory Chapter: Earthworms - The Ecological Engineers of Soil

Sajal Ray

1. A farmer's friend

Intimacy of human with earthworm has a long history. Hunter-gatherer mode of life style of primitive human faced the challenge of uncertainty of food for the alleviation of hunger. Primitive societies are thought to be solely dependent on collection of natural resources from forest, river, and other water bodies for survival. Such resources included both plants and animals with nutritional and medicinal significance. Early human invented, learnt, and improvised agricultural technologies in different regions of the planet. They observed that the silted river plains with adequate moisture content are extremely fertile for the growth of various crops. While practicing agriculture, they might have observed these moist bodied worms of soil and their casting heaps along the soil surface. During tilling, they unearthed and observed these worms, which are able to penetrate soil without much effort as and when necessary. They befriended these 'down to earth' worms, which were innocuous and beneficial for agricultural practice. Earthworms are the true friends of farmers and are capable of increasing porosity and fertility of agricultural soil by their natural activity. Traditional farmers rely on their indigenous knowledge base and experience, and care these worms for their professional interest. These slow moving, yet highly dynamic soil annelids are considered as one of the beneficial animals to human and an indicator of the general health of soil. Earthworms did not evolve vision and still are uniquely sensitive to microlevel shift in the quality of soil and other environmental parameters. Their sensitivity toward soil contaminants and selected ecological cues enabled them to act as source of biomarkers of toxin exposure to soil and water.

Intensive agricultural practices occasionally appear to be highly detrimental for the health of the soil and human [1]. Unrestricted application of fertilizers and pesticides pose a serious

ecotoxicological threat for the earthworms of many developing countries. Earthworms feed along the soil surface and thus are exposed precariously to many agrotoxins-like pesticides and fertilizers. However, authors reported the enhancement of sustainability of agriculture by the effective management of earthworms of soil ecosystem. Earthworms, in general, are reported to improve porosity and structural stability of soil facilitating the healthy yield of crop. Thus, the earthworms are claimed as 'ecosystem engineers' of soil [2]. These ecological engineers bear a high potential to influence the quality and microenvironment of soil. Scientists report 'drilosphere,' as a part of soil with burrows and casts, are rich in earthworm and microbes. An intimate relationship of earthworm with microorganisms and invertebrates often determine the general biological structure of agricultural soil. Functional interaction of earthworm with these flora and fauna affect the interspecific dynamics and biological profile of soil ecosystem. In a review [2], the author highlighted the determining role of earthworm in pedogenesis, nutrient cycling, and fertility enhancement in soil. The earthworm efficiently transforms biodegradable organic waste materials into a vermicast – a bioprocessed end product rich in nutrients [3]. During passage of egested soil, the cast accumulates enzyme, microorganisms, and hormones from earthworm's gut involving a complex and dynamic process [4]. The biochemical process of vermicomposting is a dynamic and fragile event under the growing threat of soil contamination by new generation ecotoxins. Emerging group of pollutants includes a variety of chemical agents with unknown or less known level of toxicity and stability. This group of soil contaminants encompasses pharmaceutical compounds, nanotoxins, and selective bioactive compounds of industrial origin. In recent years, toxicity of various metal nanotoxins and inorganic salts were examined in earthworms. Immunological toxicity of copper nanoparticle and copper sulphate is in report [5] in a common variety of earthworm of India. Prior to toxicity analyses, physical characterization of copper oxide nanoparticle was carried out by dynamic light scattering, zeta potential, transmission electron microscopy, and EDAX
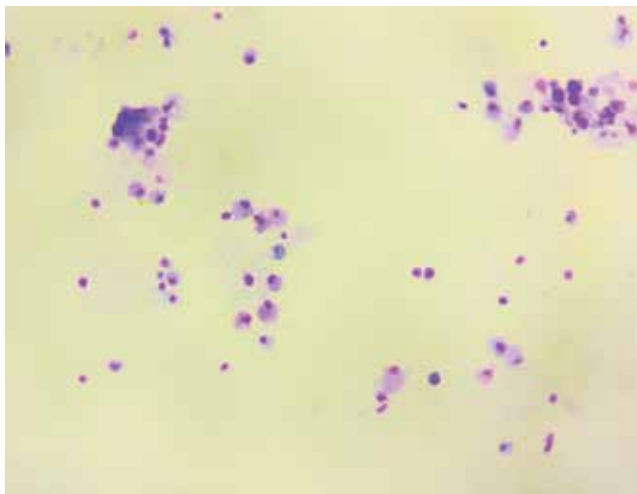
Figure 1. Polyphenotypic coelomocytes of a common earthworm *Metaphire posthuma* collected from a paddy field of India.
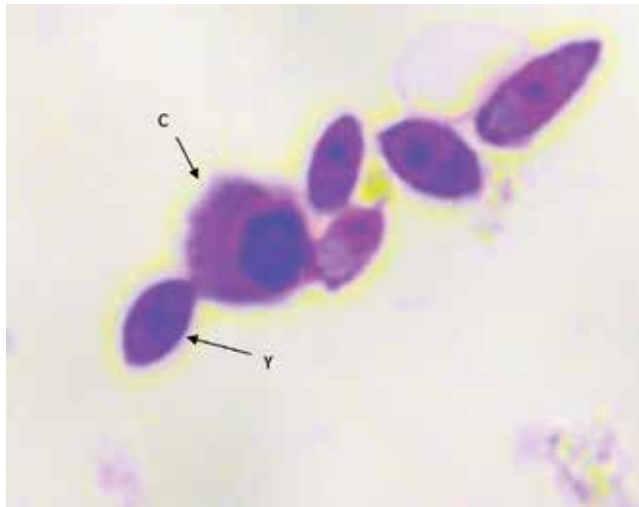
Figure 2. Formation of 'pagocytic cup' in a coelomocyte (C) of earthworm during engulfment of cultured yeast (Y). Phagocytosis is an established innate immunological response of coelomocytes against foreign particulates and toxins.

analyses of copper nanoparticle. Principal innate immune parameters like total coelomocyte (**Figure 1**) count, phagocytic response (**Figure 2**), oxidative stress were studied in earthworm exposed to environmentally realistic concentrations of sulphate and nanoparticles of copper. An undesirable shift in immune associated parameters indicated toxin induced immunological stress in earthworm. Unrestricted contamination of soil by emerging toxins like nanotoxins may thus result in prolonged immunological stress leading to population decline of these worms in agricultural soil.

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Ecology and Diversity

Abundance and Diversity of Earthworms in Managed and Non-Managed Fallow Lands of Calakmul Reserve of Campeche, Mexico

Esperanza Huerta Lwanga, Lucero Sánchez-del Cid,
Ligia Esparza-Olguín, Eduardo Martinez-Romero,
Ben de Jong and Susana Ochoa-Gaona

Additional information is available at the end of the chapter

Abstract

In Mexico, the best preserved tropical rain forest is found in Calakmul Reserve, where fallow land management has been established. Fallow lands are developed as a consequence of a successional vegetation process after clearing the primary vegetation and milpa production. Forty-nine sites were studied, where 17 were managed fallow lands, 24 non-managed fallow lands, and 8 tropical rain forests. Earthworms were collected at the end of the raining season, and four monoliths of 25 × 25 × 30 cm were developed per site according to the TSBF method. We observed how *Zapatadrilus siboney*, a native species was dominant in managed and non-managed fallow lands. Earthworm's total biomass and density were not significantly different between the managed and non-managed fallow lands. Earthworm's species richness was significantly low in non-managed fallow lands. We observed a strong correlation between earthworm density and richness with the age of the fallow lands (r^2: 0.9 and 0.7; $p < 0.05$, respectively). The management type of the fallow land seems to affect earthworm biomasses (r^2: 0.56; $p < 0.05$).

Keywords: Calakmul reserve, *Zapatadrilus siboney*, managed and non-managed fallow lands

1. Introduction

Calakmul is one of the most important biosphere reserves, where it is possible to find primary tropical rain forests, secondary vegetation, and managed lands. It has an extension of

723,185 ha, where 65% can be potentially managed (Esparza-Olguin, personal communication). Among the managed areas, fallow lands are developed as a consequence of a successional vegetation process after clearing the primary vegetation and milpa production. Fallow land management is developed by the reserve inhabitants, where wood, herbs, fruits, vegetal carbon, or honey is extracted or collected; even though it is an ancient practice, there is no information about the effects of this management over soil conditions. Earthworms constitute 60–90% of soil macroinvertebrate biomass [1], and they are considered as soil quality indicators [2]; they can inform about the degree of perturbation that can be taken place within a terrestrial ecosystem. In natural system, native earthworms are dominant [3], while exotic earthworms can inhabit managed systems due to their width range of tolerance to different pH and temperature conditions [3]. Earthworms provide soil ecosystem services such as infiltration, and they participate in soil organic matter dynamics and green house mitigation [4]. The objective of this study was to describe earthworm's communities among managed and non-managed fallow lands, in Calakmul Reserve.

2. Material and methods

Calakmul Reserve is located at 18°37′02″N and 89°33′00″W. The mean annual precipitation is 1076 mm with the mean temperature of 22.5°C. It is possible to find abundant karst outcroppings which characterize the shallow soils of the Calakmul region [5]. Forty-nine plots among nine communal settlements (ejidos) of Calakmul Reserve were selected. Within the fallow lands, 17 were managed and 24 were non-managed; fallow lands were successional vegetation belonging to evergreen tropical rain forest or subcaducifolius rain forests. Eight tropical rain forests were also sampled. Earthworms were collected at the end of the raining season, according to the TSBF method [6], where four monoliths of 25 × 25 × 30 cm per plot were done. Earthworms were preserved in 94% alcohol for further identification in the laboratory of soil of El Colegio de la Frontera Sur, Unidad Campeche. Data was normalized, and ANOVA were performed in order to observe significant differences between managed and non-managed fallow lands; also Pearson correlation was developed among variables. A canonical analysis was performed with data of managed and non-managed fallow lands from the subcaducifolius rain forest; as environmental variables, we used fallow land age, density and richness of plants, and type of management (extraction of vegetal carbon, forestall management, and apiculture); and as species variables, we used earthworm biomass, density, and richness.

3. Results

We found six earthworm morphospecies belonging to the Acanthodrilidae family; most of the individuals were juveniles. Among adults we identified one native species *Zapatadrilus siboney* and one peregrine species *Dichogaster crawi*, where *Zapatadrilus siboney* was dominant

in managed and non-managed fallow lands. Only two ecological earthworm categories were found in this study: endogeics and epigeics. Earthworm's biomass and density (**Figures 1** and **2**) were not significantly different between managed and non-managed fallow lands, but earthworm species richness was significantly different (**Figure 3**); fallow lands without management had the lowest earthworm's species number (**Figure 3**). Regarding the age of the fallow lands, we observed a significant correlation between age of the fallow land and abundance and richness of earthworms (r^2: 0.9, 0.7; $p \leq 0.05$, respectively), independently of the management and the type of vegetation (evergreen tropical rain forest or subcaducifolius rain forest). Vegetation structure between tropical rain forests and managed or non-managed old fallow lands (≥ 25 years) was not significantly different, but the vegetation composition of the tropical rain forest was significantly different, i.e., tropical rain forest had tree species with the hardest wood trunk compared with those found in non-managed fallow lands and managed fallow lands (**Table 1**). After the canonical analysis developed with data of fallow lands from the subcaducifolius fallow lands, we observed with 99% of inertia explained how earthworm density is strongly correlated with the age of the fallow land (r^2: 0.7; $p < 0.05$) and lightly correlated with vegetation richness (r^2: 0.4; $p < 0.05$, **Figure 4**). Earthworm biomass seems to be affected by the type of fallow land management (r^2: 0.56; $p < 0.05$).

Figure 1. Earthworm biomass (g.m^2) in different land uses. Managed fallow land (MFL), non-managed fallow lands (NMFL), and tropical rain forest (TRF).

Figure 2. Earthworm density (ind.m²) in different land uses. Managed fallow land (MFL), non-managed fallow lands (NMFL), and tropical rain forest (TRF).

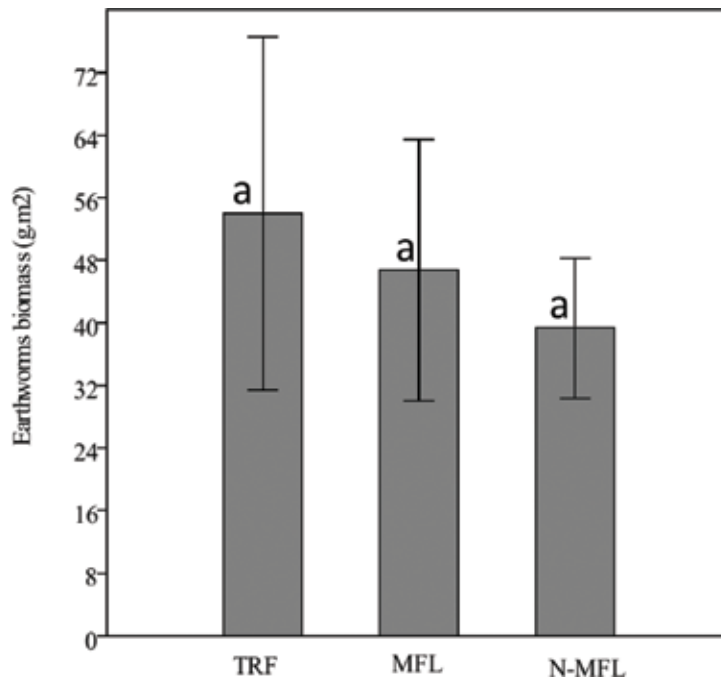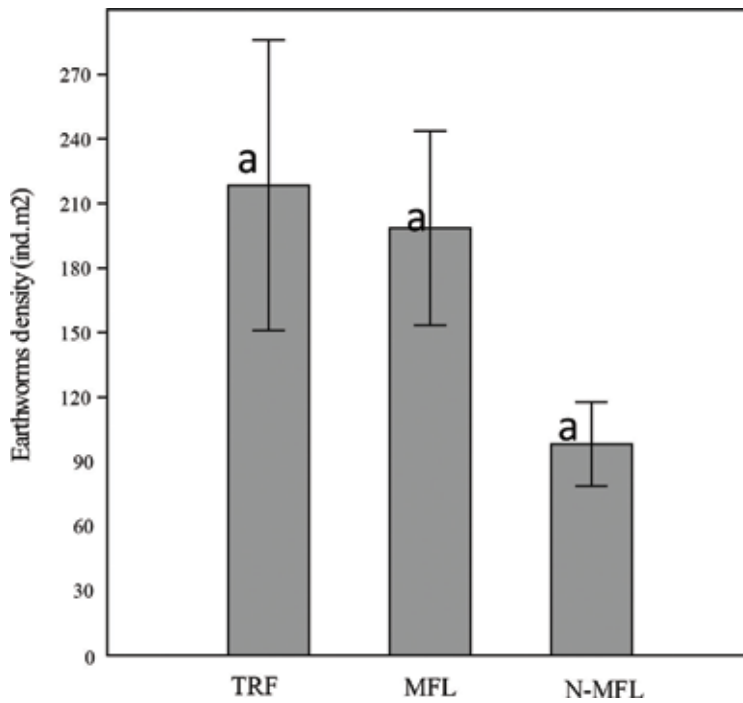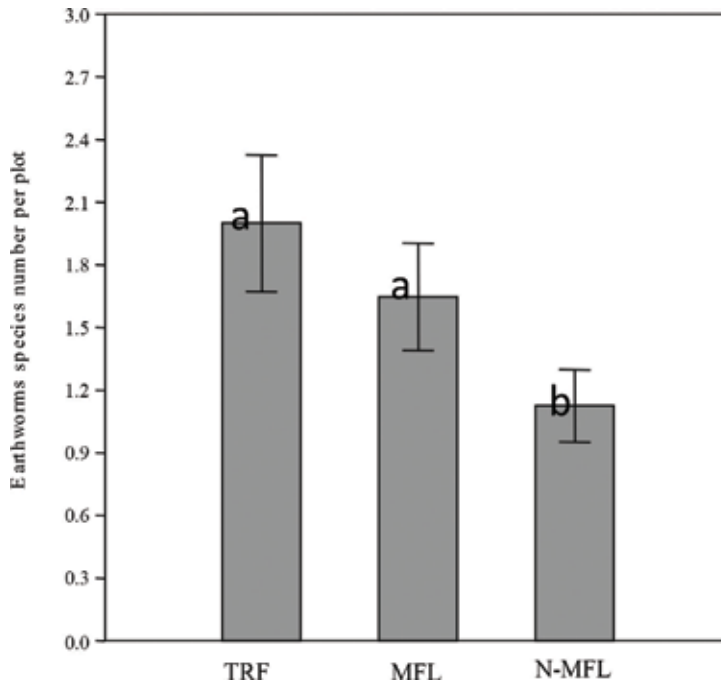**Figure 3.** Earthworm richness in different land uses. Managed fallow land (MFL), non-managed fallow lands (NMFL), and tropical rain forest (TRF).

Plot no.	Ejido name	Vegetation type	M	Age	Plant species	Zs
1	Álvaro Obregón	Fallow land SCP	Non	20	Ly, Lg, Bs, Tp, Ht, Ey, Gf	No
2	Álvaro Obregón	Fallow land SCP	With	20	Bs, Ly, Tp, Ds, Ei, Lg, Ne	No
3	Álvaro Obregón	Fallow land SCP	With	15	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
4	Álvaro Obregón	Fallow land SCP	With	12	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
5	Álvaro Obregón	Fallow land SCP	Non	15	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
6	Álvaro Obregón	Fallow land SCP	With	20	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
7	Álvaro Obregón	Fallow land SCP	With	15	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
8	Álvaro Obregón	Fallow land SCP	Non	20	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
9	Álvaro Obregón	Fallow land SCP	With	25	Bs, Ly, Tp, Ds, Ei, Lg, Ne	No
10	Álvaro Obregón	Fallow land SCP	Non	15	Ly, Lg, Bs, Tp, Ht, Ey, Gf	No
11	Km 120	Fallow land SCP	With	19	Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns	x
12	Km 120	Fallow land SCP	Non	15	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
13	Km 120	Fallow land SCP	With	17	Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns	x
14	Km 120	Fallow land SCP	Non	17	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
15	Km 120	Fallow land SCP	With	18	Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns	x
16	Km 120	Fallow land SCP	With	18	Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns	x
17	Km 120	Fallow land SCP	With	17	Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns	x
18	Km 120	Fallow land SCP	Non	18	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
19	Km 120	Fallow land SCP	Non	19	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
20	Km 120	Fallow land SCP	With	19	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
21	Km 120	Fallow land SCP	Non	17	Ly, Lg, Bs, Tp, Ht, Ey, Gf	x
22	Pueblo de Morelia	Fallow land SCP	With	17	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
23	Pueblo de Morelia	Fallow land SCP	With	18	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
24	Pueblo de Morelia	Fallow land SCP	With	19	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
25	Pueblo de Morelia	Fallow land SCP	With	19	Bs, Ly, Tp, Ds, Ei, Lg, Ne	x
26	Pueblo de Morelia	TRF SCP	Non	>25	Ly, Ey, Tp, Dl, Kf, Bs, Ba, Mz	x
27	Km 120	TRF SCP	Non	>25	Ly, Ey, Tp, Dl, Kf, Bs, Ba, Mz	x
28	Km 120	TRF SCP	Non	>25	Ly, Ey, Tp, Dl, Kf, Bs, Ba, Mz	x
29	Puebo de Morelia	TRF SCP	Non	>25	Ly, Ey, Tp, Dl, Kf, Bs, Ba, Mz	x
30	Ley de Fomento	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
31	Ley de Fomento	TRF EG	Non		Ps, Ss, Ei, Py, Mp	x
32	Ley de Fomento	Fallow land EG	Non	12	Ca, Ns, Pp, Bs, Lg, Tp, Gc	x
33	Ley de Fomento	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
34	Centauro del Norte	Fallow land EG	Non	30	Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
35	Centauro del Norte	Fallow land EG	Non	20	Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
36	Centauro del Norte	Fallow land EG	Non	12	Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
37	Centauro del norte	TRF EG	Non		Ps, Ss, Ei, Py, Mp	x
38	Narciso Mendoza	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x

Plot no.	Ejido name	Vegetation type	M	Age	Plant species	Zs
39	Narciso Mendoza	TRF EG	Non		Ps, Ss, Ei, Py, Mp	x
40	Narciso Mendoza	Fallow land EG	Non	10	Ca, Ns, Pp, Bs, Lg, Tp, Gc	x
41	Narciso Mendoza	Fallow land EG	Non	30	Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
42	Unidad de Trabajo	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
43	Unidad de Trabajo	TRF EG	Non		Ps, Ss, Ei, Py, Mp	x
44	Unidad de Trabajo	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
45	Unidad de Trabajo	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
46	Conhuas	Fallow land EG	Non	7	Ca, Ns, Pp, Bs, Lg, Tp, Gc	x
47	Conhuas	Fallow land EG	Non	20	Ca, Lg, Ll, Ns, Mz, Bt, Mb	x
48	Conhuas	TRF EG	Non		Ps, Ss, Ei, Py, Mp	x
49	Nuevo Becal	Fallow land EG	Non		Ca, Lg, Ll, Ns, Mz, Bt, Mb	x

Ejido: communal settlement where owners can or cannot sell the land. M, management; fallow land SCP, subcaducifolius forest; TRF SCP, tropical rain forest with subcaducifolius characteristics; fallow land EG, fallow land with almost evergreen characteristics (subperennifolius); TRF EG, tropical rain forest with subperennifolius characteristics. Plant species combinations according to vegetation type: Ca, Ns, Pp, Bs, Lg, Tp, Gc, *Croton arboreus*, *Nectandra salicifolia*, *Piscidia piscipula*, *Bursera simaruba*, *Lonchocarpus guatemalensis*, *Bursera simaruba*, *Nectandra salicifolia*, *Thouinia paucidentata*, *Guettarda combssi*; Ps, Ss, Ei, Py, Mp, *Pouteria reticulata*, *Sideroxylon salicifolium*, *Eugenia ibarrae*, *Piper yucatanense*, *Murraya paniculata*; Lg, Bs, Ey, Ca, Ly, Gf, Tp, Ns, *Lonchocarpus guatemalensis*, *Bursera simaruba*, *Esenbeckia yaaxhokob*, *Croton arboreus*, *Lonchocarpus yucatanensis*, *Gymnopodium floribundum*, *Thouinia paucidentata*, *Nectandra salicifolia*; Bs, Ly, Tp, Ds, Ei, Lg, Ne, *Bursera simaruba*, *Lonchocarpus yucatanensis*, *Thouinia paucidentata*, *Diospyros salicifolia*, *Eugenia ibarrae*, *Lonchocarpus guatemalensis*, *Neomillspaughia emarginata*; Ly, Lg, Bs, Tp, Ht, Ey, Gf, *Lonchocarpus yucatanensis*, *Lonchocarpus guatemalensis*, *Bursera simaruba*, *Thouinia paucidentata*, *Hampea trilobata*, *Esenbeckia yaaxhokob*, *Gymnopodium floribundum*; Ly, Ey, Tp, Dl, Kf, Bs, Ba, Mz, *Lonchocarpus yucatanensis*, *Esenbeckia yaaxhokob*, *Thouinia paucidentata*, *Drypetes lateriflora*, *Krugiodendron ferreum*, *Bursera simaruba*, *Brosimum alicastrum*, *Manilkara zapota*.

Table 1. Presence of *Zapatadrilus siboney* (Zs) along the studied sites.
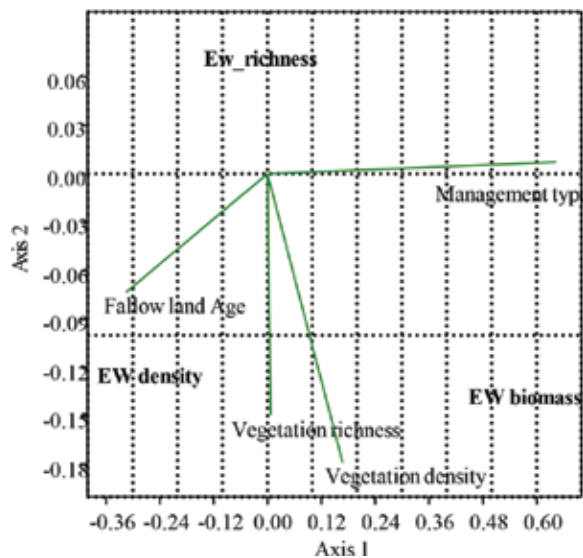
Figure 4. Earthworm community and vegetation characteristics (abundance and richness) among managed and non-managed fallow lands of subcaducifolius forests in Calakmul reserve, after a canonical analysis (99.7% of inertia explained at first axis p < 0.05).

4. Discussion

Fallow land management in Calakmul has been spread among the inhabitants of the reserve; this economical practice allows them to obtain resources (mainly economical) without damaging the environment. In this study we observed that the management of the fallow land doesn't produce an effect over earthworm communities, while the age of the fallow land is more important. The age of fallow lands informs about the plant composition, where an important succession process has been taken place within 25–30 years (>vegetation cover, >diversity, >organic matter in the soil), and earthworms respond to this process. *Zapatadrilus siboney*, endogeic species found in Cuba and north and center of Mexico, has been spread in Calakmul [7, 8] where forest and fallow lands are present. In Mexico, 102 earthworm species have been described, with 51 natives and 51 exotics; in Campeche, only 8 native species and 3 exotics have been found [8]; native species are confined to undisturbed systems. Native species, as being less tolerant to different ranges of temperature and pH [3], can be useful as indicators of soil quality [2]; in this study, *Zapatadrilus siboney* as native species was not affected by the fallow land management, which suggests us that this management can be used in other parts of Mexico where forest vegetation wants to be preserved. Earthworms, as ecosystem engineers [9], have an important role in edaphic processes [10, 11], and more studies are required in tropics, in order to study the relationship between native species and their role in soil quality enhancement.

Acknowledgements

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Earthworms and Nematodes: The Ecological and Functional Interactions

Jair Alves Dionísio, Wilian Carlo Demetrio and
Arlei Maceda

Additional information is available at the end of the chapter

Abstract

Soil invertebrate organisms are responsible for several biochemical processes indispensable for the correct functioning of ecosystems. Because of the high diversity of animals that occurs in the soil environment, some invertebrates such as earthworms and nematodes are highly important in trophic chains, with high number of species and the effect that they exert on both natural and agricultural systems. However, although numerous studies have evaluated the implications of these organisms in soil processes and their consequences on crop productivity, the interaction between earthworms and nematodes has received little attention in recent years. This chapter reviews studies focusing on the elucidation of the interaction between earthworms and nematodes in diverse situations in which they occur, for example, the vermicompost process and the native and agricultural systems. Several studies have shown that the direct and/or indirect action of earthworms can highly modify nematode populations. In addition, in the presence of earthworms, the damage caused by phytonematodes can be reduced in some crops.

Keywords: biological control, plant growth, vermicomposting, plant parasitic nematode, soil food web

1. Introduction

The first studies on earthworms were initiated by Darwin, with the classic "The Formation of Vegetable Mold through the Action of Worms, with observations on their Habits" [1]. Since then, thousands of studies related to the biology and ecology of earthworms have been performed worldwide. However, even in ancient Rome, these invertebrates had already

attracted the attention of Aristotle, who described them as "the intestines of the earth" in 340BC [2].

At present, the importance of earthworms for the functioning of natural and agricultural ecosystems is recognized [3–6]. These organisms can influence the growth of plants via several mechanisms, which were described by Edwards [2] and Scheu [7], such as increasing soil organic matter mineralization; modifications of soil porosity and aggregation that change the availability of water and oxygen to plants; production of plant growth regulators via the stimulation of microbial activity; pest and parasite control; and stimulation of symbiotic microorganisms.

However, the benefits mediated by these organisms in the soils led to erroneous interpretations, mainly because of their high diversity; there are about 3500 earthworm species described worldwide, with potential of more than 7000 species [8–10]. In addition, also it is high the diversity of earthworms occurring in an area with natural vegetation or agricultural system. This has already been noted by Steffen et al. [11], who identified about 56 earthworm species in natural and agricultural ecosystems, of which 26 were native and 30 exotic, belonging to six families. In addition, the greatest diversity of these species was related to the type of ecosystem evaluated: their richness is greater in areas of forest fragments and native fields. Brown et al. [12] evaluated earthworm populations in different land use systems and observed high earthworm abundance in conservation systems with values ranging from 116 to 179 ind. m^{-2} in no-tillage and minimum tillage, respectively. The authors suggested that the greater presence of these organisms can be attributed to the lack of soil management in no-tillage, promoting the accumulation of organic material on the soil surface, and small mechanical movement, benefiting the community of these organisms. In addition to the effect of management on earthworm populations, Tanck et al. [13] found seasonality effects in the communities of *Amynthas* spp. (exotic earthworm) under no-tillage and native forests, with densities of about 170 and 93 ind m^{-2} and biomass of 50 and 65 g m^{-2}, respectively.

The remarkable diversity of earthworm species can be divided into three distinct ecological categories: epigeic, anecic, and endogeic [14]. Epigeic earthworms comprise animals living on the soil surface, by using the litter and organic horizons as habitat, feeding on organic materials at the beginning of the decomposition process, and incapable of digging galleries in the soil; they are normally used in vermicompost processes. Conversely, endogeic species live in greater depths of soil; are geophageous, taking from the soil the food necessary for their survival; and include most of the earthworms described. The anecic earthworms are organisms that live in the soil-surface interface and are considered the most active of the three categories mentioned above [15].

These ecological categories are based on the environments in which earthworms live, ingesting and transporting organic and mineral particles at different distances horizontally and vertically in the soil profile [16–18]. Because of their size and dietary habits, earthworms also unintentionally ingest a large diversity of organisms, ranging from microorganisms such as bacteria and fungi to small animals such as nematodes [15, 19, 20].

Nematodes are highly representative invertebrates in soils, with densities ranging from 106 to 10^7 m^{-2} and biomass of up to 100 kg ha^{-1} [21]. Like earthworms, these organisms also present remarkable ecological diversity, with free-living species—bacteriophages, plant-parasitic, mycophages, omnivores, and predators—responsible for the regulation of several trophic chains in the soil, and parasitic nematodes of plants or animals [22]. Population densities of

these animals are of the order of 10^6 m^{-2} and can consume up to 800 kg ha^{-1} of bacteria [23]. However, plant-parasitic nematodes, a group with high agricultural interest, and bacterio-phages, nematodes that feed on both pathogenic and saprophytic bacteria and other beneficial species, are the most representative groups in soils [24].

Considering the small size of free-living and plant-parasitic nematodes, they are inevitably ingested by other organisms, mainly by earthworms [25]. Several studies have attempted to elucidate the interactions between these groups of invertebrates; however, because of the remarkable ecological variability already mentioned, the results have not been consistent, and these interactions have not been clearly defined [26–28]. Thus, little is known about the effects of earthworms on microbial diversity and soil microfauna [29].

In this context, a series of studies were performed in order to elucidate the interactions between earthworms and nematodes, as well as the implications of these interactions with other soil organisms and plants in natural and agricultural systems. A simplified version of these interactions is shown in **Figure 1**.
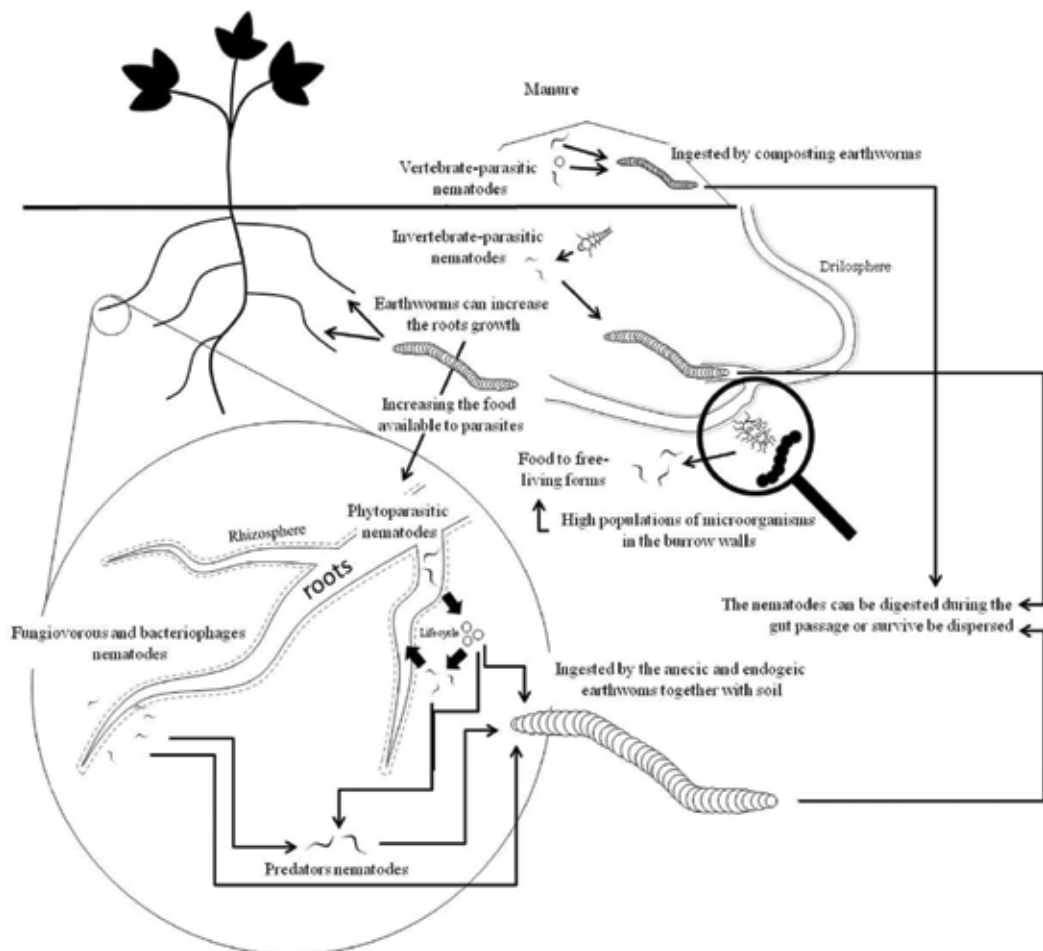
Figure 1. Interactions between earthworms and nematodes in the soils.

2. Effects of earthworms on nematode communities

The effects of earthworms on nematode communities (free living or phytonematodes) can be analyzed under four different situations. First, the effects of earthworms on the populations of nematodes during the vermicomposting process of unstabilized organic residues; second, the effects of the products generated by the action of earthworms (vermicompost) or the byproducts (vermicompost tea) as controlling agents of phytonematodes; third, when soil interaction only occurs between worms and nematodes; and fourth, when the interaction of earthworms and phytonematodes occurs in the presence of plants, the latter being more complex, with greater variability of results and thus greater difficulty of interpretation.

2.1. Earthworms and nematodes in vermicomposting process

Because of the high diversity of organisms involved and the ecological complexity of soils, the interactions between earthworms and nematodes have been completely dependent on the particularities of the surveys conducted. Domínguez et al. [28] evaluated the effects of *Eisenia fetida* (earthworms worldwide used in vermicomposting) on the population of free-living nematodes (bacteriophages and fungivorous) in cattle manure and sewage sludge. In both substrates, bacteriophage nematode populations were reduced during the evaluated period in the presence of earthworms. However, assessment of the fluctuations in nematode populations revealed that fungivorous communities were more affected by the presence of oligochaetes (**Figure 2**). The fungi represent one of the main food sources for earthworms, which might
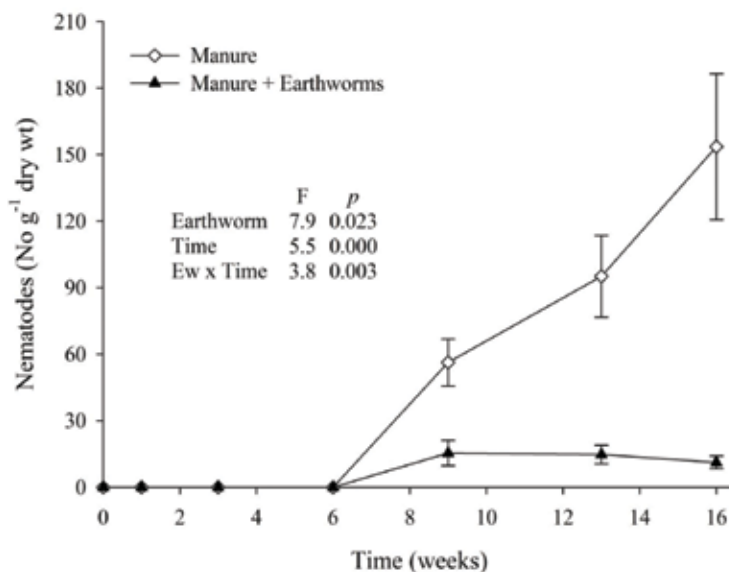
Figure 2. Fungivore nematode abundance (mean ± SE) in the presence and absence of the earthworm *Eisenia andrei* during vermicomposting of cow manure. The figure includes the results of repeated-measures ANOVA for the presence of earthworms (Source: Redrawn from [28]).

explain the greater effect of vermicomposts on fungivorous populations than on bacteriophage populations. Conversely, earthworms can also facilitate the dispersion of these microorganisms by the excretion of their spores in the coprolites [30]. However, the dispersion of nematophagous fungi by earthworms might also be responsible for the reduction of the nematode populations in the substrates evaluated [31]. Monroy et al. [32] also observed a reduction of bacteriophage populations by the activity of several earthworm species. Kokhia et al. [33] showed that the changes in nematode communities by earthworms did not occur only at the population level, but rather led to the restructuring of all biodiversity when these invertebrates were present.

The effect of earthworms on nematode populations can be attributed to the direct ingestion and digestion, or reduction by indirect effects [34]. The indirect effect is attributed to the reduction of fungal populations by integrating the diet of the earthworms, thereby reducing communities of fungivorous nematodes [30].

2.1.1. Vermicomposting and byproducts in the control of nematodes

Although the action of vermicompost earthworms shows the reduction of populations of free-living nematodes, the application of vermicompost in soils has shown to have adverse effects. Arancon et al. [35] observed a reduction of the communities of plant-parasitic nematodes after the application of vermicompost from different plant materials. However, considering the effect similar to the use of organic compounds in this experiment, the addition of organic materials to the soil was assumed to increase the availability of food for fungivorous and bacteriophage nematodes, increasing the competition between them with other groups. Gabour et al. [36] also observed this effect of vermicompost application on the populations of the plant-parasitic nematode *Rotylenchulus reniformis*.

In addition to vermicompost, recent studies have shown that the application of vermicompost tea has the potential to control plant parasitic nematodes. In this sense, Edwards et al. [37] observed a significant suppression in the number of galls caused by *Meloidogyne hapla* in tomato crop when the plants were subjected to aerated vermicompost tea (**Figure 3**).

Mechanisms of nematode control by vermicompost tea are still poorly understood. The effects of this substance are likely caused by the death of nematodes by the release of toxic substances such as hydrogen sulfate, ammonia, and nitrite produced during vermicomposting process [38]; promotion of the growth of nematode predatory fungi that attack their cysts [39]; favoring of rhizobacteria that produce toxic enzymes and toxins [40]; or indirectly by favoring populations of microorganisms, bacteria, and fungi, which serve as food for predatory or omnivorous nematodes, or arthropods such as mites, which are selectively opposed to parasitic nematodes of the plant [41].

2.2. Earthworms and nematodes in the soils

Poinar [42] reviewed several works and published a list regarding the natural relationships between oligochaetes and nematodes, with more than 150 nematode citations, also containing a brief summary of the groups of nematodes, mainly endoparasite species, found in
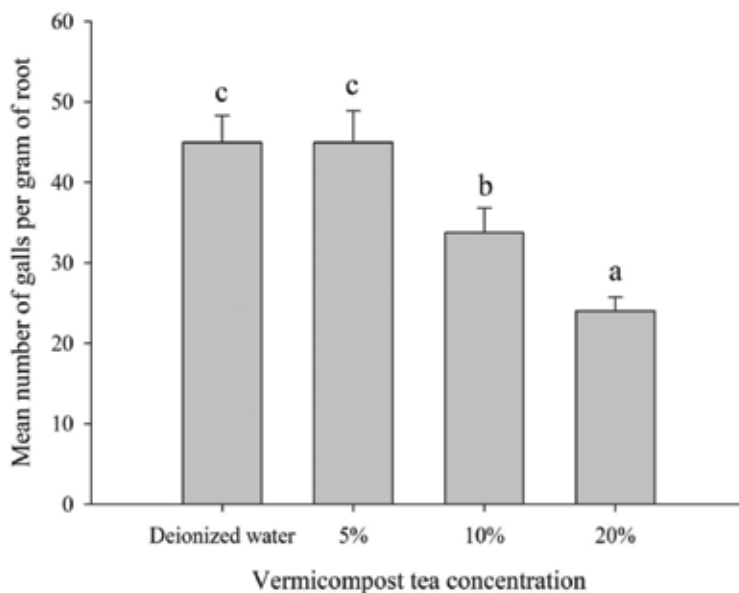
Figure 3. Mean numbers of *Meloidogyne hapla* galls (mean ± SE) on tomato roots infested with the nematodes and treated with soil drenches of vermicompost tea. Columns with different letters are significantly different ($p < 0.05$). All plants were grown in MM 360 and received all needed nutrients (Source: Redrawn from [37]).

earthworms. However, it does not present information on these endoparasites in presence of some tropical earthworm species such as *Pontoscolex corethrurus* and *Amynthas* spp. (especially *A. gracilis* and *A. corticis*), which are frequently used in studies evaluating the interaction between these organisms [26, 43–46].

The effects of geophageous earthworms on soil nematodes also differ across studies, and this variability occurs among studies that use the same worm species, which is probably related to the high diversity of these organisms, especially nematodes found in situ. Dash et al. [34] observed reductions of nematode populations in the soil in the presence of *Lampito mauritii*, an effect that occurred without the distinction of groups; however, plant-parasitic species were less affected, likely because of the low palatability of this group, which is lower than that of the free life forms. Senapati [47] also evaluated the effect of *L. mauritii* on nematode communities, but the results showed an increase in bacteriophage populations and a decrease in plant-parasitic populations, whereas Tao et al. [48] evaluated effects in the presence of *Metaphire guillelmi* in field experiments and revealed reduction of all groups of nematodes to the depth of 20 cm.

Studies by Boyer et al. [43] on *P. corethrurus*, an exotic earthworm distributed globally in tropical regions, in the laboratory by using sterilized soil showed that this species had the potential to reduce phytonematodes. They suggested that some compounds such as proteolytic enzymes released into the digestive system of earthworms seem to have an antagonistic effect on these invertebrates (**Figure 4**).

Further, Villenave et al. [46] evaluated the interaction between nematodes and *P. corethrurus* and found an increase in the population of soil nematodes, mainly of the plant-parasitic
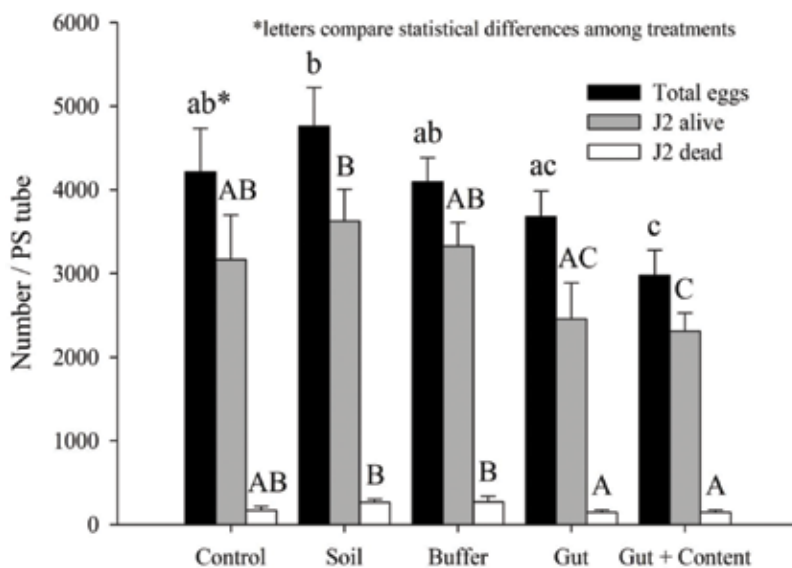
Figure 4. Living and dead J2 larvae and total eggs per polystyrene (PS) transparent tube obtained 5 weeks after exposure of J2 *Heterodera sacchari* to the *Pontoscolex corethrurus* gut contents, the *P. corethrurus* gut alone, aqueous soil extracts (Andisol), or phosphate buffer. (Source: Redrawn from [43]).

species, in a field experiment. Although these studies differed in the methodological approach, and a greater number of interactions might occur in experiments in which the substrate is not sterilized, a key factor to be observed is the earthworm density that was used in each experiment. Boyer et al. [43] used a small amount of soil (200 g) and a large number of earthworms, which would represent around 2000 m^{-2} individuals (up to 20 cm deep). However, in the experiment by Villenave et al. [46], the densities were approximately 122 earthworms m^{-2}. However, the disagreement in the results of the studies mentioned above was not necessarily an effect of the methodology used, since another factor to be considered in these interactions is the time of coexistence between worms and nematodes, which was 35 and 150 days for [43, 46], respectively. Experiments with *Lumbricus rubellus* [27] showed a reduction of the general density of soil nematodes; however, this effect occurred in a pronounced way in the first 60 days, with a reduction of bacteriophages and increase in plant-parasitic species after this period. The interaction between earthworms and nematodes, in addition to being dependent on all the variables already discussed, is also influenced by the presence of plants. Yeates [49] studied the interaction of these three components and observed the same results as those of Ilieva-Makulec and Makulec [27]. According to Yeates [49], the positive effect of earthworms on root development also increases the rhizospheric area, which is a highly complex zone in which frequent release of cells, mucilages, exudates, and lysates that contain amino acids, enzymes, proteins, sugars, carbohydrate complexes, alcohols, vitamins, and hormones [50], thereby increasing the food for microorganisms and thus for nematodes (**Figure 5**). Räty and Huhta [51] showed that some abiotic conditions such as soil pH also modify the behavior of earthworms and nematodes in the soil.

Figure 5. Effects of earthworms on the growth of bean roots (Source: Authors).

The effect of earthworms on the environment are not only restricted to the changes that occur in the soil ingested by these animals. Tiunov et al. [52] evaluated the populations of nematodes on the walls of the galleries of *L. terrestris* and found communities of bacteriophage nematodes associated with this environment. Thus, like coprolites, the walls of earthworm galleries are rich in nitrogen compounds that promote the development of microorganisms in these sites, which might also favor the development of nematodes.

In addition to all the results cited above, earthworms can also act as a transport vehicle for these small invertebrates. Shapiro et al. [53] reported the ability of *L. terrestris* and *A. trapezoides* to disperse within the soil *Steinernema carpocapsae*, the parasitic nematode of over 250 species of insects.

2.3. Interaction between earthworms and nematodes and their effects on plants

Few studies have investigated the effects of earthworm and nematode interactions on plant growth [26, 44, 45, 54].

Dionísio et al. [26] evaluated the effect of the inoculation of earthworms *P. corethrurus* and *Amynthas* spp. in tomato plants infested with the plant-parasitic species *Meloidogyne paranaensis* in a greenhouse. Six adult worms of *Amynthas* spp. or *P. corethrurus*, isolated or in the same proportion (3, 3), were inoculated in pots containing soil sterilized in a steam oven. After 1 week, tomato seedlings (Rutgers" cultivar) were transplanted into the pots, and 5 mL of a suspension of *M. paranaensis* containing 5000 eggs and/or juveniles was inoculated per pot. The authors observed a reduction in the number of galls plant per plant after 65 days in the treatments in which the earthworms were inoculated, with reduction varying from 39.2 to 55.2% for *Amynthas* spp. and *P. corethrurus*, respectively (**Figure 6**). Nonetheless, the combination of the two species resulted in the reduction of 50.0% incidence of galls.

The authors indicated that the action of the earthworms occurred probably after the inoculation of the nematodes, because tomato is highly susceptible to attack by nematodes, especially
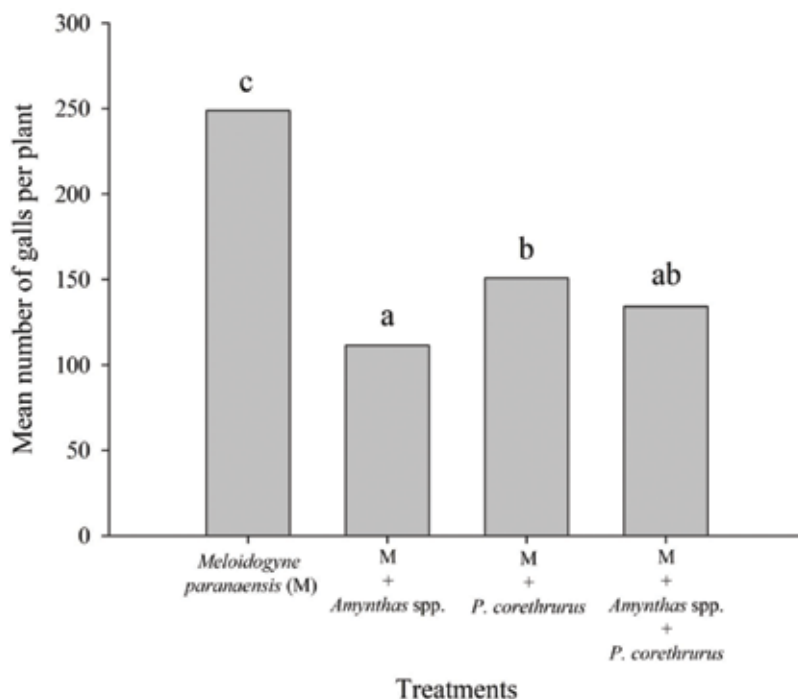
Figure 6. Galls per tomato plant (*Solanum lycopersicum* "Rutger") inoculated with earthworms (*Amynthas* spp. and *P. corethrurus*) and plant parasite nematodes. Letters indicate statistical differences ($p < 0.05$) by Tukey's test (Modified from [26]).

at the seedling stage [55]. Thus, two explanations were presented. First, the earthworms *Amynthas* spp. and *P. corethrurus* are epigeic and endogeic, respectively, and ingested a greater (*P. corethrurus*) or smaller (*Amynthas* spp.) soil quantity. Further, they might also have ingested eggs/juveniles of *M. paranaensis*, which might have been destroyed or inactivated in the passage through the digestive system, thereby reducing the possibility of gall formation in plants. Second, the eggshell of *M. paranaensis* might have been destroyed by the enzymes in the digestive tract of earthworms, mainly chitinase [30], releasing the larvae inside. Thus, the released larvae remained in the infested state in the tissues, coelom, and hemocele without essential development and, normally, without growth, what is called as paratenosis [56]. Therefore, future experiments are needed to perform parasitological tests of the earthworm tissues to better interpret the results.

Contrary results are cited by Lafont et al. [44] evaluating the effects of *P. corethrurus* and *Radopholus similis* (cave nematodes) on banana plants (*Musa acuminata*, subgroup Cavendish, "Grande-Naine"). The study was conducted in a greenhouse by using pots containing soil, which was previously frozen ($-20°C$) for 2 days to eliminate the native microfauna. The total biomass of inoculated *P. corethrurus* was 5.0 (g pot^{-1}); 4 weeks later, the plants were inoculated with a *R. similis* suspension containing 450 eggs. The results showed the absence of the control of nematodes in the soil; however, the plants developed better in the presence of earthworms (**Figure 7**) and also showed a reduction in the severity of necrosis in the root system. Similar
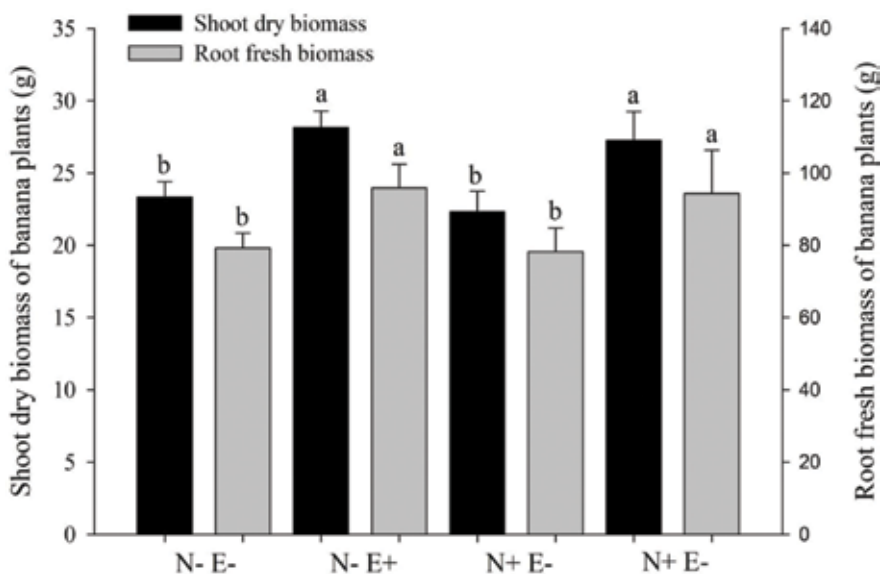
Figure 7. Shoot dry and root fresh biomass of banana plants under different treatments at the end of the experiment: N- E- Absence of fauna; N- E+ *P. corethrurus earthworms* alone; N+ E- *R. similis* nematodes alone; N+ E+ earthworms plus nematodes. Bars indicate standard errors, n = 12. For each treatment, the means with the same letter are not significantly different based on Bonferroni test at *p* < 0.05 (Source: Adapted from [44]).

results have also been reported by Loranger-Merciris [45] by using *P. corethrurus* in banana plants infected with *R. similis*, *Helicotylenchus multicinctus*, and *Pratylenchus coffeae*.

The reduction of nematode damage in plants in the presence of earthworms was also observed by Demetrio et al. [54], who evaluated the potential of the earthworm *Amynthas* spp. in reducing the infection of *Meloidogyne javanica* (worldwide parasite of tomato crop) as well as the effects of the inoculation of these organisms on some soil biological attributes. Under similar conditions as those used in [26], different densities of *Amynthas* spp. were inoculated (0, 2, 4, 6, and 8 animals per pot) in the presence of tomato plants, which received a suspension containing 3000 eggs and/or juveniles of *M. javanica*. At the end of the experiment, the increase in carbon content of the microbial biomass and positive correlation of this attribute with the dry mass of the plants was verified. The results of this experiment showed that the earthworms were not able to reduce the infection of the plant-parasitic species in the tomato roots; however, in the presence of these invertebrates, the damage caused was reduced. Further, a positive correlation was noted between the number of inoculated earthworms and the dry mass of tomato (**Figure 8a**).

The better development of plants even with the formation of galls in the presence of earthworms can be attributed to several factors: physical changes of the soil by the action of these invertebrates, since galleries formed are normally used by plants as a preferred route for root growth, in addition to facilitate the infiltration of water and oxygen throughout the soil profile [57]. Second, chemical changes, which might increase the availability of P and N mainly, because of the acceleration of nutrient cycling, as well as the continuous deposition of NH_4^+
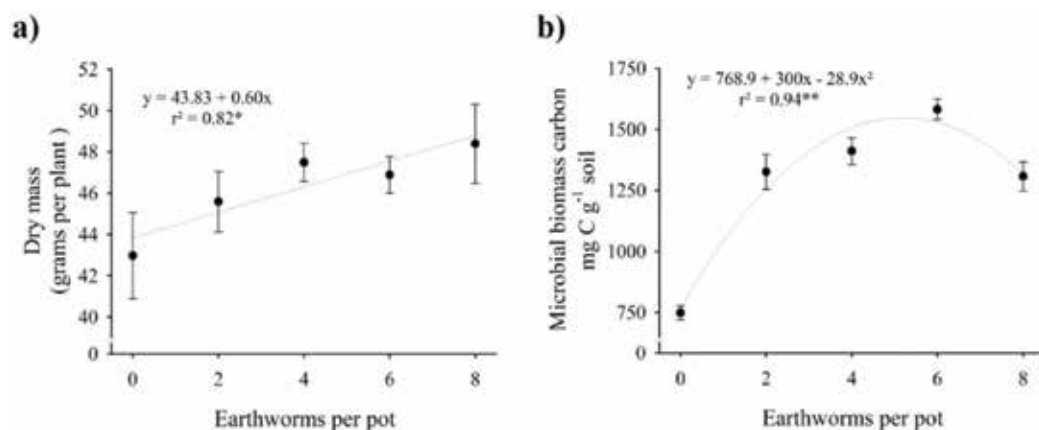
Figure 8. Effects of the levels of earthworms (*Amynthas* spp.) and nematodes (*Meloidogyne javanica*) in (a) dry mass of tomato plants; (b) soil microbial biomass (Source: Modified from [54]). *,** significance at p < 0.05 and p < 0.01, respectively.

by earthworms, both by the production of casts and organo-mineral excrements. These processes could stimulate communities of nitrifying bacteria and growth-regulating-hormone producers, as well as the deposition of mucus-rich nitrogen compounds on the walls of the galleries [7, 47, 48].

The physico-chemical variations promoted by the earthworms alter the biological component of the soil, thereby mainly stimulating the microorganisms (**Figure 8b**) that can be reflected in the colonization of the roots by arbuscular mycorrhizal fungi [58]. This contributes to the greater absorption of nutrients, mainly phosphorus; the development of plant growth-promoting rhizobacteria [59] such as *Pseudomonas* spp. fluorescents [60], which produce siderophores, that is, increase the availability of Fe^{2+} to plants; or to the production of antibiotics that inhibit the effects caused by clinical and subclinical pathogens [61]. These physicochemical and biological factors can favor the development of plants and compensate for the damage caused by plant-parasitic species in the roots.

The results of these studies showed that earthworms have a remarkable potential to be used as an alternative in the biological control of plant-parasitic species in several crops; however, further studies are needed to elucidate the mechanisms involved in this process as well as to reveal the interactions with other plants.

3. Final considerations

The complete understanding of the effects of earthworms on nematode communities requires further studies. Considering the studies performed in controlled systems, earthworms seem capable of altering the communities of these invertebrates; however, the effects of other factors such as non-sterilization of the soil and addition of vegetal components could change the number of interactions that exist in this environment, often leading to the generation of

contradictory results. The lack of adequate and standardized methodologies for determining the interaction between these organisms and the different habits of life of the nematodes and earthworm species are factors that contribute to the differences found among studies. Nevertheless, this ecological complexity is a part of the soil; therefore, it should be considered in future studies.

Because of the potential to reduce the damage caused by plant-parasitic species, studies with different ecological categories of earthworms need to be performed to understand the interactions occurring in different species and the use of these invertebrates as a tool in the biological control of plant-parasitic nematodes.

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Exploration of Earthworms of India through Online Digital Library

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Additional information is available at the end of the chapter

Abstract

Online digital library (http://earthwormsofindia.com) for identification of earthworms of India has been developed for the first time. The database comprises digital keys for identification of earthworms of India, diagnostics, and mathematical parameters to provide a useful supplement for traditional morphological taxonomists and nonexperts in this area. This will scientifically broaden the taxonomic coverage of Indian earthworms. The genomic signatures with short sequences from standardized regions of the genome for 1192 specimens of earthworms were generated. Earlier, species identification of adult earthworms was possible only by dissection of the anterior end. However, this method is labor intensive, time-consuming, and very difficult for nonspecialists, particularly when dealing with field collections consisting of several different earthworm species. Furthermore, identification is limited to adult worms, as most life stages are unidentifiable and many morphological and anatomical characteristics of earthworms are variable, consequently, and the degree of variability can differ and features can overlap the taxa. The present work appears pioneer endeavor in this direction, as there appears no work available on the digitization of earthworms of India.

Keywords: identification key, earthworms of India, diagnostics of earthworms of India, computational key to identify earthworms, genomic signature, *coi-1*

1. Introduction

The scientific exploration of earthworm's diversity in India dates back to the nineteenth century. The credit for naming the first earthworm species in the Indian subcontinent goes to Templeton [1], when he discovered *Megascolex coeruleus* from Sri Lanka. However, Perrier [2] was the first to describe earthworm species from the Indian mainland. The significant

contributions on the taxonomy of Indian earthworm are of Bourne [3], Beddard [4], Michaelsen [5, 6], Stephenson [7–10], Aiyer [11], Gates [12–20], Jamieson [21], Julka [22–27] and Julka and Paliwal [28–30] Paliwal and Julka [31], Verma et al., [32] have provided comprehensive checklists for earthworms of different regions of India. The earthworm fauna of India is well reported as compared to other Asian Countries (excluding Myanmar).

2. Database of earthworms of India

Presently, 451 valid species/subspecies of earthworms under 71 genera are known from the Indian territory, including the islands of Andaman, Nicobar and Lakshadweep which have reported 9 families described in **Table 1** (as per Brinkhurst and Jamieson's classification of Oligochaeta, and Gates [33] classification of Megascolecidae), Endemism, both at genera and species level, is very high; about 71% of genera and 89% of species are endemic. Some exotic peregrine species of earthworms are also found, and these are now widespread in disturbed habitats following deforestation and intensive cultivation practices.

Sl No.	Family	Genus (No)	Species (No)*
1.	Acanthodrilidae	03	43
2.	Almidae	02	5
3.	Eudrilidae	01	1
4.	Lumbricidae	09	16
5.	Megascolecidae	14	149
6.	Moniligastridae	03	85
7.	Ocnerodrilidae	08	17
8.	Octochaetidae	30	134
9.	Rhinodrilidae	01	1
	Total families: 9	71	451

*Species incertae sedis: 8.

Table 1. Families of earthworms of India with genera and number of species.

3. Earthworm diversity in India

India is located 8.4–37.6°N latitude with covered area 3,287,797 km^2 and is rich country as far as its biodiversity is concerned. Considering the past geological history of the Indian subcontinent excluding (Burma, Nepal, and Pakistan) has been divided into six well-defined physiographic regions depending upon topography, climate, and vegetation viz. Western Himalayas, Eastern Himalayas, and Northeast ranges, Indo-Gangetic Plains, Central high lands, Peninsular plateaus, and Western Ghats including Sahyadri and Nilgiri hills (**Table 2**).

Family	Genus	Number of valid species
Acanthodrilidae	*Microscolex*	1
	Plutellus	41
	Pontodrilus	1
	Total	43
Almidae	*Progizzardus*	1
	Glyphidrilus	4
	Total	5
Eudrilidae	*Eudrilus*	1
	Total	1
Lumbricidae	*Allolobophoridella*	1
	Aporrectodea	3
	Bimastos	1
	Dendrobaena	2
	Dendrodrilus	1
	Eisenia	2
	Eiseniella	1
	Lumbricus	3
	Octolasion	2
	Total	16
Megascolecidae	*Amynthas*	13
	Comarodrilus	1
	Kanchuria	4
	Lampito	8
	Megascolex	33
	Metaphire	10
	Nelloscolex	2
	Notoscolex	10
	Perionyx	56
	Pheretima	1
	Pithemera	1
	Polypheretima	2
	Tonoscolex	7
	Troyia	1
	Total	149
Moniligastridae	*Desmogaster*	1
	Drawida	73
	Moniligaster	11
	Total	85

Family	Genus	Number of valid species
Ocnerodrilidae	*Curgiona*	1
	Deccania	1
	Eukerria	1
	Gordiodrilus	2
	Malabaria	5
	Nematogenia	1
	Ocnerodrilus	1
	Thatonia	5
	Total	17
Octochaetidae	*Bahlia*	1
	Barogaster	3
	Calebiella	1
	Celeriella	7
	Chaetocotoides	1
	Dashiella	1
	Dichogaster	5
	Eudichogaster	6
	Eutyphoeus	23
	Herbettodrilus	1
	Hoplochaetella	19
	Karmiella	2
	Konkadrilus	6
	Kotegeharia	1
	Lennogaster	7
	Mallehulla	1
	Octochaetoides	1
	Octochaetona	15
	Octonochaeta	1
	Parryodrilus	1
	Pellogaster	3
	Priodochaeta	1
	Priodoscolex	1
	Ramiella	5
	Rillogaster	2
	Scolioscolides	1
	Senapatiella	3
	Shimodrilus	2
	Travoscolides	3
	Wahoscolex	10

Family	Genus	Number of valid species
	Total	134
Rhinodrilidae	*Pontoscolex*	1
	Total	1

Table 2. Earthworms of India with valid number of species.

4. Diagnostics of families of earthworms of India

Earthworms of India belong to 9 families that may be identified with the following key:

1	Inconspicuous male pores	2
	Conspicuous male pores	3
2	Clitellum flared into wings; zygolobic prostomium; setae in regular rows body	**Almidae**
	Clitellum not flared; irregular arrangement of setae in caudal segments	**Rhinodrilidae**
3	Male pores in front of segment 15	4
	Male pores behind segment 15	5
4	Male pores at segment 13 or 15	**Lumbricidae**
	Male pores at intersegment grove 10/11	**Moniligasteridae**
5	Without dorsal pores	6
	With dorsal pores	7
6	Thin whitish worms, female pore tiny close to mid-ventral lines or at *b* lines on segment 14	**Ocnerodrilidae**
	Robust, dark-colored, female pore large slits close to lateral margins of segment 14, Male Pore on 17	**Eudrilidae**
7	Prostate tubular, holonephric	**Acanthodrilidae**
	Prostate tubular, meronephric	**Octochaetidae**
	Prostate racemose, meronephric	**Megascolecidae**

5. Databases and genus characteristics of earthworms of India

The characteristic features of each genus with available known species and their type locality are described in the library.

5.1. Family: Acanthodrilidae

5.1.1. Microscolex phosphorus (Duges, 1837)

- Male pores minute between apertures of *a* and *b* follicles at eq/xvii.

- Female pore at or slightly median to *a*, about halfway between xiii and xiv.

- Lumbricine, widely paired setae.

- Spermathecae one pair, diverticulate.

- Size 10–35 mm.

- Single species recorded in India.

- Type locality is Montpellier, France.

5.1.2. *Plutellus Perrier, 1873*

- Male pores in xviii, xix, or xx.

- Three pairs of kidney-shaped extramural calciferous glands.

- Dorsal pores present behind clitellum.

- Intestinal origin xiv, xv, xvi, xvii, or xviii.

- Spermathecae 1, 2, 3, 4, 5, 6, or 7 pairs in some or all of vi–xii.

- 41 species recorded from India.

5.1.3. *Pontodrilus litoralis (Grube, 1855)*

- Male pores in xviii, biprostatic

- Female pores in xiv.

- Spermathecal pores at vii/viii/ix.

- Genital markings oval unpaired, median usually in xi/xx, and sometimes in xii/xiii/xiv.

- Prostate tubular.

- Spermathecae paired in vii and ix with club-shaped diverticulum.

- Without typhlosole, calciferous, and supra-intestinal gland.

- Known to occur in mud with large content of organic matter and salt on seashores and margins of estuaries and brackish water lakes.

- Single species recorded in India.

- Type locality is French Riviera.

5.2. Family: Almidae

5.2.1. *Glyphidrilus Horst, 1889*

- Male pore Inconspicuous (not clearly visible), difficult to recognize and intraclitellar behind segment xvi.

- Lumbricine

- Clitellum annular and laterally flared into wings.

- Seminal vesicles in ix–xii.

- Four species recorded in India.

5.2.2. Progizzardus varadiamensis (Nair, 2010)

- Male pore in xiii segment.

- Lumbricine

- Four pair seminal vesicle.

- Multiple Spermathecae (4-5) and adiverticulate.

- Presence of genital markings in xxi–xxvi.

- Single species recorded in India.

- Type locality is Varadiam, Thrissur dist., Kerala, India.

5.3. Family: Eudrilidae

5.3.1. Eudrilus eugeniae (Kinberg, 1867)

- Male pores minute paired on segment xvii, penes retractable copulatory chamber.

- Female pores paired, large transverse slits, close to sides of the body on segment xiv.

- Lumbricine closely paired.

- Color reddish to purple or dark purplish.

- Y-shaped gland present that opens through own porophore into the copulatory chamber.

- Dorsal pores absent.

- Only one species found in India.

- Type locality is St. Helena Island, South Atlantic.

5.4. Family: Lumbricidae

5.4.1. Allolobophoridella eiseni (Levinsen, 1884)

- Male pores in xv.

- Male tumescences confined to median half of *bc.*

- Female pores in xiv, slightly lateral to *b.*

- Holandric, seminal vesicles in xi, xii.

- Tubercula pubertatis and TP glands absent.
- Single species recorded in India.
- Type locality is Deer Garden, Copenhagen.

5.4.2. Aporrectodea (Orley, 1885)

- Male pore inconspicuous and transverse slits on usually much elevated glandular area, which occupy xiv–xvi.
- Prostomium epilobic.
- Spermathecal pore at most three pairs or pairs of groups in *cd*.
- Seminal vesicles four pairs in ix–xii.
- Tubercula pubertatis absent.
- Three species recorded in India.

5.4.3. Bimastos parvus (Eisen, 1874)

- Color reddish.
- Length 23–46 mm.
- Lumbricine.
- Clitellum saddle-shaped segments xxiv–xxx.
- Tubercula pubertatis absent.
- Male pores on segment xv.
- Single species recorded in India.
- Type locality is Mount Lebanon, New York-New England, USA.

5.4.4. Dendrobaena Eisen, 1874

- Male pores at the bottom of equatorial clefts at or bear m*bc*. Male tumescences (swellings) large protuberant confined to xv and *bc* but often slightly dislocating xiv/xv and xv/xvi.
- Female pores at xiv just lateral to *b*.
- Tubercula pubertatis longitudinal bands of translucence slightly depressed just lateral to *b* in xxxi–xxxiii.
- Spermathecal pores in *cd*.
- Color dark red to slate.
- Size 20–60 mm.
- Two species recorded in India.

5.4.5. Dendrodrilus rubidus (Savigny, 1826)

- Male pores in xv between *bc*.

- Spermathecal pores ix/x/xi.

- Clitellum covers six segments in xxvii–xxxi.

- Tubercula pubertatis xxviii–xxx or only xxix–xxx.

- Genital tumescence surrounds setae *ab* in xvi, ix, xvii, xxii–xxv, and xxx.

- Dark red, pale ventrally.

- Size 20–100 mm.

- Single species recorded in India.

- Type locality is Paris, France.

5.4.6. Eisenia Gates, 1968

- Distinctive striped appearance because of alternating segmental bands of red and yellow color along the entire length of the body.

- Male pore on segment xv.

- Clitellum covering from segment xxvi to segment xxxii.

- Tubercula pubertatis on segment xxviii–xxx.

- Spermathecal pore two pairs, close to together on the upper side, in intersegment furrow ix/x, and x/xi.

- Two species recorded in India.

5.4.7. Eiseniella tetraedra (Savigny, 1826)

- Male pores in xv at or somewhat below *c*, each at the lateral end of *a* deep

- transverse cleft.

- Tubercula pubertatis longitudinally band like, uninterrupted by intersegment furrows.

- Color reddish brown with golden tinge.

- Single species recorded in India.

- Type locality is Paris, France.

5.4.8. Lumbricus Beddard, 1895

- Dark pigmented.

- Male pore inconspicuous and without glandular area.

- Spermathecal pore of two pairs in ix/x and x/xi in *cd*.

- Seminal vesicles of three pairs in ix, xi, and xii.
- Three species recorded in India.

5.4.9. Octolasion (Oerley, 1885)

- Male pores with large glandular area which encroach xiv and xvi.
- Female pore just lateral to *b* at eq/xiv.
- Tubercula pubertatis fused to form wall.
- Spermathecal pore two pairs in ix/x or x/xi in the region of *cd*.
- Four pairs of seminal vesicles in ix–xii.
- Two species recorded in India.

5.5. Family: Megascolecidae

5.5.1. Amynthas Kinberg, 1867

- Male pore paired in xviii (rarely xix) discharging directly onto the surface.
- Female pore single rarely paired on xiv.
- Spermathecal pores paired (bithecal), occasionally numerous, or single between iv/v and viii/ix.
- Prostate racemose.
- Copulatory pouches absent.
- Spermathecae usually paired, rarely multiple or single.
- Thirteen species recorded in India.

5.5.2. Comarodrilus gravely Stephenson, 1915

- Male pores on small conical papillae, which touch each other in the middle line.
- Spermathecal pores unpaired and at the median in vii/viii and viii/ix.
- Clitellum xiv–xvii (four segments).
- Gizzard in v.
- Prostate a compact glandular mass (racemose).
- Penial setae absent.
- Length 92 mm.
- Color gray.
- Single species recorded in India.
- Type locality is Thrissur, Kerala, India.

5.5.3. Kanchuria Julka, 1988

- Male pores paired in xviii.

- Megameronephridia absent.

- Four species recorded in India.

5.5.4. Lampito Michaelsen, 1909

- Male pores in xviii (combined with prostatic pores).

- Female pore in xiv.

- Spermathecae bidiverticulate with two small digitiform diverticula arising from lateral and median faces of the duct.

- Intestinal caeca and supra-intestinal glands absent.

- Meronephric: one pair enteronephric meganephridia with preseptal funnels from the region of xx.

- Eight species recorded in India.

5.5.5. Megascolex Beddard, 1895

- Male pores in xviii.

- Female pore in xiv.

- Spermathecal pores 1–5 pairs between segments iv and ix, lateral to mid-ventral.

- Penial setae present.

- Thirty-three species recorded in India.

5.5.6. Metaphire Sims and Easton, 1972

- Male pores (combined with prostatic pores paired) within copulatory pouches on xviii rarely on xix or xx.

- Female pore single rarely paired in xiv.

- Clitellum annular xiv–xvi.

- Spermathecal pores usually large transverse slits, rarely small paired, occasionally single or multiple between iv/v and ix/x.

- Meronephric, nephridia absent from the spermathecal ducts.

- Prostate gland racemose.

- *Metaphire* is different from *Amynthas* by the presence of copulatory pouches and differs from *Pheretima* by the absence of nephridia from the spermathecal ducts.

- Ten species recorded in India.

5.5.7. Nelloscolex Gates, 1939

- Male pores in seminal grooves confined to xvii.

- Female pores anteromedian to *a* in xiii.

- Calciferous glands unstalked and not constricted off from the esophagus.

- Calciferous lamellae within lateral swellings of esophagus in viii–xii.

- Dorsal pores present from region viii/ix–ix/x.

- Pigment lacking.

- Prostate strap-shaped.

- Spermathecal pores paired lateral to mid-ventral.

- Two species recorded in India.

5.5.8. Notoscolex Michaelsen, 1907

- Male pores in xviii.

- Spermathecal pores 1, 2, or 3 pairs, the last in viii/ix, lateral to mid-ventral.

- Very close to *Megascolex* only difference is arrangement of setae.

- Ten species recorded in India.

5.5.9. Perionyx Perrier, 1872

- Male pores (combined with prostatic pores) paired on xviii.

- Female pore intraclitellar and median.

- Spermathecal pores paired, lateral to mid-ventral.

- Dorsally pigmented reddish to blackish, ventrally nonpigmented.

- Holonephric: nephridia with preseptal funnels and postseptal loops that open in their own segments to the exterior through epidermal apertures.

- Typhlosole, intestinal and supra-intestinal glands absent.

- Ovaries are fan-shaped with several egg strings.

- Fifty-six species recorded in India.

5.5.10. Pheretima darnleiensis (Fletcher, 1886)

- Male pores paired within copulatory pouches in xviii.

- Gizzard present between vii/viii and ix/x.

- Copulatory pouches present often with secretory diverticulate.

- Meronephric, nephridia always present on spermathecal ducts.

- Single species recorded in India.

- Type locality is Darnley Island, Torres Strait, Australia

5.5.11. *Pithemera bicincta (Perrier, 1875)*

- Male pores paired in xviii small on a porophore.

- Preclitellar genital markings absent.

- Postclitellar genital markings present or absent when present paired on xviii or xviii/xix.

- Spermathecal pore five pairs in iv/v/vi/vii/viii/ix.

- Spermathecae present or absent when present five pairs in vi–x with a duct as long as the ampulla, diverticulum usually shorter than duct, and ampulla combined.

- Single species recorded in India.

5.5.12. *Polypheretima Michaelsen, 1934*

- Male pores on circular porophores in xviii.

- Perichaetine, setae never excessively crowded ventrally, creeping sole absent.

- Crescentric genital markings absent.

- First dorsal pore between iv/v and xii/xiii.

- Two species recorded in India.

5.5.13. *Tonoscolex Gates, 1933*

- Male pores in xvii and in seminal grooves that extend into another segment.

- Quadrithecal pores at vi/vii–vii/viii.

- Gizzzard in vi.

- Discrete calciferous glands stalked in viii–xii, ix–xii, x–xii.

- Intestinal caeca and supra-intestinal glands absent.

- Meroic, V-shaped, exoic micronephridia on parietes from iv and especially numerous in clitella segments.

- Prostate strap-shaped extending through several segments, duct short, soft, and joined entally by the sperm duct.

- Spermathecae each with a diverticulum shorter than the main axis.

- Seven species recorded in India.

5.5.14. Troyia gundarshola Jamieson, 1977

- Male pores in *ab* near *a* of xviii relative to adjacent segments each with wide tumid lips, which constitute papillae.

- Single pair of the tubular racemose prostate in xviii.

- Three pairs extramural calciferous glands in xiii, xiv, and xv.

- Intestine begins in xvi.

- Meronephric, enteronephric tufts in v, caudally with numerous preseptal nephrostomal funnels on each side in each segment corresponding with post septal exonephric avesiculate micromeronephridia.

- Megameronephridia absent.

- Spermathecae one pair in viii with single elevate diverticulum.

- Single species recorded in India.

- Type locality is Gundar Shola, Palni Hills, Tamil Nadu, India.

5.6. Family: Moniligastridae

5.6.1. Desmogaster farina Gates, 1943

- Two pairs of male pores xi/xii and xii/xiii.

- Female pores at or just lateral to *b* and at or slightly behind xiii/xiv.

- Spermathecae adiverticulate.

- Single species recorded in India.

- Type locality in Tingpai, Myitkyina district, Myanmar.

5.6.2. Drawida Michaelsen, 1900

- Male pores at or near x/xi.

- Female pores at or just behind xi/xii.

- Spermathecal pores at vii/viii.

- Spermathecae without a stalked glandular mass in the association.

- Seventy-one species recorded in India.

5.6.3. Moniligaster Perrier, 1872

- Male pores in x/xi.

- Spermathecae with a bifid muscular atrial chamber, each horn of which bears a lobulated glandular mass.

- 11 species recorded in India.

5.7. Family: Ocnerodrilidae

5.7.1. Curgiona narayani (Michaelsen, 1921)

- Male pores in papillae just behind eq/xvii.

- Copulatory chambers in xvii into which sperm duct open.

- Gizzard in vii and ventromedian calciferous gland in ix and x.

- Single species recorded in India.

- Type locality is River Hatti at Madapur, Coorg, S. India, and is endemic.

5.7.2. Deccania alba Gates, 1949

- Male pores paired minute in seminal grooves on xviii near *a*.

- Prostatic pores paired, minute at the ends of seminal grooves on xvii and xix in

- Spermathecal pores paired inconspicuous in vii/viii/ix at *b*.

- Genital markings paired usually presetal on xx–xxi at *ab* sometimes on ix, x, xiv, and xv at *bc* or *ab*.

- Clitellum saddle-shaped.

- Length 47–66 mm.

- Single species recorded in India.

- Type locality is Baraila (near Jabalpur), MP, India, and is endemic.

5.7.3. Eukerria kukenthali (Michaelsen, 1908)

- Male pores in xvii.

- Quadrithecal pores at mAB, at vii/viii–viii/ix.

- Calciferous glands one pair in ix with a small central lumen a triangular in section.

- A solid "clear gland" with short stalk protrusible from each prostate-pore invagiation.

- Single species recoded in India.

- Type locality is Thomas Isl., West Indies.

5.7.4. Gordiodrilus Beddard, 1895

- Male pores and prostatic pores approximated xvii/xviii.

- Spermathecae without diverticulate on the duct, often with evaginations at the ectal end of the ampulla.

- Two species recorded in India.

5.7.5. Malabaria Gates, 1942

- Male and prostatic pores in xvii.

- Holonephric, large nephridia.

- Dorsal pores and pigment lacking.

- Five species recorded in India.

5.7.6. Nematogenia panamaensis (Eisen, 1900)

- Male pores in xvii.

- Seminal vesicles in xii, large.

- Paired calciferous glands in ix, each with the fairly thick wall (n large canal) and a central, vertically slit like lumen, a long and slender stalk bound in a U-loop against the esophagus.

- Single species recorded in India.

- Type locality is Panama.

5.7.7. Ocnerodrilus occidentalis Eisen, 1878

- Male pores in xvii.

- Prostomium epilobic, tongue usually open but may be closed, or even unrecognizable.

- Single species recorded in India.

- Type locality is Fresno, California, USA.

5.7.8. Thatonia Gates, 1942

- Male pores in xviii.

- Gizzard in vii, a dendritically branched system of spaces (opening into gut lumen) in the thickened ventral wall of esophagus in ix–x.

- Holonephric, avesiculate, and ducts passing parietes to b.

- Five species recorded in India.

5.8. Family: Octochaetidae

5.8.1. Bahlia albida Gates, 1945

- Male and prostatic pores paired in seminal grooves on xvii.

- Two pairs of discrete intramural calciferous glands in xi–xiii.

- Genital markings unpaired and median with two central translucent areas, post setal on xviii–xix, sometimes on xvi, xx, and xxi at aa or bb.

- Type locality is found in Allahabad, UP, India, and is endemic.

5.8.2. Barogaster Gates, 1939

- Combined male and prostatic pores paired with xviii, seminal grooves absent.

- Typhlosole lamelliform simple and "grid-like" thickening present at the posterior end of typhlosole.

- Genital markings present.

- Three species recorded in India.

5.8.3. Calebiella parva Gates, 1945

- Male pores paired in seminal grooves on xviii.

- Prostatic pores paired at the end of seminal grooves on xvii and xix.

- Typhlosole lamelliform, bifid ventrally at the anterior portion.

- Single species recorded from Partapgarh, UP, India, and is endemic.

5.8.4. Celeriella Gates, 1958

- Combined male and prostatic pores paired, on xviii.

- Intestinal caecae, supra-intestinal glands, and typhlosole absent.

- Prostates paired in xviii, extending posteriorly through several segments, and vasa deferential on each side join the prostatic duct entally.

- Seven species recorded from India.

5.8.5. Chaetocotoides montanus (Stephenson, 1920)

- Male pores paired in seminal grooves on xviii, prostatic pores paired at the ends of seminal grooves on xvii, and xix.

- One pair of discrete extramural calciferous gland present. Each gland bilobed, one lobe in xv and other in xvi, opening into gut in xv slightly anterior to septum xv/xvi.

- Single species found in India.

- Type locality is Panchgani, W. Ghats and Maharashtra, India, and is endemic.

5.8.6. Dashiella khandalaensis Julka, 1988

- Combined male and prostatic pores paired on xvii; seminal grooves absent.

- Genital markings are circular to oval, paired, and presetal on xvi and xvii at *ab*.

- Spermathecal pores in paired batteries of 1-4 (polythecal) on vi/vii/viii

- Single species found in India.

- Type locality is Khandala, W Ghats, Maharashtra, India, and is endemic.

5.8.7. Dichogaster Beddard, 1888

- Male pores paired in seminal grooves on xviii or xvii/xviii; prostatic pores two pairs at the ends of seminal grooves.

- esophagus with two gizzards anterior to septum viii/ix.

- Each calciferous gland trilobed, a vertically reniform lobe in each of segments xv–xvii with a common duct opening into gut in xvi.

- Five species recorded in India.

5.8.8. Eudichogaster Michaelsen, 1902

- Male pores paired in seminal grooves on xviii; prostatic pores paired at the end of seminal grooves on xvii and xix.

- Two pairs of discrete extramural calciferous glands in xi–xii.

- Six species recorded in India.

5.8.9. Eutyphoeus Michaelsen, 1900

- Male pore paired just posterior to prostatic pores on xvii; seminal grooves absent.

- Prostatic and male pore paired near the setal arc of xvii, discharging within vestibula or directly into the body surface.

- Typhlosole lamelliform, ending posteriorly with a short series of supra-intestinal glands.

- 23 species recorded in India.

5.8.10. Herbettodrilus bahli Julka, 2004

- Male pores paired in seminal grooves on xviii.

- Prostatic pores paired at ends of seminal grooves on xvii and xix.

- One pair of discrete, extramural calciferous glands, each gland bilobed, one lobe in xv and the other in xvi, both discharging into gut through a common duct in xv close to attachment of septum xiv/xv.

- Micromeronephridia astomate, enteronephric paired tufts in iii, few scattered on body wall in xiv and posteriad segments; paired, stomate, enteronephric megameronephridia in xv and posteriad segments, discharging into rectum through paired excretory canals.

- Single species recorded in India.

- Type locality is Herbettu, Shimoga dist., Karnataka, India, and is endemic.

5.8.11. Hoplochaetella Michaelsen, 1900

- Combined male and prostatic pores two pairs on xvii or xvii/xviii and xix or xviii/xix, seminal grooves absent.

- Genital markings present or absent when present with or without central apertures.

- 19 species recorded in India.

5.8.12. Karmiella Julka, 1983

- Male pores paired, in seminal grooves, on xviii; prostatic pores paired at the end of seminal grooves, on xvii and xix.

- Stomate megameronephridia present at least in caudal segments; micromeronephridia astomate throughout the body.

- Calciferous gland one pair, each gland bilobed one lobe in xv and the other in xvi.

- Two species recorded in India.

5.8.13. Konkadrilus Julka, 1988

- Male pores paired in seminal grooves on xviii; prostatic pores paired at the end of seminal grooves on xvii and xix.

- Discrete calciferous glands, intestinal caeca, and supra-intestinal glands absent.

- Spermathecae paired in viii and ix.

- Six species recorded in India.

5.8.14. Kotegeharia gatesi Julka, 1988

- Male pores paired, in seminal grooves on xviii; prostatic pores paired at the end of seminal grooves on xvii and xix.

- Single species recorded in India.

- Type locality is Kotegehar, Karnataka, India, and is endemic.

5.8.15. Lennogaster Gates, 1939

- Male pores paired in seminal grooves on xviii or xvii/xviii; prostatic pores two pairs at the end of seminal grooves on xvii and xix or one pair in xvii.

- Three pairs discrete extramural calciferous glands in x–xii.

- Paired stomata mega-meronephridia in caudal segments.

- Seven species recorded in India.

5.8.16. *Mallehulla indica Julka and Rao, 1982*

- Male pores paired in seminal grooves on xviii; prostatic pores paired at the end of seminal grooves on xvii and xix.

- Prostate paired in xvii and xix extending posteriorly to two segments.

- Single species recorded in India.

- Type locality is Moodabidri, Karnataka, India, and is endemic.

5.8.17. *Octochaetoides aitkeni (Fedarb, 1898)*

- Male pores paired on xviii in seminal grooves, prostatic pores paired at the end of seminal grooves, on xvii and xix.

- Spermathecal pores paired in viii and ix, each with a tubular diverticulum arising from about the middle of the duct.

- Single species recorded in India.

- Type locality is Travancore, Kerala, India, and is endemic.

5.8.18. *Octochaetona Michaelsen, 1922*

- Male pores paired on xviii in seminal grooves, prostatic pores paired at the ends of seminal grooves on xvii and xix.

- Esophagus with a single gizzard in vi or in a space between septa iv/v and vii/viii or viii/ix or ix/x.

- One pair discrete extramural asymmetrical calciferous glands opening into gut close to the attachment of septum v/vi.

- Prostate paired in xvii and xix extending posteriorly to a few segments.

- Fifteen species recorded in India.

5.8.19. *Octonochaeta rosea (Stephenson, 1926)*

- Male pores paired in seminal grooves on xviii, prostatic pores paired at the end of seminal grooves on xvii and xix.

- Esophagus with a single gizzard in a space between septa iv/v and vii/viii and one pair of discrete extramural calciferous glands.

- Single species recorded in India.

- Type locality is Secunderabad, AP, India, and is endemic.

5.8.20. Octonochaeta rosea (Stephenson, 1926)

- Male pores paired in seminal grooves on xviii, prostatic pores paired at the end of seminal grooves on xvii and xix.

- Single species recorded in India.

- Type locality is Secunderabad, AP, India, and is endemic.

5.8.21. Parryodrilus lavellei Julka, 1997

- Male pores (combined male and prostatic pores) paired, large slits in line with *b* discharging directly on the body surface of xviii, at lateral ends of transverse furrow across a tumescent male field extending laterally to mid *bc*.

- Single species recorded in India.

- Type locality in Nilambur, Malappuram dist., Kerala, India, and endemic.

5.8.22. Pellogaster Gates, 1939

- Male pores paired in seminal grooves on xviii, prostatic pores paired at the end of seminal grooves on xvii and xix.

- Prostate two pairs in xvii and xix.

- Three species recorded in India.

5.8.23. Priodochaeta pellucida (Bourne, 1894)

- Combined male and prostatic pores on xviii.

- Esophagus with single large gizzard in v.

- Single species recorded in India.

- Type locality is Coonoor, Nilgiri Hills, South India, and is endemic.

5.8.24. Priodoscolex montanus Gates, 1940

- Combined male and prostatic pores on xviii.

- Three pairs extramural calciferous glands in xiv–xvi.

- Single species recorded in India.

- Type locality is Coonoor, Nilgiri Hills, South India, and is endemic.

5.8.25. Ramiella Stepheson, 1921

- Male pores paired in seminal grooves on xviii; prostatic pores paired at the end of seminal grooves on xvii and xix.

- Esophagus with a single gizzard in v or vi and calciferous lamellae in viii–xi.

- Discrete calciferous glands and supra-intestinal glands absent.

- Genital markings usually present.

- Five species recorded in India.

5.8.26. Rillogaster Gates, 1939

- Combined male and prostatic pores paired on xix; Seminal grooves absent.

- Esophagus with two gizzards in vi and vii.

- Typhlosole in the form of low ridge.

- Spermathecal pores paired in vii/viii.

- Genital markings present.

- Spermathecae paired diverticulate.

- Two species recorded in India.

5.8.27. Scolioscolides bergtheili (Michaelsen, 1907)

- Combined male and prostatic pores paired on xviii.

- Esophagus with a single gizzard between septa v/vi and viii/ix.

- One pair discrete intramural calciferous glands in xii.

- Holandric, testes, and male funnels in x enclosed in U-shaped sacs.

- Spermathecae one pair in viii, each with a median and a lateral bi or trilobed ental diverticula, duct shorter than ampulla.

- Exonephric megameronephridia in each segment posterior to the supra-intestinal glands.

- Single species recorded in India

- Type locality is Sandakphu, Darjeeling dist., W Bengal, India, and is endemic.

5.8.28. Senapatiella Julka, 2004

- Male pores paired in seminal grooves on xviii.

- Prostatic pores paired at ends of seminal grooves on xvii and xix.

- Single gizzard in vi.

- One pair bilobed extramural calciferous gland is present. One lobe in xiv and other in xv.

- Micromeronephridia astomate, exonephric.

- Megameronephridia in caudal segments.

- Prostomium tanylobic.

- Three species recorded in India.

5.8.29. Shimodrilus Julka, 2004

- Male pores paired in seminal grooves on xviii.

- Prostatic pores paired at ends of seminal grooves on xvii and xix.

- Prostomium prolobic to proepliobic.

- Single gizzard in v.

- Large extramural calciferous glands in xvi with calciferous ridges extending to xviii.

- Intestinal caeca and supra-intestinal glands absent.

- Typhlosole simple lamellicorn.

- Penial setae present.

- Micromeronephridia astomate, exonephric, small paired tufts on body wall in ii–iv on septa in v–xii, in clusters of iv–v in xiii, and posteriad segments.

- Paired stomata exonephric megameronephridia lateral to micromeronephridia in xvii and posteriad segments.

- Two species recorded in India.

5.8.30. Travoscolides Gates, 1940

- Combined male and prostatic pores on xviii.

- Esophagus with a single gizzard in v and four pairs of discrete calciferous glands in x–xiii.

- Supra-intestinal glands absent.

- Typhlosole simple lamelliform.

- Megameronephridia absent.

- Spermathecal pores paired in or close to vii/viii/ix.

- Genital markings absent.

- Holandric, seminal vesicles in xi and xii.

- Prostate paired in xviii.

- Penial setae present.

- Spermathecae paired in viii and ix.

- Three species recorded in India.

5.8.31. Wahoscolex (Julka, 1988)

- Male pores paired in seminal grooves on xviii or xvii/xviii or on porophores on xvii; prostatic pores two pairs at the end of seminal grooves on xvii and xix or xvii and xviii or one pair on xvii.

- Esophagus with a single gizzard in v and with calciferous lamellae in in xvi.

- Discrete calciferous glands, caeca, and supra-intestinal gland absent.

- Typhlosole simple lamelliform.

- One or two pair stomate enteronephric megameronephridia in each segment from about middle of the body to posterior end.

- Testes holandric.

- Spermathecae paired or unpaired.

- Ten species recorded in India.

5.9. Family: Rhinodrilidae

5.9.1. Pontoscolex corethrurus (Muller, 1856)

- Male and spermathecal pores minute and inconspicuous.

- Female pore small transverse slit at left side of mid-ventral line just in front of intersegment furrow xiv/xv.

- Lumbricine in regular furrow, but enlarged and located in alternate positions on adjacent segments in tail region.

- Clitellum saddle-shaped generally covering segments xv–xxii/xxiii.

- Dorsal pores absent.

- Calciferous glands visible through the body wall in live specimen.

- Digestive system with paired "Panicled tubular" calciferous glands in vii–ix.

- First Segment and snout (prostomium) often elongated as a long thin proboscis when the worm is exploring the area.

- Body pale, light pink in head.

- Single species found in India and is exotic in origin.

- Type locality is found in Itajai, Brazil.

6. Conclusions

First systematic comprehensive earthworm database of India developed for identification of earthworms to provide a useful supplement for traditional morphological taxonomists and nonexperts in this area.

Acknowledgements

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Vermicomposting

Earthworms and Vermicomposting

Jorge Domínguez

Additional information is available at the end of the chapter

Abstract

Vermicomposting, the conversion of organic waste into vermicompost, is mediated by the combined action of earthworms and microorganisms. This interesting and attractive alternative to regular composting turns organic waste into a substrate that can be used as a soil amendment and as a growing medium for use in horticulture. Soil is not required in vermicomposting as the organic matter acts as both the substrate and food, and therefore only epigeic earthworms can be used in the process. Several earthworm species have been evaluated for their potential use in vermicomposting, including *Eisenia fetida* (Savigny), *Eisenia andrei* (Bouché), *Dendrobaena veneta* (Rosa), *Dendrobaena hortensis* (Michaelsen) *Eudrilus eugeniae* (Kinberg), and *Perionyx excavatus* (Perrier). The species most commonly used in vermicomposting and vermiculture facilities worldwide are *Eisenia andrei* and *Eisenia fetida*. This chapter reviews and updates the controversy surrounding the taxonomic differentiation between *E. andrei* and *E. fetida*, and between *D. veneta* and *D. hortensis*, showing that these are all different species and emphasizing the importance of maintaining pure cultures in vermicomposting systems. In the final section, methods of cultivating epigeic earthworms to ensure high rates of growth and reproduction are described.

Keywords: earthworms, *Eisenia andrei*, *Eisenia fetida*, *Dendrobaena veneta*, *Dendrobaena hortensis*, vermicomposting, vermiculture, epigeic, red worms, tiger worms, earthworm culture

1. Introduction

Earthworms (Crassiclitellata) are terrestrial oligochaetes that usually live in the soil. These invertebrates constitute the largest animal biomass in most temperate ecosystems, where they strongly influence the physical, chemical, and biological properties of soil. They play a key role in modifying soil structure and accelerating the decomposition of organic matter and

nutrient cycling, ultimately shaping the structure and composition of the aboveground plant community.

Earthworms have a burrowing lifestyle and simple body structure, leading to the commonly held belief that there is only one type of this not very pretty soil creature. However, earthworms constitute a highly diverse group of burrowing annelids, including more than 6000 extant species. For the vast majority of these, only the name and morphology are known, and nothing is known about their biology and ecology. Different species of earthworms have different life strategies and occupy different ecological niches. Earthworms have thus been classified on the basis of their feeding habits and the part of the soil profile that they inhabit into three main ecological categories: epigeic, anecic, and endogeic. These categories can be difficult to establish and some species cannot be accurately assigned to any of them. In agricultural soils, earthworms usually burrow deeper than they do in grasslands and forest soils. Epigeic earthworms live in the organic horizon, on or near the soil surface, and they mainly feed on decaying organic matter such as vegetable and animal debris. They are usually small, pigmented, and have high metabolic and reproductive rates that allow them to adapt to the changing environmental conditions of the soil surface. They also display high rates of consumption, digestion, and assimilation of organic matter and play a key role as litter transformers, producing holorganic casts. Epigeic lumbricids include the species *Dendrobaena veneta*, *Dendrobaena hortensis*, *Dendrobaena octaedra*, *Eisenia fetida*, *Eisenia andrei*, *Dendrodrilus rubidus*, *Eiseniella tetraedra,* and *Allolobophoridella eiseni*. By contrast, endogeic earthworms live deeper in the soil profile and feed mainly on soil and the associated organic matter. These worms have little pigmentation and construct highly branched horizontal galleries, which become filled with excrement as the worms move along the organic-mineral horizon of the soil. Endogeic earthworms have lower reproduction rates and longer life cycles than epigeic earthworms and are more resistant to unfavorable conditions such as drought and lack of food. Most earthworms, including *Aporrectodea caliginosa*, *Aporrectodea rosea,* and *Octolasion lacteum*, belong to this category. Anecic earthworms live more or less permanently in vertical galleries, which can extend for several meters throughout the soil profile. These species surface at night to feed on litter, feces and decomposing organic matter, which they transport to their galleries. They deposit their excreta on the surface, at the opening of their galleries, in the form of conspicuous earthworm casts. These earthworms are usually large and dark brown in color. They have relatively low reproductive rates and long life cycles. The night crawler *Lumbricus terrestris* is a typical anecic earthworm.

Vermicomposting, the transformation of organic waste into vermicompost, is a biooxidative mesophilic process in which detritivorous earthworm species interact with microorganisms, strongly affecting decomposition processes, accelerating the stabilization of organic matter, and greatly modifying its physical, chemical, and biological properties [1–4]. Vermicomposting and vermiculture are well established worldwide and are important for economic and environmental reasons [5]. As organic matter acts as both the substrate and food in vermicomposting, and soil is not involved, only epigeic earthworms can be used in the process. Among the epigeic earthworms, *Eisenia andrei* and *Eisenia fetida* are the species most widely used in vermicomposting and vermiculture facilities worldwide.

In nature, epigeic species occupy unpredictable and unstable habitats, characterized by highly variable environmental conditions, food availability, and predation pressures. When conditions are unfavorable, epigeic earthworms suffer high mortality, the population density oscillates widely (**Figure 1**), and the reproduction rate increases greatly [6]. Under these
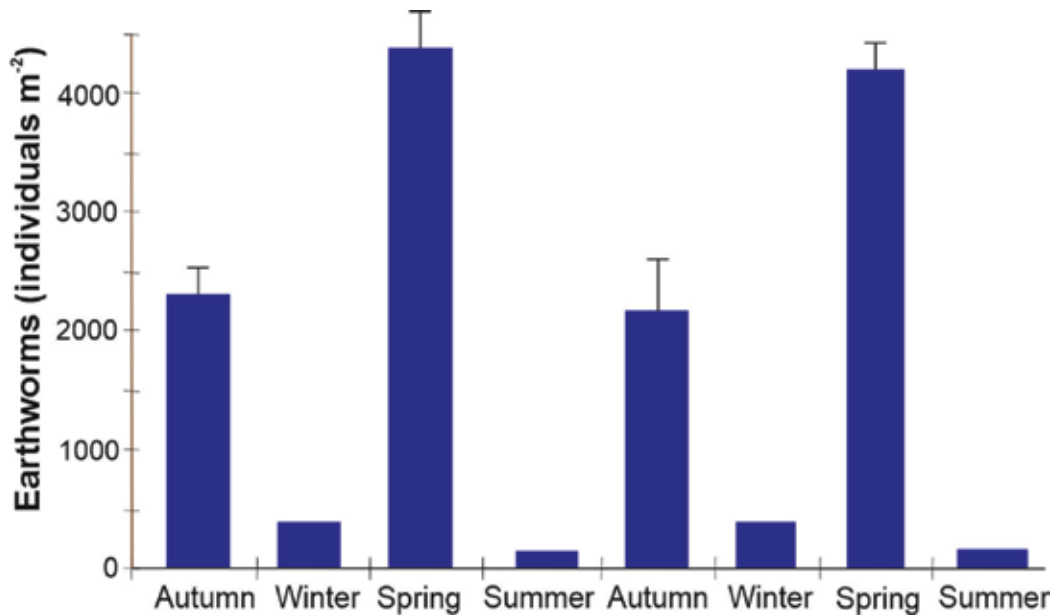
Figure 1. Seasonal fluctuations in earthworm density in a field population of the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae) in Vigo (Pontevedra, Spain).

circumstances, the ability to grow and reproduce exponentially is critical. From the point of view of their life history, epigeic earthworms are typical "r-strategists" or fast developers in the slow-fast continuum. Fast or r-selected organisms have typically short life cycles, are small, attain sexual maturity rapidly, and have high metabolic rates. Under unfavorable environmental conditions, high reproduction rates will ensure population survival, and the formation of cocoons may enable the worms to resist until conditions become more favorable, thus explaining the fluctuations in population density.

The favorable, stable conditions, and high reproduction rates enable earthworm populations to reach extremely high densities in vermicomposting facilities (more than 20,000 individuals m^{-2}, [7]).

2. *Eisenia fetida* and *Eisenia andrei* are different species

The importance of taxonomy is well recognized by most scientists and, indeed, without reliable taxonomy, most ecological studies are irrelevant [8]. In many species of earthworms, taxonomic identification based on morphological characteristics is difficult due to the structural simplicity of the earthworm body plan, which lacks anatomical complex structures or highly specialized copulatory appendages [9, 10]. *Eisenia fetida* and *Eisenia andrei* (**Figure 2**) are closely related species of earthworms that are widely used in vermicomposting systems to recycle organic waste, as well as in ecotoxicological, physiological, and genetic studies. These species are widely used because they are ubiquitous, have short life cycles, high reproductive rates, are tolerant to a wide range of temperature and humidity, and are relatively easy to handle Domínguez [1] and Domínguez and Edwards [11].

Figure 2. Photographs of *Eisenia andrei* (top panel) and *Eisenia fetida* (bottom panel) collected in Vigo (Pontevedra, Spain).

Both species were originally described as different morphotypes of *E. fetida* according to differences in body pigmentation [12]. Bouché (1972) later gave these earthworms subspecific status, naming them *E. foetida foetida* and *E. foetida unicolor* [13]. Although many authors now accept that *E. fetida* and *E. andrei* are different species, the oldest literature and also much current literature refer to these species collectively as *E. fetida* or *E. foetida*, an incorrect version of the original *E. fetida* [14, 15]. *Eisenia fetida* is the striped morph and the area between the segments has no pigmentation or is yellow or pale yellow, hence its common name of striped worm or tiger worm. By contrast, *E. andrei*, the common red worm, is uniformly red in color. Apart from the differences in pigmentation (**Figure 2**), the species are morphologically similar (**Figures 3** and **4**) with no differences in biological parameters, especially in relation to reproductive potential and life cycles, although the rates of growth and cocoon production are somewhat higher in *E. andrei* than in *E. fetida* [16]. The life cycles of *E. fetida* and *E. andrei* are well known and their population biology and ecology have been investigated by several authors and summarized by Domínguez [1] and Domínguez and Edwards [11].

A long-standing research project conducted in the soil ecology laboratory at the University of Vigo has resolved the problem of the taxonomic status of these two species; however, in much of the current literature, both species are still indiscriminately referred to as *E. fetida*, and it
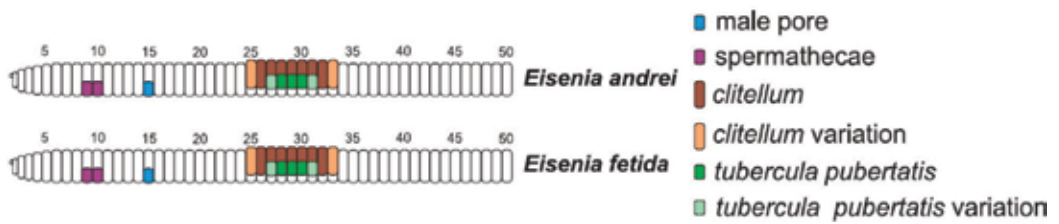
Figure 3. Diagram of the external morphology of *Eisenia andrei* and *Eisenia fetida*, showing that the two species are morphologically similar.
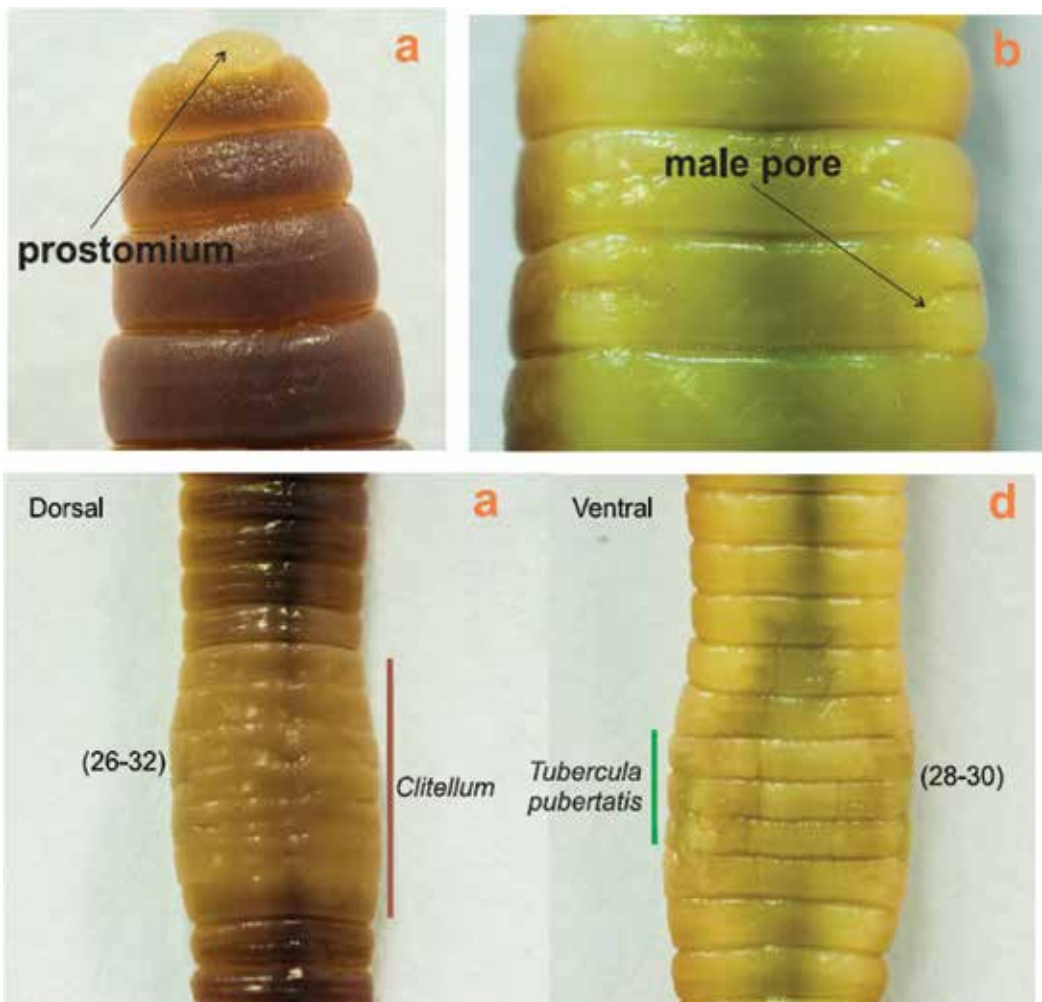
Figure 4. External morphology of the red worm *Eisenia andrei*. (a) Dorsal view of prostomium, peristomium, and first segments. (b) Male pores in the ventral side of segment 15. (c) Dorsal view of the clitellum in segments 26–32. (d) Ventral view of the tubercula pubertatis in segments 28–30. These external morphological characters are commonly used to distinguish between earthworm species.

is often not clear which of the two species is actually being considered. The objective of our research was to determine whether *E. andrei* and *E. fetida* are biologically and phylogenetically different species. We conducted laboratory experiments to determine the existence of any prezygotic or postzygotic reproductive barriers by comparing cocoon and hatchling production in interspecific and intraspecific crosses of the two species. We then used molecular phylogenetic methods data based on mitochondrial and nuclear DNA sequences to identify any differences between populations of *E. fetida* and *E. andrei*.

Four different populations of worms were used to study reproductive isolation: one population of *E. fetida* from Vigo (northwestern Spain) and three populations of *E. andrei* from Vigo, Madrid, and Brazil. Juveniles (<150 mg f.w.) were maintained in individual Petri dishes until maturity, to ensure that worms did not store sperm from previous copulations. The worms were supplied with food in the dishes, which were held in incubated chambers at 20°C and relative humidity 90%. When the worms reached sexual maturity, individuals from different populations were crossed. Individuals were randomly assigned for crossing, although the weight of the partners at each crossing was similar. Each pair of worms was held in a Petri dish for 7 days. Each worm was then placed in its original Petri dish, and cocoon production, incubation time, viability rate, and the number of hatchlings per cocoon were recorded weekly for 15 weeks. For the phylogenetic delimitation, 20 individuals of *E. andrei* from 4 populations (Brazil, Ireland and Spain [Vigo and Madrid]) and 11 individuals of *E. fetida* from 3 populations (Ireland and Spain [Vigo and Santiago de Compostela]) were used. Six individual specimens of *E. eiseni* (Levinsen, 1884) from Spain (Vigo and Santiago de Compostela) were used as outgroup.

The biological definition of a species is a group of individuals that can reproduce with one another in nature and produce fertile offspring. The crossbreeding experiment demonstrated that *E. fetida* and *E. andrei* are reproductively isolated as their crosses do not produce viable offspring (**Figure 5**). Although there were no significant differences in the rate of cocoon production in the intra and interspecific crosses of *E. fetida* and *E. andrei*, there were significant differences in cocoon viability. Thus, only the intraspecific crosses of both *E. fetida* and *E. andrei* produced viable cocoons (i.e., cocoons that produced hatchlings) [8].

In another crossbreeding experiment (*E. andrei x E. fetida*, *n* = 15; food: cow manure) carried out in the laboratory in 2016, the interspecific crosses did not produce cocoons. The study findings reject the possible existence of a single polymorphic species of *E. fetida* (including *E. andrei*), and we suggest that, as both phenotypes can be easily distinguished, the "good species" status can be applied to the studied taxa. Furthermore, our findings reveal that the reproductive isolation between *E. andrei* and *E. fetida* occurs post copulation and is probably postzygotic, with no efficient mechanism preventing interspecific copulations. In fact, both the interspecific and intraspecific crosses of the species produced similar numbers of cocoons, revealing that there are no mechanisms preventing copulation or cocoon production.

Although they are very similar, *E. andrei* and *E. fetida* are biologically different species and, as a consequence, the coexistence of both species in mixed cultures inevitably leads to poorer functioning of the vermicomposting system. The abundance and frequency of citations in the specialized and nonspecialized literature that indiscriminately refer to *E. andrei* and *E. fetida* as different names for the same species suggest that mixed cultures of both species are also quite common. In mixed cultures, the reproduction rate and biological efficiency will be much lower than in pure cultures because earthworms will waste energy in carrying out unsuccessful copulations.
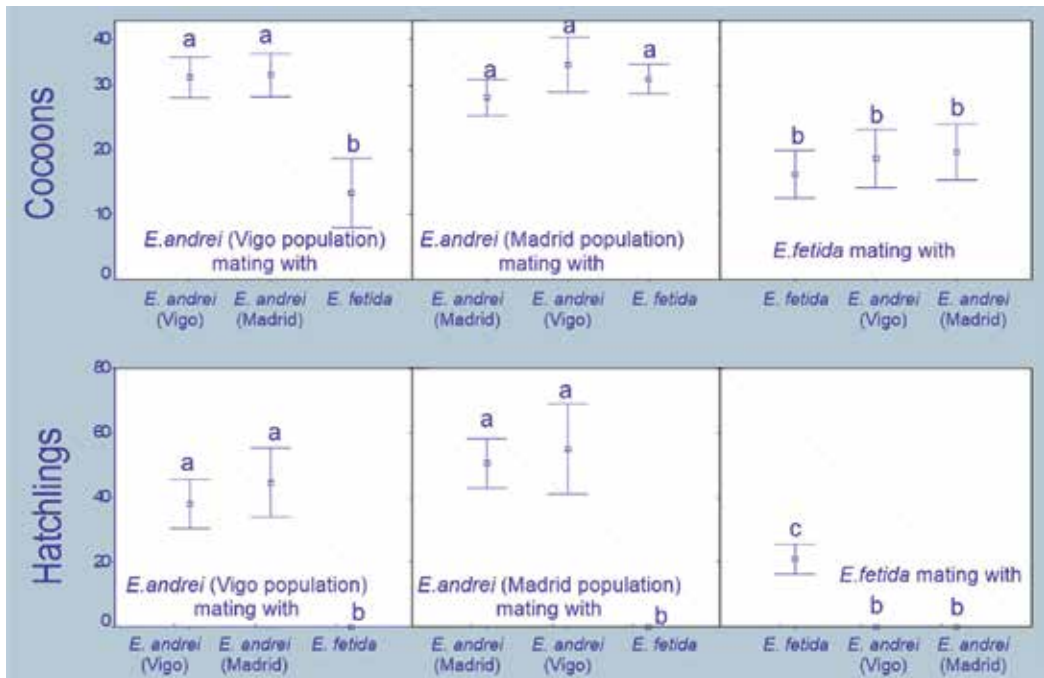
Figure 5. Results of crossbreeding experiments with the red worm *Eisenia andrei* and the tiger worm *Eisenia fetida*. Upper panel: Cocoon production (number of cocoons per earthworm) over a period of 15 weeks in the intra- and interspecific crosses. Lower panel: Hatchling production (number of hatchlings per earthworm) over a period of 15 weeks in the intra- and interspecific crosses.

This argument also applies to another two earthworm epigeic species often used in vermiculture and vermicomposting: *Dendrobaena veneta* and *Dendrobaena hortensis*. Although the names are often considered synonyms, *Dendrobaena hortensis* and *Dendrobaena veneta* are actually phylogenetically different species (see **Figure 8**; [17]). *Dendrobaena veneta* is two times larger (50–150 mm) than *D. hortensis* and the body color is also different. The dorsal side of *D. hortensis* has red-violet stripes and the ventral side is pale red, whereas *D. veneta* is uniformly red and is not striped. However, apart from the differences in pigmentation and size, both species are morphologically similar (**Figure 6**) and their biological parameters are not well known, mainly due to this taxonomic confusion.

These species can also be confused with *E. andrei* and *E. fetida* on examination by the naked eye. When more than one species coexist in vermicomposting systems, the reproduction rates

Figure 6. Diagram of the external morphology of *Dendrobaena veneta* and *Dendrobaena hortensis*, showing the morphological similarities between the two species.
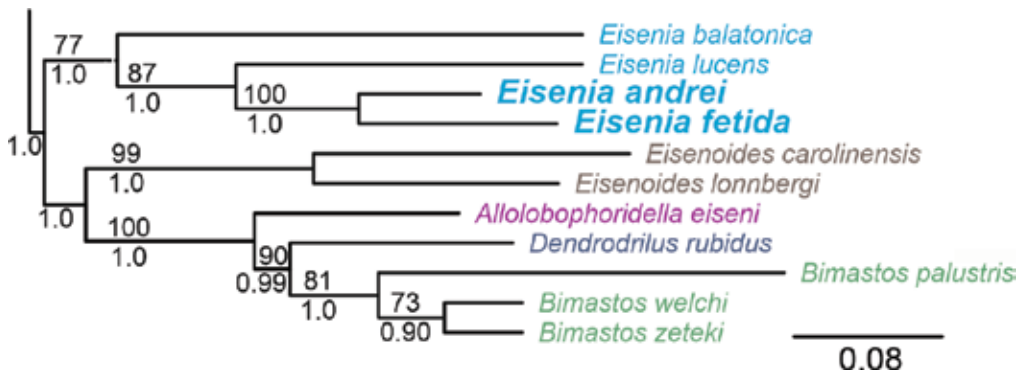
Figure 7. Clade including the species *Eisenia andrei* and *Eisenia fetida* extracted from the maximum likelihood molecular tree of the family Lumbricidae. The genus *Eisenia* is monophyletic and *E. andrei* and *E. fetida* are phylogenetically different species. Modified from [17].
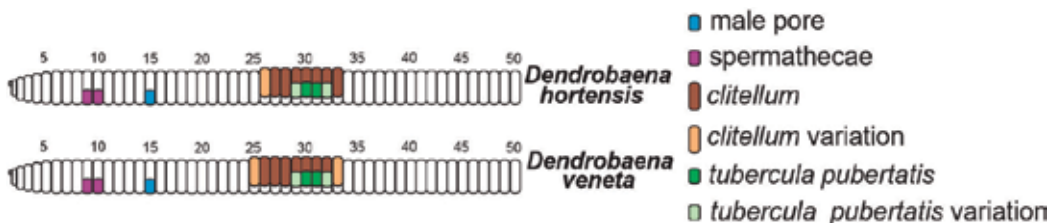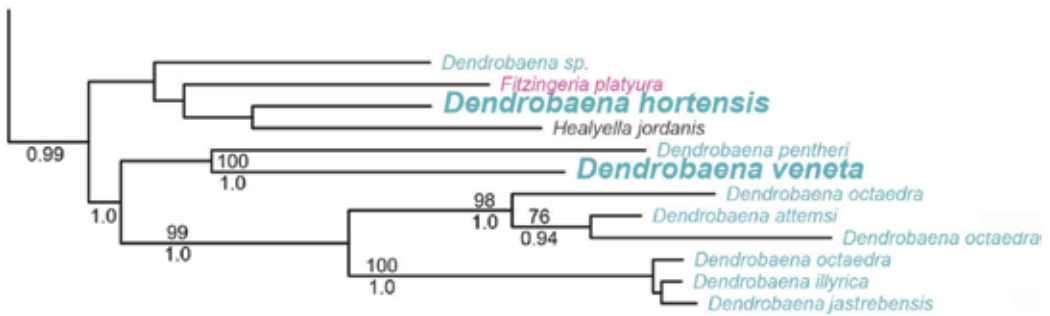

Figure 8. Clade including the species *Dendrobaena hortensis* and *Dendrobaena veneta* extracted from the maximum likelihood molecular tree of the family Lumbricidae. The genus Dendrobaena is not monophyletic and *Dendrobaena hortensis* and *Dendrobaena veneta* are phylogenetically different species. Modified from [17].

and ultimately the functioning of the process will be much less efficient. In summary, it is very important to determine which species are present in the cultures and to prevent the existence of mixed earthworm cultures.

The phylogenetic study demonstrated that *E. fetida* and *E. andrei* are phylogenetically different species. Phylogenetic analysis of maximum parsimony, maximum likelihood, and Bayesian (BMCMC) of the sequences of genes 28S and cytochrome c oxidase I (COI) and of the combined sequences (28S-COI) showed *E. fetida* and *E. andrei* to be monophyletic [18]. These results have been confirmed by other authors and by our group in a genus-level phylogeny of the family Lumbricidae (see **Figures 7** and **8**, [17]) and through a DNA barcoding study [19].

3. Laboratory culture of epigeic earthworms

Laboratory culture of epigeic earthworms should be rapid and easy to carry out, thus enabling (1) study of earthworm growth and reproduction; (2) identification of the demographic parameters of populations of different species and in different types of organic matter and organic

Figure 9. Laboratory culture of the epigeic earthworm *Eisenia andrei* (the red worm).

waste; (3) determination of the rate of consumption of organic matter; and (4) collection of casts to study the changes that take place in the organic matter during transit through the earthworm intestine (**Figure 9**).

Culture and maintenance of epigeic earthworms is quite simple and can be carried out in different ways and at different scales. However, it is important to establish some standard conditions to ensure success in culturing different species of epigeic earthworms.

3.1. Moisture and temperature

Epigeic earthworms require a substrate with a relatively high moisture content. High growth rates will be ensured by a moisture content of between 80 and 85%, which can be determined manually: the substrate should be damp, but when a handful is squeezed by hand, scarcely any water should escape. The temperature of the substrate should be between 20 and 25°C for optimal development of the vast majority of epigeic earthworms. The worms will also breed successfully under these conditions. However, they will not tolerate large variations in temperature, and the use of controlled temperature chambers is recommended. If this is not possible, the cultures should be maintained at a relatively constant temperature, and variations in temperature should be recorded with a minimum-maximum thermometer.

3.2. Culture dishes, recipes, and boxes

Different types and sizes of containers can be used for culturing earthworms, depending on the purpose of the culture.

3.2.1. Stock boxes

Relatively large populations of the different epigeic species can be maintained in stock boxes for later use (for different purposes) (**Figure 10**). The size of the boxes is not limited, except for the height, which should not exceed 50 cm. The bottom of the boxes should be perforated or formed by a grid of mesh size 0.5–1 cm. The boxes should not be in direct contact with the ground, and a container of vegetable waste can be placed underneath the box to collect the leachate. To start the culture, the box should be filled with a bed of vermicompost into which the initial population of worms is inoculated. This bed should be at least 10 cm high. The food material, for example, animal manure, is then added to the box. As the worms eat, they ascend through the food/substrate. More food is added in successive layers not exceeding 5 cm in height. When the boxes are almost full, plastic netting (mesh size 1 cm) is then placed on top of the box and covered with a new layer of manure. After some time, most of the earthworms will rise above the net. The net (plus worms) is then removed and can be used to start a new culture in another box. The surface of the substrate should be covered by a perforated plastic cover to prevent light entering and to preserve the moisture.

3.2.2. Petri dishes

Petri dishes are suitable for holding individual specimens or small groups of earthworms (**Figure 11**). Plastic petri dishes allow gas exchange while also maintaining good moisture conditions in the substrate. Some vermicompost containing earthworm(s) is placed on the bottom of the plates, which are then filled with food. The food is renewed as it is consumed. Cocoon production by mature individuals can also be monitored in Petri dishes. Dishes of different diameters can be used depending on the size of the species and the number of individuals to be cultured per dish.

Figure 10. Stock culture of earthworms (*Eisenia andrei*) fed with grape marc in the greenhouse facilities of the Animal Ecology Group at the University of Vigo (Spain).

Figure 11. Left: Petri dishes containing different densities of earthworms used to study growth and reproduction. The dishes are held in a laboratory environmental chamber under controlled conditions of temperature and humidity. Right: Detail of a petri dish with an individual specimen of *Eisenia fetida*.
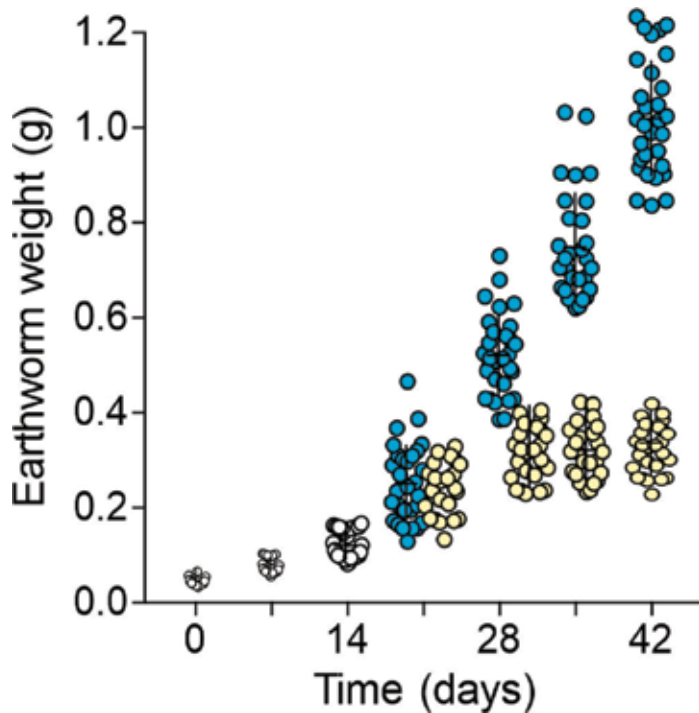
Figure 12. Growth curves of *Eisenia andrei* reared at (1) low population density (blue circles) and (2) high population density (yellow circles). High population density is usually reached when the vermicomposting system is performing at peak levels. Figure based on data from different experiments with different types of food for earthworms.

When environmental conditions are suitable and sufficient food is available, the growth of epigeic earthworm fits logistic curves, with a long phase of exponential growth (**Figure 12**, blue points). Earthworm growth is density-dependent, and individual growth and earthworm weight are lower in crowded conditions (as in vermicomposting systems) than in optimal conditions, although total earthworm biomass is greater. Earthworms reared in crowded conditions reach sexual maturity at smaller sizes than earthworm reared under conditions of low population density (**Figure 12**, yellow circles).

3.2.3. 96-well plates

Use of 96-well plastic plates to rear earthworms is recommended for studying reproduction and reproductive parameters related to cocoons, such as viability, time to hatching, and the number of juveniles hatched per cocoon (**Figure 13**). The cocoons should be washed with water and handled carefully with flat, blunt tweezers, to prevent damage. One cocoon is placed on top of moistened cotton wool in each well of the plate, each identified by a code number (e.g., A5 or F3). The plates are covered with plastic film (such as Parafilm M). The film over each well is pierced with a pin to make a small hole to allow gas exchange. In addition to reducing evaporation, the plastic film also prevents mixing among the hatchlings emerging from different cocoons.

Figure 13. Methods used to study reproductive parameters related to earthworm cocoons. (1) 96-well plates with one cocoon in each well. (2) Plates covered with covered with plastic film (Parafilm M), (3) earthworm (red worm) embryos inside the cocoon, and (4) new earthworm hatchling (red worm) emerging from the earthworm cocoon inside the well.

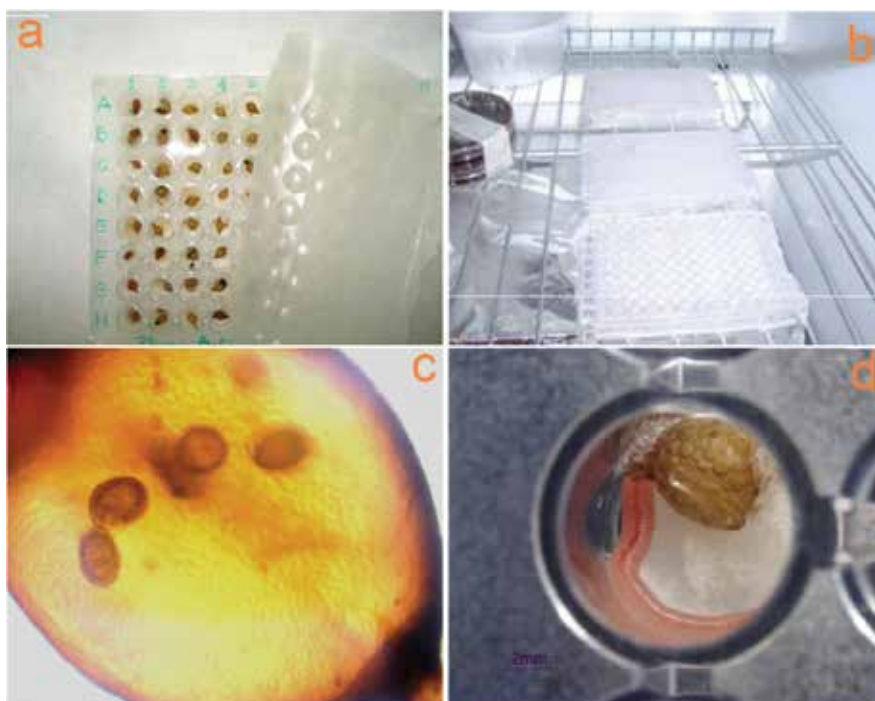The plates are checked daily to monitor cocoon development. Plates with cocoons should be placed in an incubated chamber at a temperature between 18 and 22°C in darkness until they hatch, which in the case of the red worm takes place between 18 and 26 days after cocoon production, with 2–3 new hatchlings typically emerging per cocoon [11]. A cocoon is considered viable when it produces at least one earthworm. The newly emerged hatchlings are then placed in Petri dishes, with food provided ad libitum, to study the first stages of growth (**Figure 13**).

4. Conclusions

The ideal earthworm species for rapidly transforming organic waste into vermicompost, from the point of view of the rapid return of nitrogen to the ecosystem and adjustment of the C/N ratio of the waste, should combine a short life cycle with a high metabolic rate. *Eisenia fetida* and *Eisenia andrei* are suitable for use in vermicomposting as both species are small, r-strategists, and have a short life cycle and high reproductive rates. Indeed, these are the most widely used earthworm species in vermicomposting and vermiculture facilities throughout the world because they are ubiquitous, naturally colonize diverse types of organic waste, tolerate wide temperature and humidity ranges, and they are strong, resistant, and easy to handle.

Eisenia fetida (tiger worm) and *Eisenia andrei* (red worm) are phylogenetically and biologically different species and do not interbreed. *Dendrobaena veneta* and *Dendrobaena hortensis*—other species used in vermicomposting—are also separate species. As these differences are not generally known, the existence of mixed cultures is quite common in commercial and domestic earthworm culture facilities. The presence of more than one species in mixed cultures leads to lower reproduction rates and a less successful vermicomposting system.

In summary, for optimal functioning of the vermicomposting process, the earthworm population should comprise a single species, optimal environmental conditions should be maintained, and food should be provided ad libitum.

Acknowledgements

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Environmental Influence of Soil toward Effective Vermicomposting

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Additional information is available at the end of the chapter

Abstract

Vermicomposting is a process by which the organic waste is converted into manure with the help of earthworms. Growth rate, onset of maturity (clitellum development), rate of reproduction (cocoon production) and population buildup of earthworm during vermicomposting have been depend upon the conditions like temperature, moisture and physico-chemical properties of the feed mixtures. *Eisenia fetida* was superior to other epigeic species and tolerate wide range of temperature, moisture and pH. Endogeic species produced lesser cocoon than epigeic species and cocoon production decreased at low temperature. Maintenance of temperature and moisture content is the critical step for vermicomposting. Growth and maturation of earthworms was best at 20–25°C temperature with 80–85% moisture content. Increase temperature upto 30°C accelerated growth rate of earthworms and lessened the time to sexual maturity. Earthworms can survive in the soil contaminated with heavy metals by accumulating heavy metals in their tissues.

Keywords: earthworm, growth rate, physico-chemical properties, heavy metals, vermicomposting

1. Introduction

Over the past few years disposal and management of organic solid wastes has become more problematic and rigorous due to rapidly increasing population, intensive agriculture and industrialization. Aristotle, the Greek philosopher, documented very early the importance of earthworm in the ecosystem, and called them "intestines of the earth". After that, Darwin[1] highlighted the role of earthworm in breakdown of animal matter as well as dead plant. Transformation

of organic waste through vermicomposting is of multifold interest as along with checking pollution of the environment, it results in production of a rich, more stable and homogenous product as compared to composting [2, 3]. Vermicompost acts as a buffer, has a significantly lower volatile solid content and high N, P, K content in the plant available form [4, 5].

Out of the 3200 species of earthworm reported from the world over, 509 species belonging to 67 genera are known to occur in the Indian subcontinent and about 374 species have been reported from different habitats of India alone [6, 7]. Certain epigeic earthworms, which have natural ability to colonize organic waste; show tolerance to wide range of environmental factors; short life cycle; high reproductive rates; high rates of feedstock consumption, digest and assimilate organic matter shows good potential for vermicomposting [8]. Few surface feeders earthworms species which contain all these characteristics and widely used in vermicomposting of a wide variety of organic wastes are *Eisenia fetida* (Savigny), *Eudrilus eugeniae* (Kinberg), *Eisenia andrei* (Bouche), *Dendrobaena veneta* (Rosa) and *Perionyx excavatus* (Perrier). Other species like *Dichogaster modigilani, Drawida nepalensis, Lampito mauritii, Lumbricus rubellus* and *Dendrobaena rubida* have also been used to some extent [9, 10]. Growth rate, onset of maturity (clitellum development), rate of reproduction (cocoon production) and population buildup were recorded as the indices of suitability of a particular mixture for the worms.

2. Suitable species of earthworm used for vermicomposting

Epigeics species are useful for biosolid waste management as these worms can hasten the composting process to a significant extent and produce better quality of vermicomposts, compared with those prepared through traditional methods [11]. *Eisenia. fetida* is used throughout the world for this purpose as it is ubiquitous, can tolerate a wide range of temperature and can live in wastes with good moisture content [8, 12]. *Eudrilus eugeniae* and *Perionyx excavatus* is the other commonly used worm. *Eudrilus eugeniae* is large in size, grows rapidly but has poor temperature tolerance, hence, may be suitably used in the areas with less fluctuation of temperature (tropical areas).

Lampito mauritii, Drawida willsi, Dichogaster bolani and *Pheretima elongata* are some others useful species used in the vermicomposting [13–16]. There is a species specific variation in food preference and accordingly the time taken for bioremediation also varies. Kaviraj and Sharma [16] compared the efficiency of exotic *Eisenia fetida* and local *Lampito mauritii* in vermicomposting of municipality solid waste and showed that *Eisenia fetida* was much better in vermicomposting as compare to *Lampito mauritii* in terms of C:N ratio reduction, TOC reduction, increase in EC and TK but *Lampito mauritii* was able to modify the soil characteristics. Tripathi and Bhardwaj [11] also observed that both the species of earthworms increased the N, P, K content while decreased the C:N and C/P ratios after 150 days. Moreover, the average number of hatchlings and cocoons produced by *Eisenia fetida* were also much more than *Lampito mauritii*. Thus, they concluded that *Eisenia fetida* was a better adapted species for vermicomposting of kitchen waste mixed with cow dung under tropical conditions. Dominguez et al. [17] found that *Eudrilus eugeniae* is also be a suitable species for vermicomposting. Out of the Indian species *Perionyx excavatus, Dichogaster modigilani, Drawida nepalensis* and *Lampito mauritii* could

be exploited for vermicomposting because of their continuous breeding, high rate of cocoon production, short development time and high hatching success [18].

Earthworm species *Eudrilus eugeniae, Eisenia fetida, Perionyx sansibaricus, Pontoscolex corethrurus* and *Megascolex chinensis* were compared for their efficiencies in biodegrading organic wastes and *Eudrilus eugeniae* was found to be superb of all these [10]. *Eudrilus eugeniae* inoculated in waste also diminish the toxic heavy metals and might be helpful in obtaining a clean environment [19]. Some scientists recommend that vermicomposting with polyculture gives faster results over monoculture [13, 20]. When three earthworm species, i.e. *Eisenia fetida, Perionyx excavatus* (epigeic) and *Lampito mauritii* (anecic) were used, the reactor with polyculture performed better than the traditional monoculture vermireactors [21]. On the other hand Elvira et al. [22] found that mixed cultures of epigeics species viz. *L. rubellus, E. fetida* and *D. rubida* did not show any advantage over pure cultures. They reported that *E. andrei* showed higher growth rates in mixed cultures while the growth rate of *L. rubellus* and *D. rubida* decreased slightly in mixed cultures as compared to pure cultures.

3. Effect of temperature and moisture on earthworm

Reinecke and Kried [23] and Reinecke and Venter [24] concluded that *E. fetida* could survive well even in harsh environmental conditions, especially temperature (5–43°C) and fluctuating moisture conditions. However, growth and maturation of earthworms was best at 20°C and 85% moisture content under laboratory conditions [11, 25]. Dominguez and Edwards [26] found that 80% moisture was optimum for vermicomposting of pig manure and hostel kitchen waste by *Eisenia anderi*. Lalander et al. [27] observed that if the temperature of the vermibed or unit was more than threshold temperature, then earthworm moves to the edges and top levels, leaving unprocessed material in the center.

Edwards et al. [28] studied the life cycle of *Perionyx excavatus* in a variety of organic wastes under various population density pressures and temperatures between 15 and 30°C. They observed that an increase in temperatures up to 30°C accelerated the growth of earthworms but decreased the time to sexual maturity. However, the highest rates of reproduction occurred at 25°C both in cattle solids and sewage sludge. Earthworms grew at similar rates in cattle solids, pig solids and aerobically digested sewage sludge, but the earthworms did not grow well in horse solids and grew poorly in turkey wastes. Edward [29] in another study evaluated the optimal conditions for breeding of *E. fetida* with different range of animal and vegetable waste under aerobic condition with temperature ranged from 15–20°C, moisture 80–90%, ammonia content <0.5 mg/g, salt content <0.5% and pH in the range of 5–9. He found that the population density of earthworms per unit volume or weight of a waste was very important in affecting rate of growth and reproduction.

Dominguez et al. [17] made an observation on the biology and population dynamics of *Eudrilus eugeniae* in cattle waste solids by growing them in groups of 1, 2, 4, 8 or 16 hatchlings in 100 g of waste in incubators at 15, 20, 25 and 30°C. They found that earthworm biomass production was temperature dependent, maximum production being attained at the two highest population

densities and highest temperature (30°C). The highest organic waste to earthworm ratio of 10:1 (10%) was recorded at the most dense earthworm population.

Bhattacharjee and Chaudhuri [9] studied the reproductive biology of seven Indian species of earthworm, viz. *Lampito mauritii* Kinberg, *Polypheretima elongata* (Perrier), *Perionyx excavatus* Perrier, *Pontoscolex corethrurus* (Muller), *Dichogaster modiglianii* (Rosa), *Eutyphoeus gammiei* (Beddard), and *Drawida nepalensis* Michaelsen in different seasons (natural variation in temperature). The peregrine earthworms such as *Dichogaster modiglianii, Perionyx excavatus, Polypheretima elongata* and *Pontoscolex corethrurus* were continuous breeders with high fecundity. Native *Lampito mauritii* and *Drawida nepalensis* were found to be semi-continuous, whereas, *Eutyphoeus gammiei* was a discrete breeder. There was a dramatic increase in cocoon in the summer and monsoon months with peaks during April and July while cocoon production decreased during winter. Temperature also affected the incubation period of cocoons. With increase in temperature within a temperature range of 28–32°C under laboratory conditions, incubation period increased in the endogeic worms (*Pontoscolex corethrurus, Polypheretima elongata and Drawida nepalensis*) and decreased in the epigeic worms (*Perionyx excavatus* and *Dichogaster modiglianii)*. There was a significant ($P < 0.05$) positive correlation between number of hatchlings per cocoon and incubation period in *Lampito mauritii* only. Edwards and Arancon [30] reported that an increase in temperatures up to 30°C accelerated the growth of *Perionyx excavatus* and reduced the time for attaining sexual maturity. However, the highest rates of reproduction occurred at 25°C both in cattle solids and sewage sludge.

Elvira et al. [22] studied the growth rate and reproduction of the epigeic species *Lumbricus rubellus* and *D. rubida* in cow dung and their possible interactions with *E. andrei*. The mean growth rate of *D. rubida* was 3.84 mg/day, reaching sexual maturity at 54 days and producing an average of 1.45 cocoons per week. After collection, 85% of the cocoons of this species were viable, incubation took an average of 21.7 days and an average of 1.67 worms emerged from each cocoon. On the other hand, the mean growth rate of *L. rubellus* was 8.02 mg/day, maturity at 74 days, with a mean weekly production of 0.54 cocoons. After an incubation period of 36.5 days, 64% of the cocoons hatched and one worm emerged from each. The mixed cultures tested did not present any advantage over pure cultures; *E. andrei* showed higher growth rates in mixed cultures, while the growth rate of *L. rubellus* and *D. rubida* decreased slightly in mixed cultures as compared to pure cultures. On the other hand, maximum biomass gain by *P. excavatus* was 292 mg per gram cattle waste at 25°C. Increasing temperatures up to 30°C accelerated the growth of earthworms and lessened the time to sexual maturity [29].

Soobhany et al. [19] resulted that moisture content within the feed mixture decreased with an increase in temperature by employing *E. eugeniae* in municipal solid waste. Initially they set the starting temperature of municipal solid waste in the range of 28–30°C and moisture content within the range of 81.8–83%. During vermicomposting of municipal solid waste, a sharp increase in temperature was recorded by them which mainly due to heat evolution resulting from rapid breakdown of organic matter and nitrogenous compounds by microbial activities which cause the water evaporation from the feed mixture and moisture content was decreased upto 50–55%. So thus maintenance of temperature and moisture content is the critical step for vermicomposting.

4. Growth rate of earthworm

The earthworms grow best in easily metabolizable organic matter and non-assimilated carbohydrates; these also favor their reproduction [31]. There was a positive correlation between Growth and reproduction with volatilable solid content of the waste [29]. Earthworm growth slows down when C: N ratio and temperature is high [32, 33]. The biomass gain by *E. fetida* during vermicomposting was found to depends on the population density and food type [34]. Viljoen and Reinecke [35] observed that single raised worm began to gain biomass at a higher rate than those raised in batches. While Dominguez et al. [36] reported a decrease in worm biomass even when additional feed was provided to worms every week. So the physico-chemical or the nutrient characteristics of the waste might be related to the growth of earthworm along with temperature, pH and moisture content. The interaction of these physico-chemical organic waste palatability and strength of feeding by earthworm is directly related to the interaction of these parameters and consequently it affects growth and reproduction of earthworm [37]. Hartenstein et al. [38] reported the regression equations for *Eisenia fetida* with respect to its age and observed that 50% of the earthworm population became clitellate at 25°C in relation to population density in activated sludge and in horse manure. A mean particle size of 0.3 mm of horse manure proved superior in supporting a weight gain (+45%) than a particle size of 0.5 or 1 mm. Pure cellulose, newspaper or wood shavings as substrate were ingested by *E. fetida* but failed to result in weight gain. Neuhauser et al. [39] and Neuhauser et al. [40] also reported a weight loss in *E. fetida* for a longer duration in the waste. This might be due to the transformation of most of the substrate to vermicompost, which could not further support their growth. Gunadi et al. [41] reported that *E. fetida* and *E. anderi* grew much faster in tea leaf wastes pre-composted for 1 week as compare to fresh waste because of the high protein content. However in fresh cattle solids, death of *E. fetida* was observed after 2 weeks by Gunadi and Edward [42]. They attributed death of earthworms to the anaerobic conditions which developed after 2 weeks in fresh cattle solids. Mature worms were not able to adapt to the medium as compared to 20 day old worms. Rates of growth and cocoon production were slightly less in a defined medium (7% organic content) than in a cow manure control medium (70% organic content) [43].

Reinecke and Viljoen [44] observed that cocoon production by *Eudrilus eugeniae* was much more at 25°C in different types of substrates. Evans and Guild [45] however, observed a peak production of 3.8 cocoons per week per individual of *Eisenia fetida* at 13°C. On the other hand Venter and Reinecke [46] observed clitellum for the first time in *Eisenia fetida* after 60 days. A positive correlation was observed by Satchell [47] between number of cocoons and the zone of soil inhabited by worms. The species of the deeper soil layer produced less cocoons as compare to species living near the surface due to adverse environmental condition. Olive and Clark [48] reported that temperature more than optimum level decreased cocoon production in earthworms. Lavelle [49] found a positive relationship between the size of the adult and cocoons produced by the earthworms but Senapati and Sahu [50] reported that the size of worms bore a negative relationship with the number of cocoons. They asserted that greater rate of cocoon production by small to medium sized epigeic earthworm *Dichogaster modiglianii* and *Perionyx excavatus* and top soil endogeic worms *Pontoscolex corethrurus* and

Lampito mauritii was due to exposure to the high mortality risk environment. Lee [51] and Edwards and Bohlen [52] proposed that size of cocoon was not always correlated with size of worms as cocoon production and time for maturation varied with species, population density and external factors such as temperature, moisture and energy content of the available food. Barne and Striganova [53] noticed higher mortality rate with increase in density of worms but Jager et al. [54] reported that growth curves were hardly affected by changing the density of earthworm *Eisenia veneta* but had an unexpected effect on reproduction. At higher density, the earthworm produced cocoons at larger body size and the maximum reproduction rate was lower. Reinecke and Viljoen [44] found no significant correlation between cocoon size and number of hatchlings produced. Hatching success of cocoon produced by worms younger than 60 days was low, the rate of hatching increased as the worms grew. Gunadi et al. [55] reported that the numbers of cocoons were less with increasing time of pre-composting but there was no clear pattern of effect of pre-composting on the number of hatchlings produced in cattle solids. Weight of hatchlings varied from 2.5 to 2.6 mg/cocoon.

5. Changes in physico-chemical quality of the feed wastes during vermicomposting

The physio-chemical composition of the vermicompost is known to be influenced by the different kind of feed given to the animal, bedding material used and the way the waste is collected, stored and handled before utilization [56]. A detailed review of various changes in physico-chemical parameters of feed material during vermicomposting is given in the following section.

5.1. pH and electrical conductivity (EC)

The differences in the pH of vermicompost are directly dependent on the type of raw materials used for vermicomposting. Different substrates used for vermicomposting resulted in different types of intermediates products which shows a different behavior in pH shift. The neutral pH throughout the vermicomposting is ideal for the growth of earthworm [57]. The occurrence of acidic environment may be attributed to the bioconversion of organic acids or higher mineralization of the nitrogen and Phosphorus into nitrites /nitrates and orthophosphate, respectively [42, 58–60]. The pH of cow dung and sheep manure vermicompost came out to be 8.48 and 8.6 [60], cattle manure had a pH of 6.0–6.7 [61, 62], pig manure had a pH of 5.3–5.7 [63, 64] and the one derived from sewage sludge had a pH of 7.2 [65]. The lower pH of the final vermicomposts might be due to production of CO_2 and organic acids by microbes during the process of bioconversion of different substrates in the feed given to earthworms [66, 67]. The decline in pH might be due to reduction in quantity of different types of volatile solids and to the growth of earthworm's biomass. The larger the increase in biomass growth, there was greater the reduction in volatile solids and hence the more shift toward the acidic condition [68, 69]. A decrease in pH might be an key factor in nitrogen retention as this element is lost as volatile ammonia at higher pH value. The lower pH was due to production of fulvic acid and humic acid during decomposition [70].

The change of mesophilic to thermophilic condition changes pH from acidic to alkaline due to conversion of organic –N- to NH_4^+ [71–74]. Rynk et al. [75] suggested that the excess of organic nitrogen not required by microbes was released as ammonia which got dissolved in water and increased the pH of the vermicompost. Datar et al. [76], Singh et al. [77], Goswami et al. [78], Huang et al. [79] and Lleo et al. [80] also reported an increase in pH during vermicomposting of solid waste, beverage biosludge, tea factory coal ash, fruit & vegetable waste and home waste respectively. They asserted that an increase in pH during composting and vermicomposting process was due to progressive utilization of organic acids and an increase in mineral constituents of the waste. On the other hand Song et al. [67] and Ravindran et al. [81] observed decrease in pH during vermicomposting of fermented tannery waste and animal manure spiked with mushroom residue respectively. They attributed that production of CO_2, organic acids and joint action of earthworms and microbes lead to low pH of the vermicompost.

Electric conductivity (EC) is a good indicator of the suitability and safety of vermicompost [82]. The reports regarding electrical conductivity during vermicomposting process are contradictory, some workers reported decrease in electrical conductivity [77, 83–85] and others an increase in electrical conductivity [67, 69, 86, 87]. The decrease in pH might be due to decrease in ions after forming a complex, whereas the increase in pH might be due to the degradation of organic matter to release various types of cations of different mineral salts in available forms such as phosphate, ammonium and potassium [88, 89] or may be due to loss of organic matter [16].

5.2. Nitrogen

Earthworms may influence microbial N transformation such as mineralization, nitrification and denitrification through their interaction with soil biota and increase concentration of ammonia in the fresh vermicasts [90]. Nitrogen generally declines during aerobic composting due to use of nitrogen by the rapidly multiplying heterotrophic bacteria but it increases during vermicomposting [69, 77, 91]. Chaudhuri et al. [66] reported the decrease in potassium and nitrogen content during the vermicomposting of kitchen waste with the help of *P. excavatus*. This might be due to NH_3 volatilization, incorporation into earthworm tissue and leaching into bedding material with as well as without earthworms or due to release of ammonia [92]. Although, nitrogen content increased during the process of vermicomposting of various materials [93–97] but final TKN content in vermicompost was always dependent on the initial nitrogen present in the feed material and the degree of decomposition [98–100]. Decrease in pH may also have an important effect in nitrogen retention as nitrogen is lost as volatile ammonia at high pH [89]. There is also might be good relation between nitrogen and C/N ration of the initial feed mixture because less the C/N ratio the greater will be the decomposition rate of the organic waste and hence the greater the increase in nitrogen [101]. Casting and burrowing behavior of earthworms increase C and N mineralization due to nitrogen fixing bacteria [102]. According to Needham [103], Tillinghast [104] and Viel et al. [105] loss in organic carbon may be the critical factor for nitrogen addition. Mucoproteins in the mucus secreted by epidermal glands, urea excreted through nephridia and ammonia through the gut with cast materials also helped in enhancing the nitrogen content in the vermicompost. Dead worms and their decaying tissues also adds a significant amount of nitrogen to the vermicomposting system.

Whalen et al. [106] found that microbial biomass was responsible for maximum of nitrogen released from decomposing earthworm tissue. Whalen et al. [107] observed that juvenile of *L. terrestris* excreted significantly more nitrogen as compare to adults at 10°C but in *Aporrectodea tuberculata* nitrogen excretion was significantly greater for adults as compare to pre-clitellate individual at 18°C. There is a high concentration of NH_4-N, NO_3-N in soils incubated with earthworms than soil incubated without earthworms for 48 h. Amador et al. [108] reported that organic nitrogen released by dead earthworms reached to 21.1–38.6 t/h/year. Kumar et al. [109] revealed that the content of nitrogen was decreased during vermicomposting which may be due to denitrification, NH_3 volatilization and ammonification. The decrease in content of nitrogen was also supported by Benitez et al. [110] with 36% loss of total nitrogen during vermicomposting of sewage sludge.

5.3. Organic carbon and C:N ratio

The C:N ratio is one of the most common indicator used for estimating compost maturity [111]. A decline in C: N ratio <20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes but a C:N ratio ≤15 is preferred for agronomic application [10, 112]. According to Song et al. [67], C:N ration <12 indicated that vermicompost had the preferable properties for field application. Speratti and Whalen [113] observed that mean N_2O and CO_2 fluxes during the study period tended to be greater from enclosures with added earthworms than the control (no earthworms added), but were non-significantly different due to the low survival rate of introduced earthworms. Better control of earthworm populations in the field is required to fully assess the impact of earthworms on CO_2 and N_2O fluxes from temperate agro-ecosystems. Similar results was also reported by Tognetti et al. [114] and observed that the rate of CO_2 production from vermicompost was much higher as compare to traditional compost. Cabrera et al. [115] reported faster decline in C:N ratio during vermicomposting as compared to compost without earthworm.. However, Atiyeh et al. [116] reported that the C:N ratio of the manure with or without earthworms decreased progressively.

The loss of organic carbon may be mainly due to high CO_2 emission via strengthened carbon mineralization due to respiratory activity of earthworms and microorganism [117] which cause faster reduction in carbon and lowering of C:N ratio during vermicomposting. The total organic carbon reduction ranged from 10 to 45% during vermicomposting of organic waste [118] while Singh et al. [82] observed increased in organic carbon content from soil to vermicast. The C:N ratio of vermicompost reduced to 12–17:1 from 21–69:1 [11, 16, 55, 119, 120]. Saha et al. [121] and Pramanik [122] observed that decrease in C:N ratio attributed to an increase in earthworm abundance which leads to rapid decrease in organic carbon due to enhancement in organic matter oxidation. Aira and Dominguez [123] reported that rise in microbial biomass during vermicomposting increase carbon losses. Briones et al. [124] suggested that calciferous organs of worms provided a mechanism of CO_2 regulation and both environmental and metabolic CO_2 could be fixed by this organ.

5.4. Phosphorus

Phosphorus is an important nutrient for growth of plants and is used for protein formation, metabolism, photosynthesis, seed germination and flower and fruit formation [125]. However,

phosphorus in soil is in mineral form which was readily available for plants but the potential activity of earthworm and phosphate solublising microorganisms increases phosphorus availability for plants [120, 126].

Gomez et al. [85]; Pramanik [122]; Lim et al. [127]; Singh et al. [128]; Hanc and Chadimova [129] asserted that the rise in total Phosphorus during vermicomposting was probably due to mineralization and mobilization of phosphorus as a result of bacterial and fecal phosphatase activity of earthworms. When organic matter passed through the earthworm gut, some amount of phosphorus is converted into more available form due to enzyme phosphatase and further release of might be attributed to the phosphorus solublizing microorganisms present in the cast [20]. In 1999, Patron et al. [130] noted that earthworm activity accelerated transformation of organic Phosphorus to plant available phosphorus form. Lim et al. [127] and Bayon and Binet [131] observed an increase of phosphorus by 25% and 2.4–49.5% by employing E. fetida and *E. eugeniae* for vermicomposting of paper waste sludge and rice husk respectively. Ghosh et al. [132] observed that vermicomposting of different waste materials resulted into 12–20.9% increase in easily extractable phosphorus.

According to Kaviraj and Sharma [16], organic matter decomposition by microbes resulted into acid production which is the major mechanism for solubilization of insoluble phosphorus and potassium. Therefore, presence of a huge number of gut microbes in earthworm might play an important role in increasing phosphorus content in the vermicompost. Mba [133] and Wan and Wong [134] studied the effects of *Bacillus megaterium* (phosphate solubilizing bacteria) and earthworm *E. fetida* and *Pheretima guillelmi* on Phosphorus turn over and transformation in soil. They found that the number of *B. megaterium* was increased in all the treatments with earthworm. The activity of acid phosphatase increased in the treatments having earthworm *Pheretima guillelmi* along with a significant increase in both inorganic and water soluble phosphorus. Acid phosphatase promoted the hydrolysis rate of organic phosphorus into inorganic phosphorus and the *B. megaterium* found in the worm casts of *E. euginae*. According to Pramanik [122], the higher phosphorus content might pertain to the higher adsorption rate of NO_3^- anions and replacement of PO_4^- ions from humic colloids. Hanc and Chandimova [129] resulted that total phosphorus content in the final vermicompost was 11% higher than control. They also observed that enhanced number of microflora in the earthworm gut might have played an important role in release of available phosphorus.

5.5. Potassium

There are contradictory reports regarding the total potassium content in vermicomposts obtained from different substrates due to the differences in the chemical nature of the initial raw materials [135]. Song et al., [67], Gomez et al. [85], Benitez et al. [110], Lim et al. [127] and Bhat et al. [136] have reported higher potassium concentrations during vermicomposting process. Increase in potassium content in vermicompost suggested that earthworms have symbiotic gut microflora with secreted mucus and water to degrade the ingested substrate which cause release of easily assailable metabolites [89]. Garg et al. [83], Singh et al. [128] and Elvira et al. [137] reported that total potassium concentration decrease in vermicompost. This decrease in concentration of potassium may attributed to the variation in chemical

composition of initial feed mixture or due to leaching of potassium because of low water holding capacity of the vermicompost [128].

Guerra-Rodriguez et al. [72], Delgado et al. [138] and Suthar [139] revealed that mineralization process significantly enhanced the concentration of exchangeable potassium during vermicomposting. Suthar [20] and Nahrul Hayawin et al. [96] also reported higher potassium content in the vermicompost produced from distillery sludge, oil farm waste and food industrial sludge respectively. Lim et al. [127] observed 15–121.4% increase in potassium content by using *E. eugeniae* for vermicomposting of rice husk. Huang et al. [84] cultured hatchlings, juveniles and adults *E. fetida* into three different culture tubs to study the changes in bacterial and fungal community composition. They observed that potassium concentration in vermicompost cultured with juveniles was more as compare to vermicompost cultured with adults earthworms. Vermicompost cultured with juveniles has 33.3% more potassium as compare to vermicompost cultured with adults.

5.6. Bioaccumulation of heavy metals and its effect on earthworms

The increasing exploitation of natural resources by human beings during the past few centuries has adversely affected the global balance of heavy metals causing a gradual increase in the concentration of metals in the soil ecosystem [140]. In order to maintain the environmentally sound soil quality, investigators are seeking methods to reduce the mobility of heavy metals from wastes to soil ecosystem. Metal mobility and availability can be reduced by raising the soil pH [141]. Phyto-remediation is known as the most viable and environment friendly technology. But, a limited number of plants have been found to have phyto-accumulation ability and a very less number can be used for field phyto-remediation because of low biomass production. Therefore, earthworms appears to be a valuable substitute for control of metals in contaminated soils [142]. According to Hopkin [143], the earthworms have capacity to control metals, particularly trace metals, such as Cu and Zn, in their bodies. Earthworms can also be used as bioindicators for assessing the level of soil contamination with agricultural runoff, heavy metals, acid rain, pesticides etc. [144].

The capability of earthworms to mitigate the heavy metal toxicity and to increase the nutrient profile of organic wastes might be useful in sustainable land restoration practices [20]. Heavy metals have the capability to bind with ligands of the tissues and thus leads to their bioaccumulation in the food chain [145]. A positive correlation between metal concentrations in the earthworms and those in the soils were observed with differences in bioaccumulation factors for different metals, this could be due to a variable metabolic requirement of earthworms for metals [146]. The effects of sub lethal concentrations of lead nitrate on reproduction and growth of *P. excavatus* was studied by Maboeta et al. [147]. The growth was affected negatively by the presence of lead while maturation rate and cocoon production was not affected.

Earthworms are have the capability to inhabit and survive in sites contaminated with metals [148] and have the ability to accumulate heavy metals in the cells of yellow tissue [149]. Earthworm populations may develop mechanism by which they can tolerate or resist the effect of metal induced stress. Such tolerance or resistance acquired by earthworms either through a variation in their genetic structure or reversible changes in an earthworm's physiology.

Toxicity tests done by various authors have shown that heavy metal pollution negatively affects life-history of earthworms such as growth, reproduction and survival [150]. Beyer et al. [151] studied the bioaccumulation of methyl-mercury in the *E. fetida* and its effect on regeneration after excision of the caudal end. They found that earthworms treated with 25 ppm or more methyl-mercury did not survive while the survival rates in other concentrations were 97% in control, 92% in 1 ppm and 79% in 5 ppm after 12 weeks. All surviving earthworms in the control regenerated but 29% of earthworms in 5 ppm group only healed without regenerating their tail end. An opposite results were reported by Boudou and Ribeyre [152] and Burton et al. [153] that the absolute concentration of total mercury and monomethyl-mercury bioaccumulated in *E. fetida* were higher in the earthworm exposed to the higher mercury soils and lower in the less mercury contaminated soils. The bioaccumulation factors for total mercury and monomethyl-mercury were larger in earthworms exposed to less contaminated soils and smaller in more mercury contaminated soils. Zhang et al. [154] reported that Bioaccumulation factors of methyl-mercury from soil to earthworms were much higher than those of total mercury, which suggested that methyl-mercury might be more easily absorbed by and accumulated in earthworms because of its lipid solubility.

Maenpaa et al. [155] showed that the treatment of high Phosphorus significantly reduced lead, zinc and cadmium bioavailability to the earthworm which was due to formation of metal–phosphate complex in the soils. This amendment reduce ecological risk to soil-inhabiting invertebrates exposed to heavy metal contaminated soils. Malley et al. [156] reported that earthworm act as an indicator for heavy metals toxicity that are present in the materials and are bioconverted, giving an indication of potential environmental hazard. The capacity of earthworm to uptake and redistribute heavy metals in their body lead to a balance between uptake and excretion which helps them to survive in metal contaminated soil. Kızılkaya [157] observed that the earthworm *L. terrestris* had the capacity to accumulate significant levels of zinc, and thus earthworm ingestion may result in zinc transfer to higher trophic levels. He observed that the cast and earthworm bodies receiving the highest Zn dose showed significantly higher Zn content than the non-treated soil. The effect of earthworm (*L. mauritii*) activity on mobility of Pb^{2+} and Zn^{2+} in the soil (DTPA-extractable) and its composting potential in the presence of these metals was investigated by Maity et al. [158] and suggested that the use of *L. mauritii* in amelioration of metal contaminated soil. Liua et al. [159] noticed that earthworm treatment increased the biomass of cabbage and decreased the bioaccumulation of Cd and Cu in the cabbage plants.

Udovic and Lestan [160] reported that bioavailability of Pb and Zn before and after soil leaching with EDTA with two earthworm species, *L. rubellus* and *E. fetida*, actively regulated soil pH, but did not significantly change Pb and Zn fractionation in remediated soil. Sivakumar and Subbhuraam [161] reported the effects of Cr (III) and Cr (VI) on the survival, behavior, and morphology of the earthworm, *E. fetida*, in water at pH 6, 7 and 8 and their toxicity in 10 different soils and an organic substrate. A decrease in the pH of water resulted in increased toxicity of Cr to the earthworm. In water, both Cr species produced behavioral changes and morphological symptoms. Wei-bao and Hong-qiang [162] elucidated role of earthworms and microbes in improving soil structure and controlling bioavailability of soil nutrients including heavy metals through bio-absorption, enrichment, precipitation, dissolution, and oxidation–reduction.

The influence of salinity on partitioning of, uptake in and toxicity of zinc to earthworms was studied by Owojori et al. [163] by exposing *E. fetida* in the laboratory for 28 days in OECD artificial soil spiked with either NaCl (experiment 1) or a combination of Zn and NaCl (experiment 2) and observed that the cocoon production was significantly affected by increased NaCl and Zn administered as individual substances, and the effects were more severe when both substances were present together. It was concluded that an increase in salinity had an additive to synergistic effect on influencing the toxicity of Zn to these earthworms. Frank et al. [164] noted slight increase in the metal contents in worm castings except for Cr and Zn over the worm feed mixture. This could be explained by the fact that organic matter was being reduced on passage through the gut of worms but actually worms did not appear to bioaccumulate metals within their tissue. Singh et al. [77], Kaur et al. [91] and Deolalikar et al. [165] reported an increase in heavy metal content in the vermicompost of paper mill sludge. The increase was more appreciable for Fe and Cu. The weight and volume reduction due to breakdown of organic matter during vermicomposting might have been the reason for increase in heavy metal concentrations in vermicompost. A 2% increase in Cu and a decline in the concentration of Mn, Zn and Pb in vermicompost were reported by Khwairakpam and Bhargava [166].

Song et al. [67] conducted a pilot scale trial to investigate the response of heavy metals and nutrients changes to composting animal manure spiked with mushroom residues with and without earthworms. They resulted that composting without earthworm have high concentration of heavy metals, that is, As, Pb, Cu, Zn, while that in vermicompost concentration of heavy metals decreased significantly relative to the compost. The decrease of metals concentrations in the vermicompost occurred for at least two reasons. First, vermicompost processed by earthworms had high level of humic acid which posed a stronger sorption effect on formation of stable metal humus complex especially for Cu and Zn [167]. Second, bioaccumulation of heavy metals by earthworms tissues with the help of epithelial layer and body fluids [168]. Singh et al. [82] and Kharrazi et al. [120] also observed decrease in concentration of heavy metals in the final vermicompost material. Soobhany et al [19] concluded that the reduction in toxic heavy metals by inoculating earthworm in the organic waste might be helpful in gaining clean environment.

6. Conclusion

Growth rate of earthworm, clitellum development, cocoon production and population buildup of earthworm were depend upon the physico-chemical composition of the feeding materials, types of feed mixture and environmental conditions like temperature, moisture and pH determine the sexual maturation in earthworms. Out of the various species of earthworms, *Eisenia fetida* is the most preferred species as it is hardy, prolific breeder and accepts a wide variety of food. Thus vermicast egested by the earthworm is a good source of N and P which is easily available to the plants and it has many advantages as compared to fertilizer and compost. The bioaccumulation of heavy metals by earthworms may be helpful to reduce the metal from organic waste. The feeding and casting activity of earthworm can stabilized the soil structure and change its physico-chemical properties and thus played an important role in sustainable agriculture.

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Edited by Sajal Ray

This book in two sections represents the current trend of research in ecology and biology of earthworms. In section "Ecology and Diversity" the authors reported the ecological and geographical uniqueness and diversity of earthworms in different environmental terrains of Siberia and Mexico. Functional interaction between earthworms and soil nematodes was elucidated with reference to vermicomposting and agricultural systems. Importance of digital library was highlighted for inventorization and taxonomical identification of earthworms. In section "Vermicomposting" the importance of maintaining pure cultures was discussed from the viewpoint of growth rate and the reproduction of composting species. This section includes article describing the management-related issues like roles of physicochemical parameters of soil and feed mixture on growth and reproduction of commercially important species of earthworm.

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