Rediscovering Earthworms

C.S.K. Mishra and Suryasikha Samal

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Cambridge
Scholars
Publishing

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This book first published 2021

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-6286-7
ISBN (13): 978-1-5275-6286-8

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PREFACE

Earthworms have attracted widespread attention due to their significant influence on an array of soil processes and in sustaining soil health. Since Aristotle described these animals as "The Intestines of the Earth" and Charles Darwin's notable observation on the role of earthworms in nature, a good number of scientists have studied the diverse roles earthworms play in the ecosystem and devised methods to utilize their services for a beneficial purpose.

Earthworms inevitably constitute a major share among the dominant soil macrofauna. Their species richness, abundance and distribution pattern reflect edaphic and climatic factors of the geographical zone. Their horizontal and vertical stratification and density contribute to soil formation and sustaining the soil profile. Earthworm population density is a key indicator of soil quality. One of the major contributions of earthworms in soil is their vital role in facilitating the decomposition of organics. These animals significantly enhance aeration of topsoil, thus inducing the growth and activities of aerobic microbes, the primary decomposers. Besides, they increase aggregation and water infiltration in the soil alongside their roles in maintaining a stable carbon to nitrogen ratio and increasing the level of essential nutrients like calcium, phosphorous and potassium.

For the last several decades, earthworms of diverse species have been successfully used in producing vermicompost from biodegradable wastes. With the gradual realization of the harmful impacts synthetic agrochemicals inflict on ecosystems, people all over the world are switching over to organic food. Organic farming demands the production of high-value manures for field applications. Besides conventional compost, the demand for vermimanure has increased consistently over recent years. Vermimanure at present is recommended for the majority of agricultural and horticultural crops. The advantage of vermicomposting is that in addition to producing vermimanure, it facilitates vermiculture to increase the earthworm population.

The potentiality of earthworms as a cost-effective substitute protein supplement in formulating fish and poultry feed has been realized over the last few years. Earthworms are rich in protein, amino acids, fat, minerals, vitamins, etc. and therefore could be successfully used as a substitute for fish meal and soybean meal. Many researchers have reported the remarkable growth of fish and poultry fed with substitute diets that have earthworms as a major component. Earthworm protein has immense nutritional value for humans too.

Due to their permeable skin, earthworms can easily absorb toxic metals and chemicals from the soil and accumulate these in their tissues. Beyond a threshold level, the animals indicate specific alterations in morphology, histology, physiology and behaviour and therefore are considered ideal bioindicators of soil pollution. Researchers have identified several sensitive markers in earthworms, which could be immensely helpful in contamination diagnosis. Earthworms have been successfully used as bioremediating agents.

The book provides information on the diversity and functional role of earthworms in natural terrestrial ecosystems and their multiple utilities as agents for the production of organic manure, an ideal source of nutrition and indicators of ecological perturbations. The authors sincerely believe that the book will be useful to students and faculties of biological and agricultural sciences, researchers working on earthworms and anyone interested in knowing more about this lowly but immensely useful animal.

The authors have referred to many books, research papers and websites to collect and compile information besides their published research results, which have been duly acknowledged. They are grateful to friends, colleagues and family members for their sincere help, constructive criticism and encouragement for writing this book.

CHAPTER ONE

INTRODUCING EARTHWORMS

Earthworms in Darwin's last manuscript

Introducing earthworms in their natural habitats to humankind is not a strange concept. These small animals have already drawn the attention of a large group of scientists. Aristotle once described the earthworm as "The Intestines of the Earth". In early times, when humans began to learn about agricultural practices, the invention of the plough constituted one of the landmark events. However, long before this invention, the soil was naturally and regularly ploughed by earthworms. The importance of earthworms in the soil came to light after publication of Darwin's finding in his book titled *The Formation of Vegetable Mould through the Action of Worms, with Observations of their Habits* in 1881. The book was published six months before his death. Earthworms are fondly called "Darwin's plough". Before that, no one had ever perceived the role of earthworms in soil fertility. Up to the mid-19th century, most people believed that earthworms were pests and harmful to the plants. Darwin was assured about the beneficial role of worms for turning over the soil by moving up and down through the soil layers, chewing it up and excreting it, thereby making it more fertile. After a long period of close observations of earthworms and their habits, Darwin published his findings in the book. His publication demonstrated the influence of earthworms in nourishing the soil by the breakdown of organic matter. Although the book did reach the height of maximum sales at that period, over time it lost its identity. However, with the changing tides, earthworms and their application to boost agricultural production have drawn the attention of a considerable number of soil biologists.

In his book, Darwin (1881) described earthworms as nocturnal animals, and at night they start crawling in large numbers. They can also stay underwater for a long period. Earthworms stay close to the mouth of their burrows and easily become the prey for predator birds. Darwin also described the worms as lacking eyes and ears but able to respond to light, touch and vibrations.

Darwin's book further reveals his observations on earthworm habits and effects on soil (Fig. 1.1). Darwin learned that the meandering activity of worms aerates the soil and influences the chemical composition by rendering soil and plant matter into fertile pellets. Worms are responsible for depositing the topsoil as a by-product of their movements and gut contents. It is estimated that earthworms can move an average of 8 tons of earth for a single acre of cultivated land to produce a new layer of topsoil rich in nitrogen, phosphorous and calcium. Soil biologists also agree with the fact that the juice of earthworm gut contents collected beneath compost bins is an ideal organic fertilizing agent for garden farming. Darwin also experimented and noticed the typical tendency of earthworms to pull down leaves on lying on the soil into their burrows. Although the worms do not have any sense organ, Darwin noticed the degree of intelligence of these animals use to find the best way to drag the leaves into the narrow burrow. He simultaneously studied the sensitivity of these tiny animals towards odours, vibration and light intensity (Edwards, 1981; Feller et al., 2003).

During the period of Darwin, earthworms were described as slimy, ugly and senseless creatures because of their surface casting, and having little use except for fish-bait (Graff, 1983). After the publication of Darwin's book, certain noble behaviours of worms, such as their intelligence and role in soil weathering processes were unfolded. According to Darwin, worms monitor the rock weathering process both physically and chemically. Several stone particles or grains of sand with the mixture of hard calcareous concretions formed by the calciferous glands of earthworms showed the grinding action of their gizzards to facilitate the physical weathering of soil. The demonstration of the chemical weathering process by earthworms was carried out by Darwin by putting earthworms into a pot filled with red oxide sand. After some time, he noted the red sand in the casts of worms along with digested leaves. The transformation of the colour of the sand was possible due to the intestinal secretions of the earthworms. He also believed in the similarity of earthworms' digestive acids to soil humic acid. The gut enzymes of worms can digest the ingested organic matter with the mutualistic help of microorganisms (Martin et al., 1987; Lavelle et al., 1995). However, the spectrum of enzymes released from the intestine of different species depends upon their ecological category (Brown et al., 2000).

a b

Figure 1.1: a) Man is but a worm - caricature of Darwin's theory in the Punch almanac for 1882 CUL T992.b.1.45, Cambridge University Library, b) Charles Darwin as an earthworm scientist: caricature from the journal *Punch*, published in the year 1882 (Adapted from Kutschera& Elliott, 2010)

As described by Darwin, the consumption and partial digestion of leaves and rotation of casts by earthworms maintained the dark colour of topsoil. He experimentally demonstrated the formation of the humus layer by the casting and burrowing activities of earthworms in an earthen pot with sand and leaves inside. A large number of leaves buried by earthworms was also mentioned at several places in his book. He further emphasized the catalysis of the decomposition process by earthworms. The last two chapters of Darwin's book described the contribution of earthworms to geomorphology and landscape evolution. He also mentioned the significant role played by earthworms in the erosion–sedimentation cycle and how their action on fine particles in surface casts encourages the movement of particles by wind and water. The erosion of castings and the movement of drain water help the short-term formation of topsoil. The ability of worms to penetrate concrete floors and walls was also mentioned in the book (Feller et al., 2003).

Evolution and classification of earthworms

Earthworms were as old as mammals and dinosaurs and probably lived over 209 million years ago. The earthworm is not preserved in fossil records and very few trace fossils exist. There are over 7000 earthworm species found on all continents except the cold terrains of Antarctica. Based on the distributions, comparative morphology, anatomy and molecular profiles of earthworm species, attempts have been made to find their possible affinities and origins. From the current report of Anderson et al. (2017), it is evident that the introduction of approximately one-third of the earthworm species in North America was from Europe or Asia. Certain species have also been introduced into the previously earthworm-free areas of northern forests since the end of the last ice age ~11,000 years ago. The earthworm classification tree has been categorised into two major branches, both with subgroups in the Northern continent of Laurasia and Southern landmass of Gondwana. The precursors of present-day Oligochaeta (earthworms) were away from the branching of the classification tree. One of these groups consists of at least three families, and another branch contains nearly all familiar European species, which includes the northern subgroup, Lumbricidae, and the southern subgroup, the Megascolecoidae family. Around 178–186 million years ago, the divergences of the two major branches of earthworms occurred between the Northern and Southern Hemisphere, coinciding with the partition of the supercontinent Pangaea 180–200 million years ago during the Triassic period. A continental breakup influenced the early diversification of earthworms. This proposition also supports the fact that earthworms most likely inhabited Antarctica before the continent's southward drift. Sims (1980) supported the continental drift theory and diversification of widespread earthworm superfamilies. Michaelsen (1910) proposed the appearance of worms in the upper Jurassic period, whereas Stephenson (1930) believed that the earthworms evolved in the Cretaceous period. The evolution of earthworms still instigates many theories, but the actual record is still unclear.

Many scientists have classified earthworms, but broadly they are included under the phylum Annelida, class Clitellata and order Oligochaeta. The taxonomy and phylogenetic classification of earthworms again bring controversy according to different taxonomists. Certain taxonomists have placed earthworms in the underclass Oligochaeta (Fig 1.2). The order of earthworms may be classified as Haplotaxida or Lumbriculida. The major terrestrial group of soil animals that come under Oligochaeta is known as earthworms. Worms are classified based on different features such as anatomy, morphology, phylogeny, behaviour and ecology. Michaelsen grouped earthworms into 11 families, and later Stephenson arranged them into 14 families. He proposed that the common ancestor of the terrestrial Oligochaeta belonged to the aquatic Lumbriculidae. The classification of Oligochaeta by Jamieson (1978) has been widely accepted. The Moniligastridae family is considered to be the most primitive, whereas Megascolecidae and Eudrilidae show advanced characteristics. The families such as Glossoscolecidae, Lumbricidae, Hormogastridae and Microchaetidae have few primitive characters and may be considered to have evolved later than the other families. The Lumbricidae probably is considered the most recent family. According to modern taxonomy, the earthworm represents more than 7000 species, and around 18 families are found in all regions except Antarctica (Table 1.1).

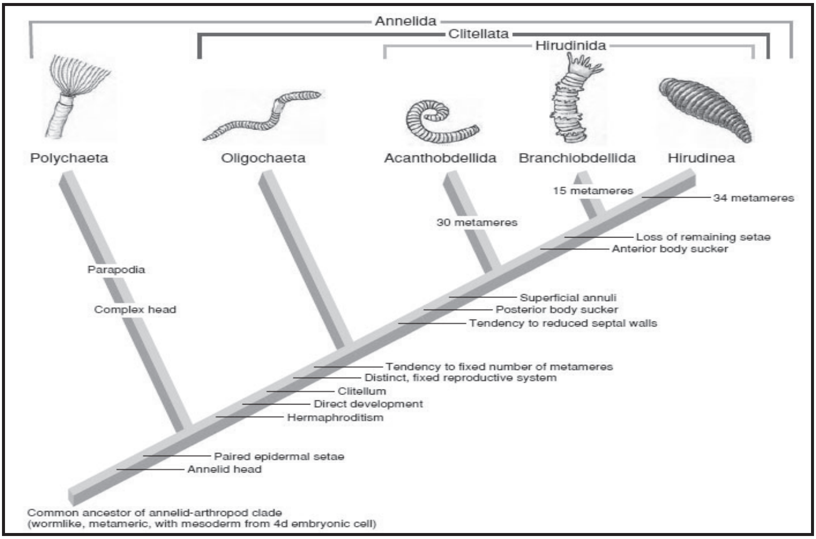

Figure 1.2: Classification of Annelida (Adapted from B.S Media, 2017)

In India, nine earthworm families have been categorised by Julka, 1988, the Glossoscolecidae, Moniligastridae, Lumbricidae, Ocnerodrilidae, Acnthodrilidae, Octochaetidae, Megascolecidae, and Eudrilidae families. The classification has been based on the reproductive features of worms:

- Families with inconspicuous male pores: Almidae, Glossoscolecidae

  - Families with conspicuous male pores: Moniligastridae, Lumbricidae, Ocnerodrilidae, Acnthodrilidae, Octochaetidae, Megascolecidae, Eudrilidae
- Further, they have been grouped based on the position of male pores:
  - Families with male pores on or in front of segment 15: Moniligastridae, Lumbricidae
  - Families with male pores behind segment 15: Ocnerodrilidae, Acnthodrilidae, Octochaetidae, Megascolecidae, Eudrilidae

Table 1.1: The regional distributions of the ten recognized major families of terrestrial earthworms (Hendrix & Bohlen 2002)

Family of earthworm	The geographical region of origin
Ailoscolecidae	**Europe**
Eudrilidae	**Africa**
Glossoscolecidae	**Central America, South America**
Hormogastridae	**Mediterranean**
Komarekionidae	**North America**
Kynotidae	**Madagascar**
Lumbricidae	**Europe, North America**
Megascolecidae	**Africa, Central America, North America, South America, Asia, Madagascar, Oceania**
Microchaetidae	**Africa**
Ocnerodrilidae	**Africa, Central America, South America, Asia, Madagascar**

General description of earthworms

Body plan and morphology: Earthworms are elongated, streamlined, soft-bodied invertebrates having ring-like structures (annuli) all over the body. This simple, coelomate and cylindrical animal has segments separated externally by rings and internally by septum. Earthworms have neither any skeletal system nor visible external appendages. These animals have pointed anterior and posterior ends. They lack a true head and the anterior segment is known as peristomium, whereas the posterior segment is known as pygidium. Earthworms have a mouth and a small fleshy projection from the peristomium above the mouth called the prostomium. The last segment contains the anus (Fig 1.3). The pattern of the prostomium is often used as a tool in earthworm taxonomy. Based on the position, prostomium has been categorised into four different types, prolobic, epilobic, tanylobic and zygolobic (Fig 1.4, 1.5). The upper surface or dorsal side of the worm is dark in colour, and the lower surface or ventral side is light in colour. The absence of external locomotory appendages in earthworms is compensated by tiny bristle-like structures, called setae (Fig 1.6, 1.7). Setae are present all over the body except the first and last segment, and the arrangement is either in four pairs (lumbricine) or ring-like structure (perichaetine). The setal arrangement is key for the identification of earthworm species. Setae are closely associated with the outer cuticle of the body and can be detached only by alkali treatment. The retractor and protractor muscles support the motion of setae (Sharma et al., 2009; Dash, 2012).

Size and colour: The size of earthworms varies considerably within individuals and different species from few millimetres to metres. The species *Dichogaster bolaui* is about 1.5 mm long. The earthworm *Megascolides australis* is around 2–4 metres long (Fig 1.8) and *Terriswalkeris terroereginae* is 2 metres long and secretes luminescent mucin. *Drawida nilamburensis*, another Indian species from the state of Kerala, measures about 1 metre.

Earthworms show diverse colours and pigmentation. The pigmentation of the body is related to their ecological niches. Body colours such as grey, purple, red, maroon, brown, and black are commonly found in different species. Some species show heavy pigmentations and glow in the dark, whereas some worms are unpigmented. Protoporphyrin and protoporphyrin methyl esters are the fluorescent compounds deposited in the tissue and may be responsible for pigmentation in worms.

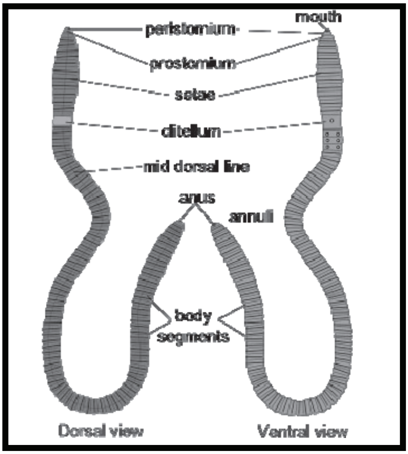

Figure 1.3: External features of earthworm (Adapted from Earthworm: External morphology, Body Wall, Coelom, Locomotion & Digestive System | Study&Score, 2018)

Figure 1.4: Scanning electron microscopic photograph of prostomium in *Eisenia fetida* (Adapted from Earthworms of India, Identification key and digital library)

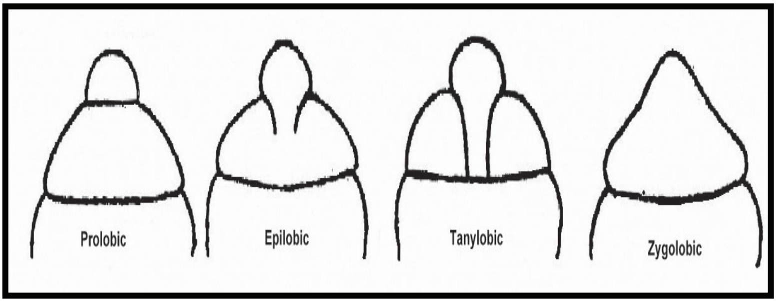

Figure 1.5: Types of prostomium in earthworm (adapted from Earthworms of India, Identification key and digital library)

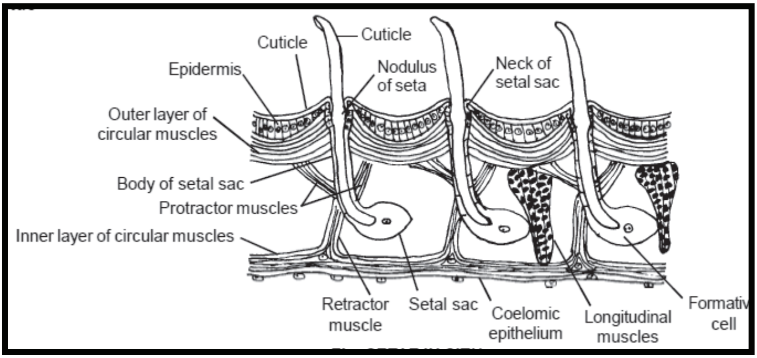

Figure 1.6: Transverse section of earthworm body wall showing setae

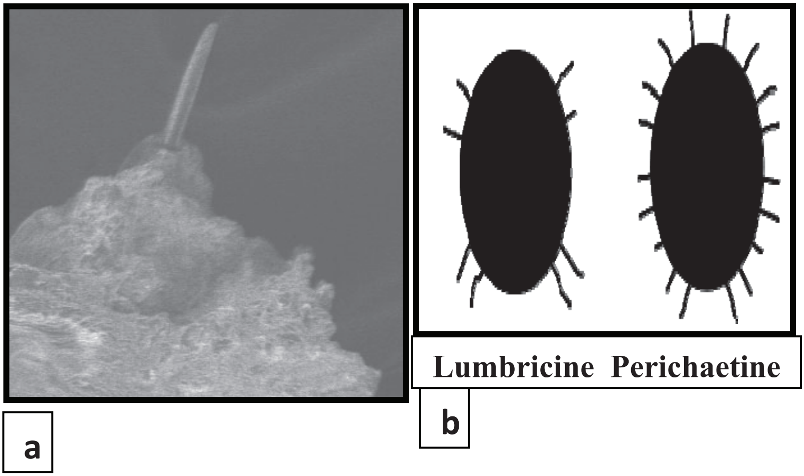

Figure 1.7: a) Scanning electron micrograph showing a) seta in *Eudrilus eugeniae*, b) setal arrangement

Figure 1.8: Photograph of *Megascolides australis* adapted from (*Megascolides australis,* Giant Gippsland Earthworm) - Emily Havlena Classification Project, 2020)

Dorsal pore and Nephridiopore: Dorsal pores are small openings lying along the midline on the upper surface of the earthworms and a passage to expel coelomic fluid from the body to the exterior. The worms throw out the fluid through dorsal pores to keep the skin moist in dry conditions. Hence, the pores are absent in worms of aquatic habitats. Nephridiopores, invisible to the naked eye, are the openings of the nephridia.

Coelom and coelomic fluid: The earthworm has a tube within a tube plan, and the space between the body wall and digestive system is called the coelom. The coelom of earthworm contains coelomic fluid, which acts as a hydrostatic skeleton and helps in burrowing and locomotory activities. The coelomic fluid also protects the internal organs from external jerks and destroys foreign pathogens. The fluid maintains the moist skin of animals for cutaneous respiration. In some species, the fluid has an unpleasant smell to give protection from predators. It contains various types of cells, such as phagocytes, mucocytes, small circular nucleated cells and chloragogen cells. These cells actively participate in the immune system of earthworms (Fig 1.9).

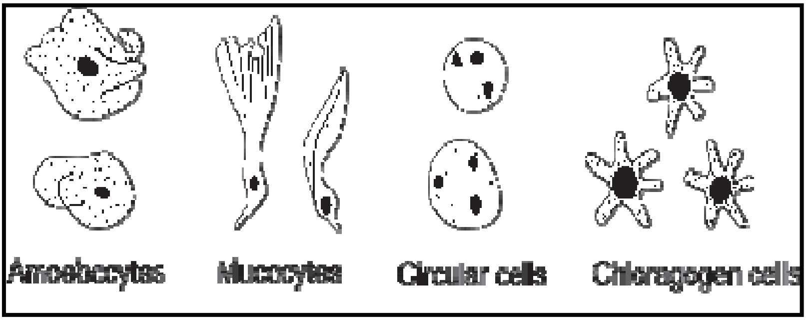

Figure 1.9: Coelomic corpuscles of *Pheretima posthuma* (adapted from Earthworm: Body Wall, Coelom, Locomotion & Digestive System | Study&Score, 2020)

Clitellum and genital apparatus: A glandular girdle-like thick band appears in sexually mature earthworms, restricted to certain segments, known as the clitellum. The position, shape and colour of the clitellum may vary in different species, hence this feature is useful in the identification of earthworms (Fig 1.10). The clitellum is present between 10-13 segments in Moniligastridae, 15-23 segments in Glossoscolecidae, 24-33 segments in Lumbricidae. The shape also varies, such as annular in Megascolecidae and saddle-shaped in *Dichogaster qffinis.* The colour varies from orange to yellow and red. In adult earthworms, certain areas on the ventral side may be depressed or swollen, called genital markings or tubercula pubertatis, respectively. Genital markings around genital setae and copulatory setae are called genital tumescences and copulatory tumescences, respectively. The external openings of the reproductive organs are known as genital pores. The openings of the vasa differentia (male pores) and oviducts (female pores) vary in different families and hence are useful taxonomic characters. The position of male pores may be on segments 13, 15 or 18 and the female pores on segment 14. In some families such as Megascolecidae and Octochaetidae, male pores are coupled with prostatic pores (Dash, 2012).

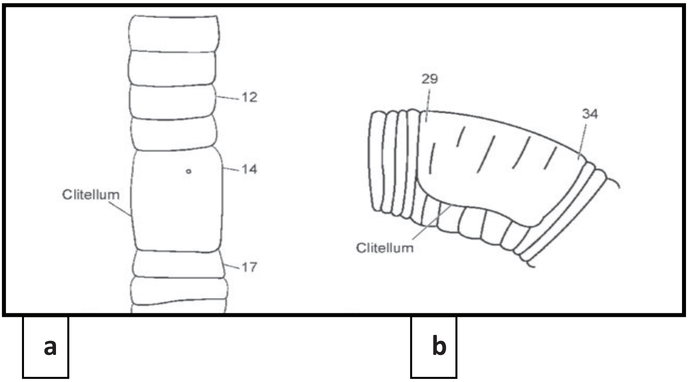

Figure 1.10: Schematic diagram of different shaped clitellum, a) annular (b) saddle shaped (adapted from Plisko & Nxele, 2015)

Reproduction and life cycle

Earthworms are hermaphrodites. Although the organisms have both testis and ovary, they prefer cross-fertilization. Copulation may occur above the ground or below the soil surface. Two mature worms come side by side with the alignment of the anterior prostomium region opposite to each other for the mutual exchange of sperm. The setae of the pairing worms help to hold the animals together during the mating process. The spermathecal opening of one worm is fitted with the male gonopore of the other. In certain species, such as *Lampito, Perionyx, Drawida*, the male pore is close to the spermathecal opening of another individual. Still, in *Eisenia* and *Bimastos*, the male pore is situated at a distance and the movement of sperm occurs through longitudinal grooves (Fig 1.11, 1.12). As the body moves, the spermathecae of each worm are filled with the sperm of the partner. The copulation lasts for hours and ends with the separation of partners. Each individual produces a coat-like structure in the clitellar region after receiving male gametes from another individual.

The coat is produced from the jelly-like substance secreted by the clitellum and becomes hard after exposure to air, forming the cocoon. The worm wriggles out, and the cocoon collects the sperm from the spermathecal opening along with the eggs—the two ends of the cocoon close, carrying both sperms and eggs inside to initiate fertilization. Cocoon shapes, sizes and colours are different in different species, and the cocoon contains albuminous fluid to nourish the developing worms (Fig 1.13). The structure may be egg-shaped, as in *Lampito mauritii*, spherical as in

Drawida calebi or spindle-shaped, for example in *Perionyx excavatus*. The colour of the cocoon varies from white to cream. The developing time for cocoon is known as the incubation time, and it is species-specific. The incubation time is also affected by soil moisture and temperature. The average period of incubation is about 7–10 days in a warmer climate and longer in colder regions. The emergence of a juvenile from the cocoon is followed by sexual maturation, thus initiating the life cycle (Dash, 2012). Earthworms continue to grow throughout their life by proliferating segments to form a growing zone just in front of the anus (Fig 1.14).

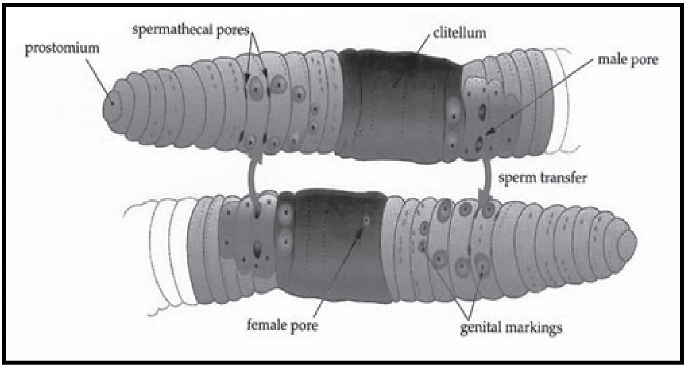

Figure 1.11: Genital pore of earthworm (adapted from Pokhrel, 2016)

Figure 1.12: Mating of earthworm (adapted from www.Sciencescore.com)

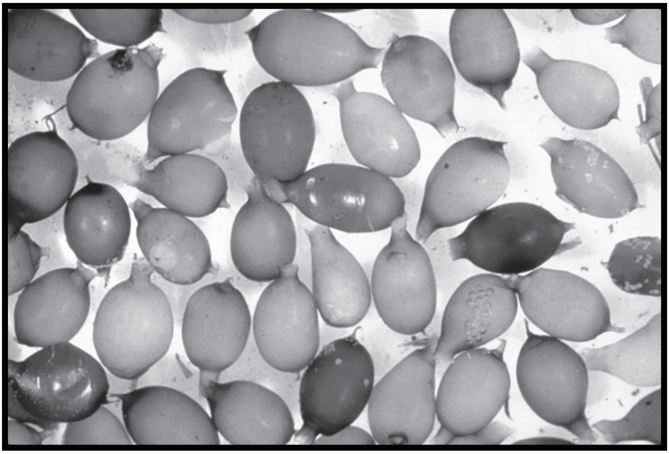

Figure 1.13: Cocoons of earthworms (adapted from www.earthwormsoc.org.uk)

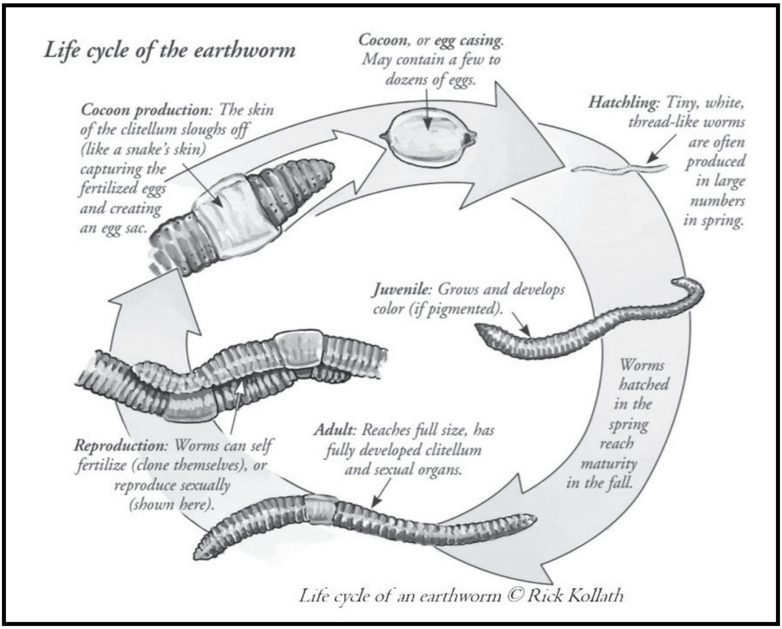

Figure 1.14: Lifecycle of earthworm (adapted from www.earthwormsoc.org.uk)

Food, predator and parasitic relationships

In general, earthworms are omnivorous, consuming both plant materials and animals like protozoa. They feed on organic matter, dead grass and also sometimes on berries and vegetables. They may also feed on algae, bacteria and fungi. The daily ingestion of food varies from 100 to 300 mg/g of a worm's body weight. According to a study, earthworms can uptake 8 to 20 g cattle dung/year as food (Dash, 2012).

Earthworms can be a good source of food for many vertebrate predators, such as fish, birds and mammals, and are an easy target for many organisms, such as centipedes, ants, carabid and staphylinid beetles, and their larvae. Worms are also attacked by various internal parasites, including protozoans, platyhelminthes, rotatorians, nematodes and dipterans. The protozoan *Spirochaeta* sp., bacterium *Bacillus* sp. and fungal pathogens live in different parts of the earthworm's body as parasites. The most common sites for parasites include the alimentary tract, blood vascular system, testes, spermathecae, seminal vesicles, coelom and also the cocoons of the worms. Several platyhelminthes and nematodes also cause infection to the worms (Dominguez & Edwards, 2010).

Behaviour of earthworms

Darwin's book made the worms popular for their interesting behaviours. He described the intelligence of worms in food collection and the protection of their burrows through his self-declared experiments. Darwin also demonstrated the plugging of burrows by earthworms to protect themselves. However, the intelligence of worms discussed in his published book is a controversial argument for soil biologists. Several workers on earthworms claim that an invertebrate having a cerebral ganglion might not be able to possess intelligence rather than instincts. The learning process of worms in reward or punishment patterns could be a reflection of instincts. The criticism of Darwin's observations is continuing as further research is required for a better understanding of earthworm behaviour (Brown et al., 2003).

Some worms show an alarm response by secreting mucus to escape predators (Ratner & Boice, 1971). Avoidance behaviours of earthworms due to xenobiotic exposures is a well-documented phenomenon. Worms prefer to escape from a habitat contaminated with toxic agrochemicals. Certain worms also adopt coiling behaviour to overcome the stress induced by physical and chemical factors (Mishra et al., 2017).

Unfavourable environmental conditions in the habitat may compel earthworms to become inactive in a process called aestivation or diapause. For their survival, they move deeper into the soil, reduce their metabolic rate and secret protective mucus. They remain in a coiled ball to sustain in adverse conditions of water loss until conditions become favourable. An interesting behaviour of earthworms has been studied in *Eisenia foetida* showing that these worms could communicate and influence each other's behaviour to travel in the same direction by forming active herds (Fig 1.15). The behaviour of earthworms will require future research to address unanswered questions.

Figure 1.15: Herds formed by earthworms (adapted from California Academy of Sciences, 2020)

Anatomy of earthworms

Digestive system: The alimentary canal of an earthworm is a long tube consisting of a mouth, pharynx, oesophagus, gizzard, stomach, intestine and anus. The mouth leads to the buccal cavity and pharynx. The muscular pharynx is enriched with pharyngeal glands that secrete proteolytic enzymes, mucin and carbohydrates. The pharynx leads to the oesophagus, which bears a thick-walled gizzard running up to the stomach and intestine. The calciferous gland associated with the oesophagus is found to release excess calcium in the form of calcium carbonates (Fig 1.16b). The elimination of calcium helps to maintain the acid-base balance of the body. The intestine is a long tube containing a pouch-like structure called the intestinal caeca. Many enzymes, for example, amylase, lipase, invertase, etc., are secreted and regulated by the stomach and intestine. The internal surface area of the intestine may be increased by the presence of a large dorsal fold, the typhlosole. The shape of the typhlosole varies from a low ridge to a well-developed lamella, either bifid or trifid. The intestine of earthworms can be divided into three distinct regions, namely, the pre-typhlosolar region, typhlosolar region and post-typhlosolar region. Several pairs of supraintestinal glands are located on the posterior wall of the typhlosole. The circular opening in the last segment is called the anus, and it releases the undigested food materials in the form of a worm cast. The structural components of the alimentary canal, the gizzard, calciferous gland, intestinal caeca, supra-intestinal glands and typhlosole, are important in the classification of earthworms (Fig 1.16).

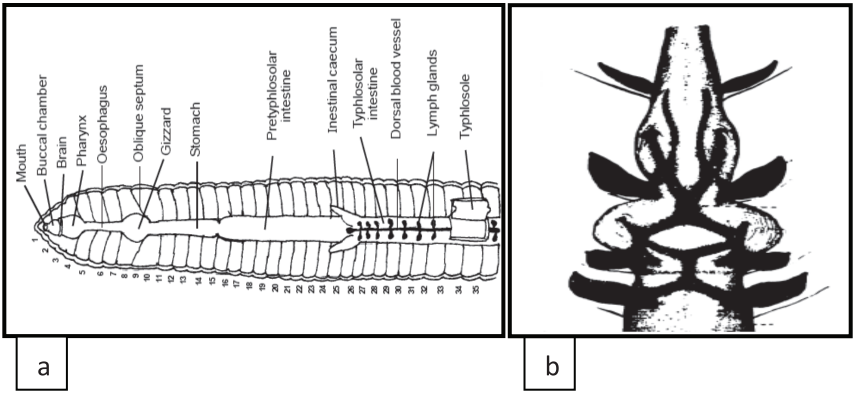

Figure 1.16: a) Digestive system of earthworms (adapted from *Earthworm, Chapter Notes, Class 11, Biology EduRev Notes*, 2016), b) Calciferous gland (Julka, 1988)

Excretory system: The excretory organs of earthworms are coiled tubes known as nephridia, which excrete the nitrogenous wastes present in the coelomic fluid. The presence of different types of nephridia in different species or their absence in the first few segments provides a clue about the identification of earthworms. The nephridia may be one pair, holonephridia, or more than one pair, meronephridia. There are three types of nephridia based on their location, namely pharyngeal, integumentary and septal nephridia. Pharyngeal nephridia contain neither nephridiopore nor nephrostome; integumentary nephridia contain nephridiopore but not the nephrostome. Septal nephridia are attached to both sides of septa in each segment and bear the nephrostomes. Either type of nephridia may be open (stomate) if nephrostomes persist or closed (astomate) in the absence of the nephrostomes. The duct of nephridia opens to either the exterior (exonephric) or into the alimentary canal (enteronephric) (Fig 1.17). The nephridia consist of three parts: nephrostome, body and terminal duct. Nephrostome is a funnel-shaped externally ciliated structure and opens into the coelomic cavity. The funnel leads into a narrow neck, which is continued into the body of the nephridium. The end part of nephridium is known as the terminal duct. Septal nephridia release the waste products through canals and ducts into the lumen of the intestine, whereas pharyngeal nephridia discharge the nitrogenous metabolic waste directly into the buccal cavity and pharynx from where these are passed outside with undigested food through the anus. Integumentary nephridia discharge the wastes products directly to the exterior of the body through nephridiopores.

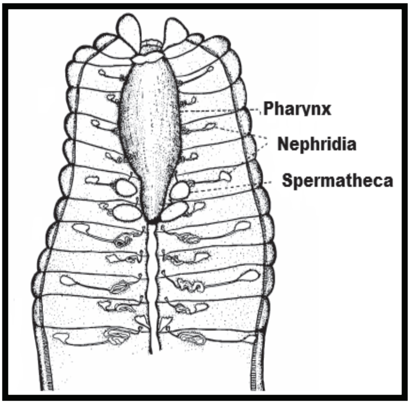

Figure 1.17: Excretory system of earthworms (adapted from Julka, 1988)

Circulatory system: The circulatory system of an earthworm is a closed type and consists of thick muscular blood vessels called the heart or pseudoheart, loops and blood vessels. The dorsal vessel may be single or double and is closely associated with the mid-dorsal line of the alimentary canal. The ventral vessel is located just below the digestive tract and is suspended from it by a mesentery. The subneural vessel lies beneath the nerve cord. The supra-oesophageal vessel is either single or double and important for taxonomic characters and runs along the dorsal wall of the gut in the anterior segments. The extra-oesophageal vessel is situated anteriorly, and latero-parietal vessels are on the latero-ventral side of the gut. The paired commissural vessels connect the ventral vessels with the dorsal or supra-oesophageal vessels. The heart is known as a lateral heart when it opens into the dorsal vessel and oesophageal heart when it opens into the supra-oesophageal vessel (Fig 1.18). The number and position of hearts have taxonomic importance. The blood of earthworms is red due to the presence of haemoglobin and contains colourless nucleated amoebocytes in the liquid plasma.

In general, the earthworms lack respiratory systems, but exceptions are seen in species like *Brachiodrilus hortensis* which has large areas called gills for gas exchange. Respiration in worms occurs through their highly vascular skin. Earthworms directly absorb oxygen from the environment and also release carbon dioxide to the outside.

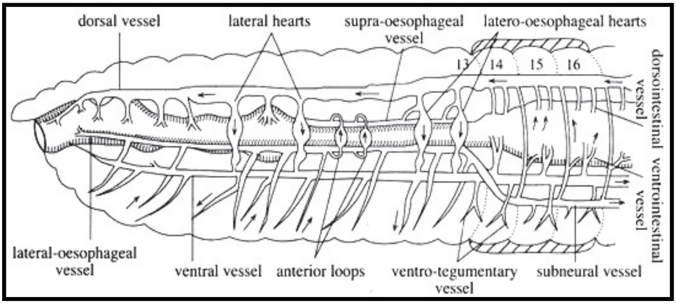

Figure 1.18: Circulatory system of earthworms (adapted from https://www.notesonzoology.com/earthworm/circulatory-system-in-earthworm**)**

Nervous system: The nervous system of an earthworm includes a pair of cerebral ganglia, called the brain, a ventral nerve cord and a pair of circumpharyngeal connectives (Fig 1.19). The nerve collar is formed by the connection of the brain to these connectives. The ventral nerve cord, situated just below the alimentary canal, bears a ganglion in each segment and sends peripheral nerves to various organs. The peripheral nervous system regulates both motor and sensory nerves. The nervous system conducts reflex actions even after being disconnected from the brain. Earthworms lack sense organs for vision and hearing, but the apical nerve ending of the skin is sensitive to stimulus. The sense of taste and smell are controlled by special epithelial cells of the buccal sac. Pieces of evidence suggest the neurosecretory potential of some neurons in earthworms. These neurosecretory substances play vital roles in different activities such as locomotion, feeding, reproduction, osmoregulation, growth and regeneration. Neuropeptides secreted in *Eisenia foetida* are associated with rapid muscle contraction during locomotion and feeding behaviour, including salivation (De Vries-Schoumacker,1977). The neuropeptides found in various species play different roles according to their niche requirements.

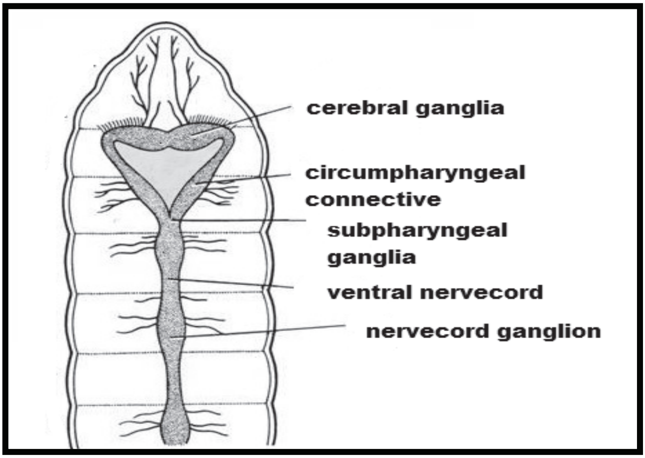

Figure 1.19: Nervous system of earthworms

Reproductive system: Earthworms are hermaphrodites having both male and female reproductive systems in one individual (Fig 1.20). The external openings of gonads are called genital pores, which lie on the ventral side of the worms. Paired testes (holandry) and paired ovaries (hologyny) are seen in megascolecoid earthworms. The number of testes and ovaries may be reduced to a single pair known as meroandry and progyny, respectively. The testes are either paired or unpaired and may lie free in their segments or are enclosed in special coelomic chambers, called testis sacs. In some species, the male pores are united with openings of accessory reproductive organs called prostate glands. The male funnels lead to the vasa deferentia, which may be straight or coiled. The ducts may unite with each other before opening to the exterior, or they may discharge separately on the body surface. The posterior end of the vas deferens is known as bulbus ejaculatrice and is enlarged into an ejaculatory bulb. Pair of seminal vesicles lie lateral to the corresponding testis and are connected to the testis sacs. The spermathecae are sac-like structures with the ampulla, one or more diverticula and a duct opening to the exterior. The structure of the spermatheca varies in different species. Setae associated with spermathecal pores are known as copulatory setae and those with prostatic pores as penial setae. The female reproductive system contains a pair of ovaries and a female gonopore. The ovaries are situated below the gut and on each side of the nerve cord. The oviductal funnel leads to oviducts, which in turn run posteriorly to meet the female gonopore.

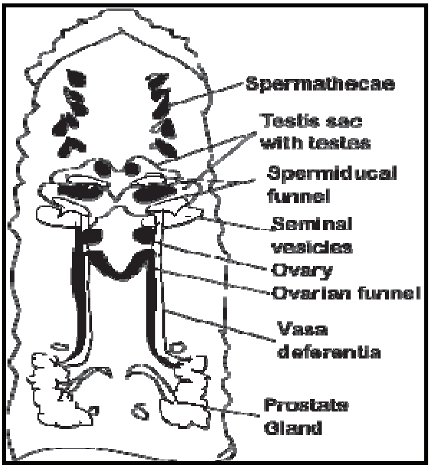

Figure 1.20: Reproductive system of earthworms

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CHAPTER TWO

ECOLOGICAL FUNCTIONS OF EARTHWORMS

Major categories of earthworms

Various species of earthworms occupy different strata in the soil ecosystem. They can be broadly divided into three categories, epigeic, endogeic and anecic, based on their ecological niche, feeding habits and burrows (Fig 2.1, 2.2). A comparative account of different parameters of different categories of earthworms is presented in Table 2.1.

Epigeic earthworms

These worms inhabit and feed on leaf litter of soil between the depths of 0–20 cm. They do not make burrows and are conventionally surface feeders. The upper surface of the soil is rich in organic matter such as leaf litter and animal excreta. These provide a suitable substrate for surface-feeding earthworms. The worms are exposed to high risk of mortality due to UV rays from sunlight as well as predation because of their surface feeding habit. These worms are heavily pigmented and, therefore, darker in colour. Epigeic worms can modify litter and accelerate microbial decomposition processes by interacting with the microbes inhabiting the upper layers of the soil horizon. They are small in size with a high reproductive rate and short life span. Because of the above-described characters, epigeic worms are generally designated as "r" selected species. This category includes species such as *Dendrobaena octaedra, Dendrobaena attemsi, Dendrodrilus rubidus, Eiseniella tetraedra, Helodrilus oculatus, Lumbricus rubellus, Lumbricus castaneus, Lumbricus festivus, Lumbricus friendi* and *Satchellius mammalis.*

Endogeic earthworms

Endogeic earthworms make horizontal burrows and are often larger, light coloured (grey, pale pink, green, blue) and less pigmented. These can be further classified into three groups, a) polyhumic endogeic (feed on high organic matter), b) mesohumic endogeic (feed on mild organic matter), c) oligohumic endogeic (feed on less organic matter). They can ingest soil and organic matter containing microbes and are designated as "k" selected species. The species in this group include *Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea icterica, Aporrectodea rosea, Murchieona muldali, Octolasion cyaneum* and *Octolasion lacteum.*

Anecic earthworms

This category of earthworms feed on litter on the soil surface and also drag it into their burrows. They produce permanent vertical burrows in the soil, which may extend up to 1–2 m inside the soil, and their casts are deposited on the surface. The burrow, which is lined with mucus and organic matter, serves as a channel for water and air. The worms are of medium size and can not tolerate wide environmental perturbations. They have the dark coloured head end and light-coloured tail. This group contains worms like *Aporrectodea longa, Aporrectodea nocturna, Lumbricus friendi* and *Lumbricus terrestris.*

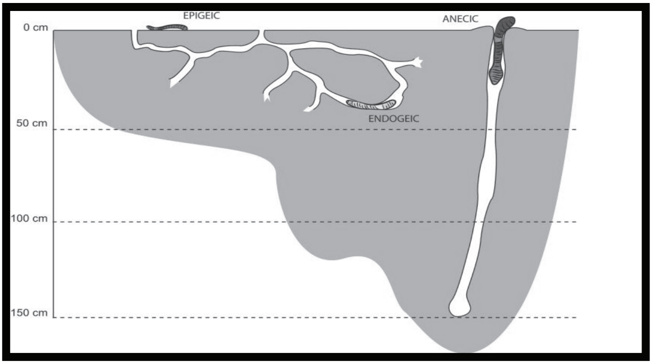

Figure 2.1: Different ecological categories of earthworms (adapted from Schelfhout et al., 2017)

Table 2.1: Comparison between different ecological parameters (Dash, 2012)

Parameters	Epigeic	Anecic	Endogeic
Habitat	Litter	Soil	Soil
Feeding	Litter , coarse particulate organic matter	Litter, humus and soil	Soil containing organic matter
Pigmentation	Dorsolateral and partly ventral	dorsally	No pigmentation
Body size	Small	Medium	Medium- large
Burrow formation	No/ negligible	Vertical	Vertical and horizontal
Life cycle	High reproductive rate, high metabolism	Low to medium reproductive rate, high metabolism	Low reproductive rate, low metabolism
Contribution	Fragmentation of litter, ingestion of soil biota	Litter fragmentation and transportation, mucus production	Mucus production, macrostructure formation, mineralization
Adaptability	Survive wide variation in environmental conditions of temperature, soil moisture, pH, high mortality	Unable to tolerate wide variation in environmental conditions	Unable to tolerate wide environmental fluctuation, more deeper in soil, hibernate and aestivate

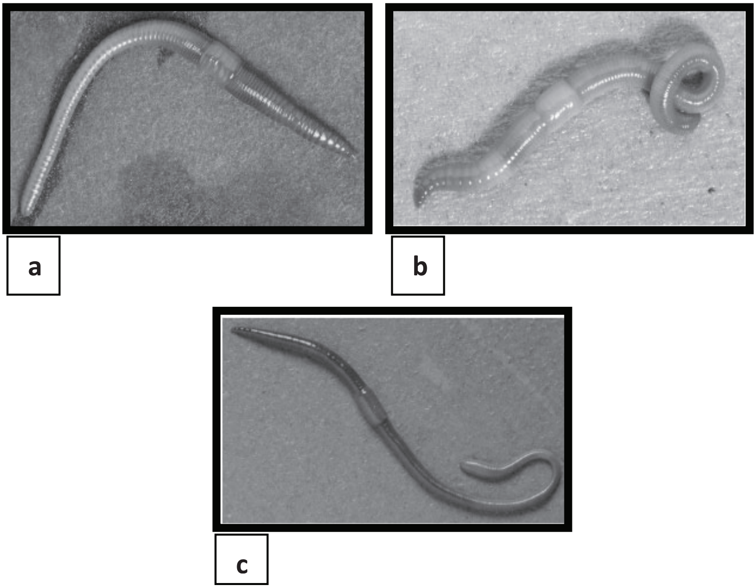

Figure 2.2: a) *Lumbricus castaneus*, an epigeic, b) *Allolobophora chlorotica*, an endogeic, c) *Aporrectodea longa*, an anecic earthworm (adapted from https://www.earthwormsoc.org.uk/earthworm-ecology)

The functional role of earthworms in soil

The soil represents one of the most important territories of terrestrial biodiversity. It contains flora, fauna and microbes along with physical and chemical components. The role of soil fauna in maintaining the ecosystem is well documented. The pedogenesis process is largely influenced by soil organisms that play pivotal roles in soil metabolism, including the decomposition of organics, and are responsible for sustaining nutrient balance. These soil faunas include larger invertebrates like earthworms, myriapods, insect larvae, protozoans, rotifers, nematodes, microarthropods and microfauna. Soil is also nurtured by both symbiotic and non-symbiotic nitrogen-fixing bacteria, actinomycetes, viruses and fungi. Based on size, soil fauna are classified into different categories, microfauna (≤ 100 µm), mesofauna (≤ 2 mm), macrofauna (> 2 mm) and megafauna (> 20 mm).

The fundamental question for ecologists is whether all the species in the soil are equally important or if certain species occupy the top place. The majority of ecologists, however, are unanimous that earthworms are the most abundant and dominant species and important for soil processes.

Earthworms contribute the largest part of invertebrate biomass in most soils and are also considered as an important decomposer. These animals are popular for their ability to modify soil's physical properties through the mixing of surface litter, casting, and burrowing activities. The life cycle of worms may impact on the soil properties and also facilitate the water flow through soil profiles to deeper layers. The activity of earthworms not only helps in water filtration and soil porosity but also accelerates the nutrient transformation and uptake by plants. Earthworms can consume a large amount of soil and produce casts containing a mixture of organic nutrients. This process could maintain the organic soil horizon with deeper minerals on the upper layers. The ability of organisms to move through the soil and to create organo-mineral structures as faeces (casts) and burrows is commonly referred to as "bioturbation”. Animals that do this accelerate nutrient cycles and organic breakdown in soil by feeding on organic materials. The worms feed on low C/N ratio organic materials to leave a pool of high C/N ratio materials. Earthworms induce the decomposition of surface litter and alter the C/N ratio of that litter. Soil with a high number of worm population shows less litter loss and low C/N ratio. The decomposition of litter by earthworms in agro-systems helps to manage soil erosion (Bohlen et al., 1997). It has been observed that endogeic species add intestinal mucus to the ingested soil that stimulates microbial activity. The mucus is entirely metabolized in the latter part of earthworm's gut and the microorganisms start to degrade the soil organic matter into assimilable form. Irrespective of different ecological groups, earthworms increase the availability of inorganic nitrogen in the soil and promote plant growth (Lemtiri et al., 2014). Although they generally increase the mineralization of carbon in certain cases, the breakdown of carbon is prevented under the stable aggregation of the soil by the worms. The reduction in soil carbon occurs in the first two years of earthworm invasion, and gradually it leads to carbon stabilization, which is a very difficult phenomenon to observe due to the background carbon content of the soil. According to a study, earthworms encourage carbon sequestration through unequal amplification of carbon stabilization (Zhang et al., 2013). The elevated amount of inorganic nitrogen in an earthworm's cast stimulates its mineralization. The ammonium component in earthworm excreta exerts a stimulating effect on nitrification processes in the soil. The feeding activity of worms converts nutrients like phosphorus (P) and potassium (K) into available forms for plants. On the other hand, the report says the invasion of the earthworm *Lumbricus rubellus* in sugar maple forests lowered P availability compared to the control. The nutrient management by earthworms in soil depends upon the type of species and the history of the land (Suárez et al., 2004). The worm gut releases K from the non-exchangeable form to the useful form for plant growth as the soil material passes through the worm gut. The calciferous glands in earthworms actively secrete mucus rich in calcium carbonate, which leads to the elimination of excess calcium (Ca) ions through casts. This process significantly increases Ca availability in soil. Apart from all the above functions, earthworms considerably influence the soil respiration process because they facilitate the growth and activity of aerobic microbes (Sinha et al., 2010; Bhadauria & Saxena, 2010; Blouin et al., 2013).

Earthworms regulate the water-holding capacity of soil. The movement of water through burrows depends on the morphological characteristics of the burrows and is related to the different ecological groups of earthworms. Water infiltration and conductivity activity generally increase with the increasing diameter of burrow and inter-connectivity. However, the conductivity decreases with an increase in the branching rate.

Darwin initially studied the contribution of earthworms in the chemical weathering process of soil formation, but he did not investigate the role of worms on mineral weathering. Pop (1998) reported that the acceleration of clay mineralogy and the formation of illite in the soil are possible by the earthworm *Octodrilus*. Another study also claims that the epigeic earthworm *Eisenia veneta* helps in weathering and transformation of anorthite, biotite, smectite and kaolinite to produce a new mineral phase (Carpenter et al., 2007). The process of mineral weathering during soil formation, whether solely controlled by earthworms or by the collective action of both worms and the microbes in their gut, is still a topic for open debate. In addition to contributing to mineral weathering, worms actively participate in humus formation. Earthworms carry out the rotation of organic matter. These animals bring soil particles from deep soil to the surface and facilitate the burial of surface litter such as leaves and twigs derived from natural vegetation or crops in the soil. Earthworm feeding and burrowing activities greatly influence the soil's chemical and biological properties—the ingested soil particles and organic matter result in the formation of the egesting cast. The different amounts of cast produced annually in various locations are presented in Table 2.2. The casts on soil can be eroded by wind or deposited as a stable aggregate. Alternatively, these surface casts are responsible for the migration of stones into the soil profiles and become a part of soil formation. The rate of surface cast deposition depends on the number of earthworms present along with the soil type, climate and vegetation.

Earthworms generally improve aeration by burrow or soil aggregate formation. The dynamic role of earthworms in maintaining the compactness of soil is different in various species. Certain compacting species such as *Reginaldia omodeoi*, decrease soil porosity by 3%, whereas species from Eudrilidae, having a de-compacting nature, increase it by 21%. Some earthworms decompact the soil due to the destruction of macroaggregates formed by compacting worms. Worms reduce the loss of surface nutrients by increasing the rate of water infiltration into the soil. The growing literature about the impact of earthworms on climate change is fragmentary. But certain studies indicate the connection between important greenhouse gases (CO_2, N_2O and CH_4) and carbon sequestration, denitrification and N_2O production by earthworms.

The impact of earthworms on plant growth might be both positive and negative. These animals help plant growth by the stimulation of microbes and making nutrients and water available. Despite the undeniable effects of earthworms, they might harm plants and soil. Earthworms may increase erosion by removing the protective cover of surface litter. Other adverse effects of earthworms include the pathogenic transmission of microbes to plants and loss of nitrogen through the leaching and denitrification process. Some earthworms cause damage to plants by seizing the leaves of growing plants. The burrowing activity also affects the germination process to some extent. The casts become hard on exposure to air and affect the normal percolation of water in the soil. Worms also cause soil erosion on hill slopes by bringing fine soil particles to the surface. Certain earthworm species in the United States of America have been reported to cause a nuisance on golf courses by making burrows below the grass cover, thus softening the surface, which makes the area unsuitable for the game. Critics have also argued that enhanced soil respiration facilitated by earthworms could release a significantly high amount of CO_2 (a major greenhouse gas) into the atmosphere, thus aiding global warming. However, the beneficial effects of earthworms in sustaining soil quality outweigh their negative roles (Julka, 1988; Kooch & Jalilvand, 2008).

Table 2.2: Earthworm casts produced in various locations (Kooch & Jalilvand, 2008**)**

Environment	Location	Cast (mg ha^{-1})/year
Arable	Germany	92
Arable	Nigeria	50
Arable/ Flood plain	Egypt	268
Pasture	England	19-40
Grassland	United State	24-94
Grassland	India	4-78
Savanna	Columbia	10-50
Temperate forest	Germany	7-60

Earthworms and other soil biotas

Earthworms significantly influence the microbial activity in the soil. They boost microbial functions in their gut by the ingestion of soil. These animals also provide favourable habitats for the growth and multiplication of microorganisms. Earthworms can consume several bacteria but fail to digest many fungal species such as *Thievia vasinfecta* and *Neocosmospora vasinfecta* due to their multilayered thick spore walls. These fungal species come out with casts. Not surprisingly, habitat invasion by earthworms modifies the microbial community and their enzymes to a larger extent in comparison to a habitat not invaded by these animals. The growing interest in the relationship between the microbial community and earthworms may raise several questions. For example, one may ask whether the worms stimulate microbial abundance in soil or the colonies fall as earthworms consume them. It is also not clear if they feed on selective microbial groups. The impact of worms on soil microbial communities is critical and different for diverse ecological groups.

Epigeic earthworms influence the soil microbial populations either in an increasing or decreasing way. A few pieces of literature show that the number of microorganisms remained unaffected by their action. Certain studies conducted in the laboratory taking epigeic earthworm species such as *E. andrei* and *E. foetida* demonstrated high microbial biomass in the transformed substrate made up of casts in the initial period, which subsequently got reduced. Studies have also been done on the interaction between microbes and endogeic earthworms. The endogeic *Aporrectodea caliginosa* did not show any significant impact on soil microbial biomass (Medina-Sauza et al., 2019). Most studies report that anecic earthworms have a positive or neutral impact on the microbial population in the soil. Earthworms also affect the population density of other invertebrates. The multitrophic interactions of worms either directly or indirectly affect the other living beings connected with the soil ecosystem. Worms alter the performance of herbivore insects by inducing changes in leaf chemistry and also get affected by aboveground predators, with potential feedbacks on plants and herbivores.

Distribution and diversity

A total of 7000 species of earthworms have already been identified, which are distributed worldwide. A single square metre of land can accommodate more than 150 individuals. Earthworms are also highly diverse, and anecdotal evidence says many species are yet to be identified and described. Although the worms are highly sensitive to various climatic factors, it is not clear how they have dispersed globally irrespective of wide environmental variations. Most of the boreal and subarctic regions are predicted to have low species diversity. Earthworms also show low species richness in subtropical and tropical countries, such as Brazil, India, and Indonesia (Fig 2.3).

Figure 2.3: Global distribution of earthworm total abundance (Phillips et al., 2019)

Distribution and Diversity of Earthworms in North America, Europe, Australia and Africa

The varying soil and climatic conditions of North America mean its territory has various temperate earthworm species. Reynolds and Reynolds (1992) reported 19 species and *Sparganophilus eiseni* was identified for the first time in Canada. Scheu and McLean (1993) presented the first report of earthworm distribution from southern Alberta and reported eight species including *Lumbricus rubellus.* Later, species like *Bimastos parvus, Dendrobaena octaedra and Dendrodrilus rubidus* were reported in Canadian territories. The cornfield, hayfield and forest systems of south-western Quebec, Canada show varying spatial and temporal distributions of earthworms. Certain earthworms like *Allolobophora chlorotica, Aporrectodea longa, Aporrectodea trapezoids, Aporrectodea tuberculata, Lumbricus terrestris* and *Octolasion tyrtaeum* are widely found there. The forest habitat of Central New York, North America is characterized by species such as *Aporrectodea tuberculata, A. turgid, A. trapezoids, Bimastos parvus, Lumbricus rubellus, L. castanea, L. terrestris, Octolasion tyrtaeum* and *Octolasion* sp.

The forest floor situated in the Osbroek woodland near Aalst (Belgium) is characterised by various groups of epigeic earthworms such as *Lumbricus castaneus, Dendrobaena mammalis, Dendrodrilus rubidu, Eisenia foetidas;* endogeic species *Allolobophora limicola, Nicodrilus caliginosus caliginosus, Octolasion tyrtaeum* and anecic species *Lumbricus terrestris* and *Lumbricus rubellus.* The dark coniferous forests in Pechora-Ilych nature reserve, Russia maintains the species diversity of the Lumbricidae family and species like litter dwelling *Dendrobaena octaedra, Dendrodrilus rubidus tenuis*, and *Eisenia foetida*; soil-litter dwelling *Perelia diplotetratheca, Eisenia atlavinyteae, Eisenia nordenskioldi, Lumbricus rubellus;* and soildwelling *Aporrectodea caliginosa, Aporrectodea rosea* and *Octolasion lacteum.*

Australia has an unrealized natural resource of earthworm diversity. The records from the territory of Australia states include major earthworm families like Moniligastridae, Ocnerodrilidae, Acanthodrilidae, Octochaetidae, Megascolecidae and Eudrilidae and characterised by commonly found earthworm species such as *Pithemera bicincta*, *Polypheretirm brevis*, *Polypheretima elongate, Polypheretima taprobanae, Aporrectodea tuberculata, Lumbricus terrestris, Octolasion cyaneum, Bimastos corutrictus, Bimastos parvus*, etc. Two species, *Aporrectodea caliginosa* and *A. longa,* were first recorded in Tasmanian pastures. Evidence claims *A. longa* was introduced to Australia, along with other countries like North America (e.g., California), Mexico, South America, the Maghreb, Asia, from southern France. Nxele et al. (2015) reported various indigenous and exotic species in the forest of South Africa, where the earthworm contributes important invertebrate diversity. Authors recorded 282 indigenous earthworm species (most endemic) belonging to three families: Microchaetidae, Tritogeniidae and Acanthodrilidae and 44 exotic species from six families. The grassland of Queen Elizabeth Park, South Africa contains mostly three species, *Dichogaster* sp., *Amynthas cortices* and *Amynthas rodericensis*, and is rich in indigenous *Tritogenia howickiana* species.

Distribution of earthworms with special reference to India

Ecologists have identified 418 species of earthworms, including 44 exotic species in well defined geological regions of India such as the Himalayas (western, central and eastern), the Indo-Gangetic plains and the Decan Peninsula (Western and Eastern Ghat). The species in India includes nine families and 69 genera (Tables 2.3, 2.4, Fig 2.4). The southern plateau and hill regions contain 14% of the Indian earthworm species. The Decan Peninsula is rich in earthworm species such as *Dichogaster bolaui, Drawida willsi, Perionyx excavatus, Perionyx sansibaricus, Lampito mauritii* and many more. Thirteen unique species, namely *Chaetocotoides, Comarodrilus, Dashiella, Hoplochaetalla, Karmiella, Konkadrilus, Kotegeharia, Mallehulla, Priodochaeta, Pridoscolex, Rillogaster, Travoscolides, Wahoscolex* are confined to Decan Peninsula. The central region of India and the Decan region share some common genera such as *Lennogaster, Ramiella, Perionyx, Octochaetona* and *Eudichogaster*. Climatic conditions and soil quality determine the distribution pattern of earthworms. It has been reported that the abundance of the tropical earthworm *L. mauritii* is high in all habitats of Southern India (Sathianarayanan & Khan, 2006). The habitat of Assam, North-East India, and a part of Indo-Burma regions have 17 species belonging to six families, namely Moniligastridae, Megascolecidae, Almidae, Glossocolecidae, Lumbricidae and Ocnerodrilidae. Species like *Amynthas diffringens, Perionyx excavates, Glyphidrilus gangeticus* and *Lampito mauritii* are dominant under agricultural land use system. At the same time, *Metaphire posthuma* and *Dichogaster saliens* are common in open grassland and mixed forest system (Rajkhowa et al., 2015). Earthworm biodiversity of the state of Uttar Pradesh indicates the occurrence of *Metaphire posthuma, Lampito mauritii, and Perionyx excavatus* of Megascolecidae family, *Eutyphoeus waltoni, E. gigas, E.orientalis, E. pharpingianus,* and *E.paivai* of Octochaetidae family and *Eisenia foetida* of Lumbricidae family (Prakash, 2017).

Likewise, the North-East and western Himalayan regions include species like *Octochaetona beatrix, Eutyphoeus festivus, E. nanianus,* and *E. waltoni.* Among exotic species, Lumbricidae is more common in the Western Himalaya region. The most common earthworm family found in India is *Octochaetidae eutyphoeus*, commonly seen on the Indo-Gangetic plains. Species like *Pontoscolex corethrurus* is common in all land use zones, whereas *Tonoscolex horai, Drawida assamensis* and *Amynthas* spp. are mostly found in the forests of North India and *Lampito mauritii* in the state of Odisha. The Eastern coastal plain harbours 7%, and other regions like the Trans-Gangetic plains, Gujarat plains, Western dry regions and Islands exhibit very poor earthworm diversity of 1-4% of the total number of species. Earthworms such as *Metaphire anomala, Metaphire houlleti, Ocnerodrilus occidentalis, Dendrodrilus rubidus* occupy the region of Nanda Devi biosphere reserve, a relatively cooler area having less anthropogenic pressures (Dash, 2012). The Malabar mountain areas maintain its species such as *Celeriella, Lampito, Moniligaster, Notoscolex* and *Troyia.* Species like *Amynthas* and *Metaphire* are native to Andaman and the Nicobar Islands.

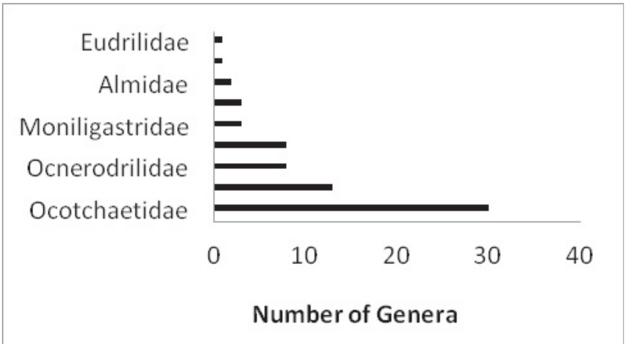

Figure 2.4: Number of genera under earthworm families in India

Ecosystem and regions of India	**Earthworm diversity**	**Density/m²**
Pastures, Berhampur, Odisha	*Lampito mauritii, Ocnerodrilus occidentalis*	18 - 88
Grassland and pasture, Sambalpur, Odisha	*Drawida calebi, D. willsi, Lampito mauritti, Dichogaster bolaui, Octochaetona surensis, Ocnerodrilus occidentalis*	0 - 272
Mixed deciduous forest, Sambalpur, Odisha	*Lampito mauritii, Drawida calebi, Rameila bishambari, Pellogaster bengalensis*	24 - 131
Humid tropical deciduous forest, Andhra Pradesh	*Amynthas alexandri, A. diffringens, Metaphire posthuma, M. Houlleti, Dichogaster* sp.	28 - 281
Shifting agriculture, North-Eastern India, Nangpoh	*Megascolides antrophytes, Drawida assamensis, Nelloscolex strigosus*	68
Shifting agriculture, North-Eastern India, Shillong	*Amynthas diffringens, Drawida assamensis, Eutyphopus festivus, Nelloscolex, Tonoscolex horaii*	4 - 47
Pasture, Kumaon Himalaya	*Amynthas diffringens, A. alexandri, Eisenia foetida*	138
Cultivated soil, Kumaon Himalaya	*Amynthas alexandri*	58
Varanasi, Uttar Pradesh	*Dichogaster bolaui, Eutyphoeus incommodus, E. nicolconi, E. waltoni, Octochaetona surensis, Ramiella bishambari, Amynthas morrisi, Lampito mauritii, Metaphire posthuma, Drawida calebi, Glyphidrilus* sp.	

Village landscape, Central Himalayas (Mid elevation)	*Bimostus parvus, Octolasion tyrtaeum, Octochaetona Beatrix, Amynthas corticis, Eutyophoeus festivus, E. nanianus, E. waltoni*	300 – 940
Cropland, pasture, garbage sites, Jharkhand, Ranchi	*Lampito mauritii, Drawida calebi, D. bollui, D. affinis, Metaphire planate, M. Posthuma, Perionyx sansibaricus, Ocnerodrilus occidentalis, Lennogaster bengalensis, Glyphidrilus tuberosus , Pontoscolex corethrurus , Pellogaster bengalensis*	75 - 7600
Village landscape, Garhwal Himalayas (Mid elevation)	*Amynthas corticis, Drawida nepalensis, Allbophora parva, Eutyphoeus pharpingianus, Octochaetona Beatrix, Perionyx* sp., *Lennogaster pusillus*	108 - 247
Nanda Devi Biosphere, Central Himalaya	*Lennogaster pusillus, Metaphire houlleti, M. anomala, Ocnerodrilus occidentalis, Dendrodrilus rubidus, Aporrectodea calliginosa, Amynthas corticis*	5 - 100
Nilagiri biosphere, Kerala	*Dichogaster affinis, Drawida modesta, D. barwelli, D. ghatensis, D grandis, Glyphidrilis annandalei, Haplochetalla sp., Lampito mauritii, Megascolex insignis, M.triangularis, Octochaetona Beatrix, Parryodrilus lavellei, Plutellus variabilis, Pontoscolex corethrurus*	2 - 294
Rubber plantation, Tripura, India	Pontoscolex corethrurus, *Eutyophoeus gammiei, E. camillahnus, E. assamensis, E. festivas, Dichogaster bolaui, D. teraffinis, Octochaetona Beatrix, Perionyx sp., Kanhuria sumerianus, Metaphire houlleti, Drawida assamensis, D. papillifer, D. nepalensis, Lennogaster chittagongensis, Gordiodrilus elegans*	108

Table 2.3: Earthworm diversity in the different ecosystems of India (Dash, 2012**)**

Earthworm families	**Distribution in India (Native/ exotic)**	**Origin**
Eudrilidae	Exotic	Central Africa
Glossoscolecidae	Exotic	Tropical America
Lumbricidae	Exotic	Europe
Megascolecidae	Native (Tamilnadu, Nilagiri)	South-East Asia, China
Moniligastridae	Native (Tamilnadu, Nilagiri, Malabar, Kerala, Odisha)	Eastern Asia
Ocnerodrilidae	Native (Peninsular India)	South America, Tropical Africa
Octochaetidae	Mostly Native (Tamilnadu, Nilagiri, Odisha, Maharastra, Western ghat, Madhyapradesh)	West Africa
Acanthodrilidae	Exotic	Australia, New Zealand, South Africa, South America, and North America
Almidae	Exotic	Africa, South Asia

Table 2.4: Native and exotic earthworm families distribution (Dash, 2012**)**

Habitat and habits

Earthworms prefer neutral and organic-rich soils, but comfort zones vary among different species. Worms can make burrows up to 3 metres in the top 45 cm of soil. They can easily migrate to nearby sites if conditions become unfavourable. The presence of adequate food and optimal moisture provides a suitable environment for the worms to increase their population. They are found in grassland, forest, cultivated lands, and caves. Some species are also seen in mountains. The major source that attracts worm is organic material such as compost, manure, forest litter and humus. They are also observed under stones and the leaves of trees (Julka, 1988). Some species, for example *Perionyx excavatus,* and

Drawida bolaui, are found in organic-rich soil, whereas *Pontodrilus litoralis* lives in saltwater marshes. Some native species like *Eutyphoeus* prefer the alluvial soils of Indo-Gangetic plains, but *Haplochaetella* is found in the laterite and red soils of the Western Ghats in India.

Earthworms are cold-blooded or poikilothermal animals as their body temperature fluctuates with the surrounding temperature. They are nocturnal in habit and avoid bright light. They come out of their burrows at night in search of food. The worms use their setae to move against the wall of the burrow. They can crawl in both forward and backward directions by shortening and lengthening their bodies. Only endogeic and anecic groups make burrows. The shape of the burrow varies from a U to Y shape. The burrow is often lined with mucous and casting materials. Earthworms disperse from soil either directly or indirectly (Marinissen & Van den Bosch, 1992). They also migrate a considerable distance in large numbers during heavy rain, due to oxygen deficiency or increased amount of hydrogen sulphide in soil.

Species like *Perionyx excavatus* and *Amynthas diffringens* from Western Himalaya of India start migrating en masse at the end of the monsoon (the rainy season). Although the reason behind it is not well understood, rapid depletion of soil moisture and low temperature might be the possible reasons. During this migration, many worms are likely to die due to desiccation or exposure to ultra-violet rays from sunlight or predator attacks. Earthworms undergo diapause during adverse climatic conditions. During burrowing activities, worms feed on soil and microorganisms and deposit it in the form of casts. The cast shape and composition vary from species to species. The casts of the epigeic group of worms contain fragmented leaf material, whereas the other two groups release organic-rich materials in their casts. Earthworms do not have eyes but have a tremendous sense of smell. They can use their sense of smell to search for food and mates. The mating of worms occurs during the rainy season. The adaptation of earthworms to escape from danger has been studied in different species. The epigeic worms take advantage of fast movement to escape from predators. *Perionyx excavatus* can jump and produce a series of whipping moments to escape from danger. Likewise, *Amynthas cortices* moves in a serpentine manner—certain species like *Hoplochaetella inornata* and *H. stuarii* lose a fragment of their tail. *Metaphire posthuma* coil their bodies, *Amynthas alexandri* pretend to be dead, and *Eisenia foetida* produce a bad smell when touched by predators (Darwin, 1881; Edwards, 1981).

How ecological factors influence earthworms

The abundance and growth of earthworms are critically affected by various environmental factors (Kale & Karmegam, 2010). Although epigeic earthworms are tolerant of a wide range of environmental fluctuations such as pH, temperature, moisture, etc., every ecological group has a certain limit to withstanding environmental stress (Table 2.5).

Temperature

Earthworms have fairly complex responses to changes in temperature. The growth and reproduction of species are controlled by temperature irrespective of regions. They prefer a temperature range of between 15 °C (59 °F) and 25 °C (77 °F). In subtropical regions, worms can tolerate a variation in soil temperature from 4 °C to 30 °C. The worms beyond this temperature range reduce metabolic activities, coil their bodies and undergo hibernation or diapause. Earthworms might sometimes migrate to deeper layers of the soil to avoid temperature fluctuations. Extreme temperatures cause large scale mortality because of moisture deprivation and consequent reduction in cutaneous respiration in the worms. Soil temperature can also impact the lifecycle and cocoon production of worms. Higher temperatures induce a short incubation period and vice versa. The earthworms in temperate regions have a long incubation period. The optimum temperature for cocoon production is 25 °C. Edwards (1988) reported the variation in tolerance to temperature on life cycles of four different species of earthworms, *E. foetida*, *D. veneta*, *E. eugeniae*, and *P. excavatus.* The optimum temperature for *E. foetida* was 25 °C, whereas *D. veneta* had a lower range. The optimum temperature for *E. eugeniae* and *P. excavatus* was around 25 °C. These species suffer heavy mortality at temperatures below 9°C and above 30°C. Cocoon production requires a lower temperature than the growth of species. The survival of organisms under prolonged exposure to freezing conditions is not possible unless they are in protective cells. Extreme low and high temperatures induce reactive oxygen species (ROS), enhancing the antioxidant enzyme level in earthworms such as *E. eugeniae* (Mishra et al., 2018). In certain species, the impact of temperature is not direct; rather, it promotes microbial activities in the substrate. The increase in microbial activities causes depletion in the oxygen amount of soil and might impact the survival of earthworms.

Moisture, pH and ammonia salt

The dermis of an earthworm is permeable to water, and the worms can hold 75%-90% water in their fresh body weight. The earthworms maintain hydrostatic pressure of the coelomic fluid for locomotion. Earthworms lack a special respiratory organ. The moist skin of the worm can uptake gases from outside. The excretion of ammonia requires water to dissolve it. Hence, the water level of the worm needs to be maintained. The soil moisture plays an important role in the survival mechanism of earthworms. Soil moisture deprivation also causes oxidative stress in earthworms and may affect stress enzymes (Mishra et al., 2020). Although the worms can withstand desiccation up to a certain extent, soil moisture deficiency could seriously impair the respiration and excretion processes of these animals. Species like *E. foetida* and *E. andrei* survive in soil moisture ranging from 50% to 90%, but the growth is maximum in soil moistures of 80% to 90%. The normal body functions of worms are disturbed at soil moisture levels of below 10–20%. During reproduction, cocoon production is affected if the soil lacks optimal moisture content. Worms are not commonly found in arid and semi-arid soil due to moisture deficiency. Some earthworms like *Glyphidrilus, Perionyx,* and *Thatonia* are hydrophilous and live in a submerged habitat.

Soil pH also influences the distribution of earthworms. Most earthworm species can tolerate pH levels of 5–9 but the optimum pH level for the earthworm is 6–8. Earthworms are highly sensitive to ammonia and cannot withstand the elevated ammonia levels in organic wastes. Excess inorganic salts cause mortality in worms.

Species	Soil temperature (°C)	Soil moisture (%)	Soil pH
Pontoscolex corethrurus	19-32	10-29	4.5-4.8
Drawida papillifer	22-32	10-29	4.5-4.7
D. assamensis	21-31	10-29	4.4-5.2
Metaphire houlleti	21-28	14-20	4.4-5.2
Eutyphoeus comillahnus	21-27	17-18	4.5-4.6
Dichogaster affinis	21-28	17-21	4.8-5.2
Octochaetona Beatrix	24-28	11.5-14	4.7-4.8
Lennogaster chittangongensis	24-30	16-18	4.7-4.8

Table 2.5: Range of temperature, moisture and pH tolerances of certain Indian earthworms (Chaudhuri et al., 2008**)**

Taxonomy of tropical and subtropical earthworms (Julka, 1988)

Order LUMBRICULIDA

1) Family Lumbriculidael

Order MONILIGASTRIDA

2) Family Moniligastridae

Order HAPLOTAXIDA

Suborder HAPLOTAXINA

3) Family Haplotaxidae
   Suborder TUBIFICINA
   Superfamily Enchytraeoidea

4) Family Enchytraeidae
   Superfamily Tubificoidea1

5) Family Tubificidae

6) Family Naididae

7) Family Phreodrilidae

8) Family Opisthocystidae

9) Family Dorydrilidae
   Suborder ALLUROIDINA

10) Family Alluroididae

11) Family Syngenodrilidae
    Suborder LUMBRICINA
    Superfamily Biwadriloidea

12) Family Biwadrilidae
    Superfamily Criodriloidea

13) Family Criodrilidae
    Superfamily Lumbricoidea

14) Family Sparganophilidae

15) Family Ailoscolecidae

16) Family Hormogastridae
    i. Subfamily Hormogastrinae
    ii. Subfamily Vignysinae

17) Family Lumbricidae
    i. Subfamily Lumbricinae
    ii. Subfamily Diporodrilinae

18) Family Lutodrilidae
    Superfamily Glossoscolecoidea

19) Family Kynotidae

20) Family Micicrochaetidae

21) Family Glossoscolecidae

22) Family Almidae
Superfamily Megascolecoidea

23) Family Ocnerodrilidae
i. Subfamily Ocnerodrilinae
ii. Subfamily Malabarinae

24) Family Acanthodrilidae

25) Family Octochaetidae

26) Family Megascolecidae

27) Family Eudrilidae
i. Subfamily Eudrilinae
ii. Subfamily Pareudrilinae

Key to the Families of Indian Megadrile Oligochaeta* (*The key does not apply to the species which may lack spermathecae, male pores and prostatic glands. Julka, 1988)

1. ....
Testes and male funnels intraseptal, male pores at the posterior margin of the segments as their corresponding tests; clitellum single-cell thick; Eggs large, yolky.. O. Moniligastrida

(Moniligastridae)

Tests and male funnels interseptal, male pores at least two segments posterior to the segment bearing testes; clitellum formed by multiple layers of cells; eggs small, not yolky................................. 2. O. Haplotaxida

(S.O. Lumbricina)

2. ....
Male pores anterior to or on segment XVl 3

Male pores posterior to segment xvi (not easily recognizable in Glossoscolecidae and Almidae) 4

3. ....

Dorsal pores present .... Lumbricidae
Dorsal pores absent .... Criodrilidae

4. ....

Prostatic glands present 5
Prostatic glands absent 9

5. ....

Spermathecal pores anterior to testis segments or mostly so located (not associated with ovaries or oviducts 6
Spermathecal pores in or posterior to the testis segments (always associated with ovaries or oviducts) .... Eudrilidae

6. ....

Last pair of hearts in segment xi .... Ocnerodrilidae
Last pair of hearts posterior to segment xi 7

7. ....

Prostatic glands racemose without central canal...Megascolecidae
Prostatic glands tubular with central canal 8

8. ....

Holonephric .... Acanthodrilidae
Meronephric .... Octochaetidae

9. ....

Extramural calciferous glands present..................... Glossoscolecidae

Extramural calciferous glands absent .... Almidae

Common earthworms of the tropics and subtropics

The characteristics of some earthworm species are described below.

1. ***Perionyx excavatus*** or Indian dung worm (Michaelsen)

Family: Megascolecidae

Habitat and feeding habit: Epigeic, commonly found in moist habitats such as animal dung, domestic waste, sewage and banks of ponds. Feed on decaying organic matter of plants and animals. Species are widely distributed in India.

Character: Colour is a deep brown with a bluish shine, there are many setae per segment, an epilobic prostomium, clitellum extends from 13 to 17 segments, the male pore is situated in pits on 18^{th} segment, the pits are close together and contains many setae, a single median female pore is on 14^{th} segment. Length: 40–80 mm and rarely up to 150 mm. Temperature tolerance: 9°C–33°C. The reproductive rate is high, cocoon production/year is in the range 55–365, the number of young per cocoon is 1–4, and the incubation period of a cocoon is 14–28 days.

2. ***Perionyx sansibaricus*** or Zanzibar worm (Michaelsen)

Family: Megascolecidae

Habitat and feeding habit: Epigeic, commonly found in moist soil surface covered with leaf litter, kitchen waste, sewage and on the banks of river and streams. Feed on decaying organic matter, native to Peninsular India.

Characters: Purple colour on the dorsal side and pale on ventral, has many setae per segment, has an epilobic prostomium and a short groove extends behind the prostomium intersegmental furrow, first dorsal pore occurs in the 2/3/4/5/6 furrows, clitellum covers 13 to 17 segments, male pore situated on 18^{th} segment, three pairs of spermathecal pores are close together and the female pore is on 14^{th} segment. Length: 32–65 mm. Cocoon production/year: 70.

3. ***Perionyx ceylanensis*** or Ceylonese blue worm (Michaelsen)

Family: Megascolecidae

Habitat and feeding habit: Found in moist organic matter and leaf litter. Worms probably feed on leaf litter and organic matter. Found in Sri Lanka and Peninsular India.

Characters: The dorsal side is violet in colour with a darker median strip, and the ventral side is yellow, has an epilobic prostomium, the clitellum covers from 14 to 17 segments and the first dorsal pore is situated between the 4^{th} and 5^{th} segments. The male pore is present on the 18^{th} segment, three pairs of spermathecal pores in are present on segments 6/7/8/9. The female pore is on the 14^{th} segment and median placed. Length: 50–60 mm.

4. ***Dichogaster bolaui*** or Bolaui worm (Michaelsen)

Family: Octochaetidae

Habitat and feeding habit: Epigeic, native to Africa, mostly found on soil with high organic matter, compost pits, kitchen wastes, tree holes and sewage beds. Feeds on decaying plants and animal and organic waste.

Characters: Red in colour with an epilobic prostomium and the underside lacks pigmentation. There is a lumbricine arrangement of setae, the clitellum is annular and extends from the 13th to 20th segments. The male pore is on the 18^{th} segment and the female pore on the 14^{th} segment, with two pairs of spermathecal pores at the intersegmental furrows of segments 7/8/9. Length: 19–43 mm. Temperature tolerance: 20–28 °C. Cocoon production/year: more than 47, number of young per cocoon: 1–2, incubation period of a cocoon: 7–14 days.

5. ***Eisenia foetida*** or red worm/compost worm/garlic worm/European manure (Savigny)

Family: Lumbricidae

Habitat and feeding habit: Epigeic, found in the garden, forests, roadside dumps and domestic sewage rich in organic content. Feeds on dung and organic matter. Species are native to the UK and Europe.

Characters: Red and yellow body with distinct segmental bands with eight setae per segment, an epilobic prostomium and the clitellum extends from segment 26 to 32. The male pore is situated on the 15^{th} segment and the first dorsal pore is situated in between 4^{th} and 5^{th} segment. Two pairs of spermathecal pores are present between the 9^{th} and 11^{th} segments. Length: 35–130 mm. Temperature tolerance: 3°C–31°C. The reproductive rate is high, cocoon production/year: 55–300, number of young per cocoon: 2–3, incubation period of a cocoon: 30–75 days.

6. ***Lampito mauritii*** or Mauritius worm (Kinberg)

Family: Megascolecidae

Habitat and feeding habit: Anecic and epigeic, native to India, widely distributed and found in grassland, forest litter, crop fields, gardens, domestic garbage and sewage. Animals feed on decaying plant material and microbes such as fungi in soil.

Characters: Greyish to brownish in body colour with many setae per segment, an epilobic prostomium and the annular clitellum covers segments 14 to 17. The male pore is on the 18th segment and the first dorsal pore is situated in between the 10/11th–11/12th intersegmental furrow. Three pairs of spermathecal pores are located in intersegmental furrows of the 6/7/8/9th segments and a pair of penial setae project from each male pore. Length: 95–155 mm. Temperature tolerance: up to 32°C. The reproductive rate is high, cocoon production/year: 12–45, number of young per cocoon: 1–2, incubation period of a cocoon: 28–30 days.

7. ***Drawida willsi*** or willsi worm (Michaelsen)

Family: Moniligastridae

Habitat and feeding habit: Epigeic in nature, commonly found in crop fields, compost pits and kitchen waste. Feeds on leaf litter and decomposed materials.

Characters: Purple in colour with a lumbricine arrangement of setae, and a prolobic prostomium. The clitellum is red and covers segments 10 to 13. Dorsal pores are absent and paired male pores are on slightly raised oval areas in the inter-segmental furrow of the 10/11th segments while one pair of a genital pore is located in the intersegmental furrow of the 9/10th segments. Length: 40–60 mm. Optimum temperature tolerance: 24–28°C, cocoon production/year: 15, number of young per cocoon: 2–3, incubation period of a cocoon: 14–18 days.

8. ***Octochaetona surensis*** (Michaelsen)

Family: Octochaetidae

Habitat and feeding habit: Epigeic and anecic, mostly found in peripheral areas of compost pits, plant roots and agricultural fields. Feeds on microbes and soil organic matter.

Characters: Grey or brown in colour, with an epilobic prostomium, the clitellum covers segments 13 to 17. There is a minute spermathecal pore and the female pore is median, paired/unpaired. Each spermatheca contains a short stalk-like diverticulum. Length: 60–140 mm. Temperature tolerance: 20–26°C. The number of young per cocoon is one, with an incubation period of 28–30 days. The cocoon colour gradually changes from yellowish to bluish-green and deep brown.

9. ***Eudrilus eugeniae*** or African nightcrawler (Kinberg)

Family: Eudrilidae

Habitat and feeding habit: Native to West Africa and found in soil rich in organic matter. Feeds on decomposed plant and animal materials.

Characters: Reddish to dark brown and purple in colour, with four pairs of setae per segment and an epilobic prostomium. The clitellum covers segments 14 to 18 and dorsal pores are absent. Small male pores are paired at the tip of the penis and paired female pores are situated on the 14^{th} segment. Length: 90–185 mm. Optimum temperature tolerance: 7–32°C, cocoon production/year: 73–347, number of young per cocoon: 2–3, incubation period of a cocoon: 15–30 days.

10. ***Lumbricus terrestris*** or lobworm; nightcrawler (Linnaeus)

Family: Lumbricidae

Habitat and feeding habit: Native to West Europe but globally distributed in temperate to mild boreal climates, found on the forest floor and feeds on litter.

Characters: Dark brown to reddish in colour, faded pigmentation towards the posterior end with a tanylobous prostomium. Male pores are prominent on the 15^{th} segment while the clitellum covers segments 32 to 37. Setae are closely paired. Length: 110–200 mm. Optimum temperature tolerance: 15 °C.

11. ***Bimastos parvus*** or rotten wood red worm (Eisen)

Family: Lumbricidae

Habitat and feeding habit: Native to North America, epigeic in nature. These are commonly seen in kitchen garbage, leaf litter, domestic waste and manure. The species feeds on decaying organic matter.

Characters: Red in colour, with four pairs of setae, an epilobic prostomium, and a saddle-shaped clitellum covering segments 24 to 30. The male pores are on the 15th segment, and the spermathecal pore and tubercula pubertatis are absent. Length: 23–46 mm. This species prefers cool temperatures and has between one and four young per cocoon.

12. ***Pontoscolex corethrurus*** or brushytail worm (Muller)

Family: Glossoscolecidae

Habitat and feeding habit: Endogeic and anecic, the species was first reported in South America. Mostly found in the gardens, forests, rubber plantations, tree trunks and compost heaps. Feeds on decomposed organic material.

Characters: The body colour is light pink and has four pairs of setae per segment. The prostomium is elongated, and there is an absence of a dorsal pore. The clitellum is saddle-shaped and covers segments 15 to 23 and both male and spermathecal pores are minute. The female pore is situated mid-ventrally. Length: 50–100 mm. Optimum temperature tolerance: 15–29°C, cocoon production/year: 25–120, number of young per cocoon: 1 or rarely 2, the incubation period of a cocoon: 34–42 days.

13. ***Amynthas cortices*** or crazy worm/snake worm/black wriggler (Kinberg)

Family: Megascolecidae

Habitat and feeding habit: First reported in China, found in crop fields, pastures, under stones and in kitchen waste. Feeds on decaying materials and surface litter.

Characters: Body colour varies from reddish-brown to yellowish-brown and dark chocolate colour, and has a perichaetine arrangement of setae. The prostomium is epilobic and the first dorsal pore is between segments 11 and 12. The clitellum covers segments 14 to 16 and four pairs of spermathecal pores and the male pore is on segment 18, while the single female pore is present on the 14th segment. Optimum temperature tolerance: 21–27°C, number of young per cocoon: 1, incubation period of a cocoon: 30–90 days.

14. ***Amynthas gracillis*** or Alabama/Georgia jumper (Kinberg)

Family: Megascolecidae

Habitat and feeding habit: Mostly found in garden soil and under stones. Feeds on decaying plant materials.

Characters: Body colour is brown and red and this worm has many setae per segment and an epilobic prostomium. The first dorsal pore is between segments 10 and 11. The clitellum extends from segments 14 to 16, there are three pairs of spermathecal pores and the male pore is on segment 18. Small circular markings are scattered between the male pores and the female pore is present on the 14^{th} segment. Length: 60–160 mm. Optimum temperature tolerance: 21–27°C, number of young per cocoon: 1.

15. ***Eutyphoeus assamensis*** (Stephenson)

Family: Octochaetidae

Habitat and feeding habit: Found in the region of Assam, India. Feeds on decaying materials.

Characteristics: The prostomium is pro/tanylobic. The first dorsal pore is on the $11/12^{th}$ segment. The clitellum covers segments 13 to 17. Female pores are paired, and the spermathecal pore is minute. Length: 185–245 mm. The description of this species is not adequate, as male genitalia has not fully developed.

16. ***Eutyphoeus orientalis*** (Beddard)

Family: Octochaetidae

Habitat and feeding habit: Mostly found in gardens. Feeds on decaying organic matter.

Characteristics: This worm is dark grey and the clitellum is present from segments 14 to 16. The female pore is single and situated on the left side. Spermathecal pores are paired. Length: 130–250 mm.

17. ***Glyphidrilus tuberosus*** (Stephenson)

Family: Almidae

Habitat and feeding habit: Mostly found in paddy fields. Feeds on decaying matter in the soil. Native to India.

Characters: The clitellum is annular. Genital markings are small, circular to elliptical tubercles. Spermathecal pores are minute and situated between segments 13/14 and 14/15. Female pores are paired and situated on segment 14. Length: 60–118 mm.

18. ***Metaphire posthuma*** (Vaillant)

Family: Megascolecidae

Habitat and feeding habit: Native to Asia. Mostly found in paddy fields and domestic sewage.

Characteristics: The clitellum is annular and covers segments 14 to 16. The prostomium is epilobic, paired genital markings are present, and the single female pore is situated on the 14^{th} segment. Male pores are minute and present on the 18^{th} segment. Four pairs of spermathecal pores are present, and the first dorsal pore is on the $12/13^{th}$ segment. Length: 60–140 mm.

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CHAPTER THREE

EARTHWORMS AS COMPOSTING AGENTS

Vermitechnology

Over the last few decades, rapid population growth and industrialization have caused devastating environmental disasters such as agrochemical toxicity and waste disposal mismanagements. Prolonged chemical treatments and unplanned industrialization are the root of soil ill health. This problem uplifts the idea of organic farming. The progressive interest of using biota in ecological sustainability demands that the earthworm becomes a part of eco-management. The tremendous decomposing potentiality of the earthworm makes it an ideal composting agent. The earthworm is a natural aerator, crusher and mixer and stimulates microbial activities related to the composting process. Currently, farmers are realizing the worth of this biological decomposer and making efforts to involve it in various farming processes. Vermitechnology involves the application of earthworms to produce organic products. This process is eco-friendly, cost-effective and socially acceptable. Vermitechnology may include vermicomposting; vermi-remediation; vermi-agro production; vermi-protection; vermi-production (Sinha et al., 2010). However, vermiculture and vermicomposting are strong pillars of vermitechnology. The production of vermicompost involves two major steps:

1. Vermiculture that involves the culturing of worms.
2. Vermicomposting includes the conversion of municipal and domestic wastes into organic manures using earthworms.

Vermiculture

The age-old practice of culturing earthworms gained popularity after experiments conducted in Holland in 1970 and subsequently in England and Canada. The American Vermicompost Technology Company could produce tons of vermicompost per month in 1978–79. In early 1980, Dr Roy Hartenstein developed a field-scale application of compost using poultry, pig and cattle waste. Since then, several vermicomposting studies have been done in different countries like Spain, the USA, Australia, Germany, France, Italy, etc. In 1984, The Agricultural University, Bangalore in India propagated the practice of vermiculture and farming throughout the country. The popularity of vermicomposting has been established in India by the entrepreneuring organization, Mr Morarka-GDC Rural Research Foundation, Rajasthan, India.

The term vermiculture is derived from the Latin word *vermis*, meaning worm and culture, meaning farming. It describes the mass production of earthworms for waste degradation and composting. Raising and culturing worms is not a difficult task. Earthworm cultures can be maintained in a small pit with suitable organic food resources. Adequate organic residues and enough soil moisture are required for the optimal growth and multiplication of earthworms. The selection of suitable earthworm species for vermiculture depends on various criteria (NPEC, 2004; Munroe, 2007).

1. The earthworm should have high consumption, digestion and assimilation rate.
2. It should have wide adaptability to environmental factors such as temperature and moisture.
3. The growth rate and disease resistance of the earthworm should be high and it must have high interspecies compatibility.
4. The species should be an efficient converter of plant and animal biomass to body proteins and have a short inactivity period.

Vermiculture can be maintained either in a small wooden/plastic/earthen box or a large culture pit. Around 100 earthworms can be easily cultured in a small box, whereas a larger pit of 1m x 1m can hold 1000 worms. The box should be filled with sand, broken bricks, soil, rice bran or sawdust, cow dung, and organic waste in equal proportions from the bottom upwards to maintain an ideal culture. The ideal size of a culture box is 50–60 x 25–30 x 20–30 cm. The optimum soil moisture and temperature for a worm culture are 30–40% and 18–28°C, respectively. The C/N ratio of organic materials is important for the culture and varies from 30–35 in the initial period and is gradually is reduced to 10–15 in organic manures. Mostly epigeic and anecic species like *Perionyx excavatus, Drawida bolaui, D. willsi, Eisenia foetida, Lampito mauritii,* and *Eudrilus eugeniae* are suitable for vermiculture and composting.

The composition of organic waste impacts the growth of earthworms during culture. Excess carbohydrates (lignin, cellulose, etc.) in the feed material might create problems during the culture of worms since these cannot be processed. Epigeic worms can directly break down certain organic materials, but some need pre-treatment. Among the different types of organic wastes used in vermiculture, cattle solids, and horse manure are good for earthworm growth. During waste processing through vermicomposting, non-degradable solid materials are removed from the organic waste. Pig and poultry wastes, including chicken, duck and turkey manures, contain ammonia and inorganic salts, hence there is a need for processing before use in the culture medium. Nevertheless, these wastes are rich in nutrients and ideal organic materials for vermiculture. Domestic wastes such as potato peel, tree leaves, grass, and food wastes can be easily consumed and processed by earthworms after partial decomposition by microbes. Agricultural wastes like sugar cane thrash, biogas sludge/slurry, horticulture wastes, dry flowers, leaves and amended cow dung are also suitable for vermiculture (Sharma et al., 2009; Dash, 2012).

Participation of earthworms in composting

The production of manures by microbial breakdown of organic wastes is known as composting. Under suitable environmental conditions, microorganisms such as bacteria, fungi and actinomycetes excrete enzymes onto the organic substrate, which catalyses the conversion of complex compounds into simple forms. Certain groups of soil fauna such as earthworms, nematodes and arthropods facilitate this process. This process is called decomposition. The composting of organic wastes involves the process of decomposition with a certain degree of facilitation. Composting requires optimum temperatures, moisture contents and aeration to maximize decomposition. The end product of this process is an organic fertilizer rich in nutrients and suitable for plant growth (Ansari & Ismail, 2012). The composting process is of two types i) aerobic and ii) anaerobic, based on the requirement of oxygen uptake.

Heterotrophic microorganisms break down the polymers of organic compounds into simple, intermediate compounds like alcohol and organic acids and finally convert these to simple sugars. The end products of this degradation process are mineral nutrients like nitrates, sulphates and phosphates required for plant growth. The decomposition process brought by soil microorganisms and pedo-fauna also produces carbon dioxide and humic acids (Fig 3.1). Carbon is the energy source for plants and is also needed for the synthesis of protoplasm.

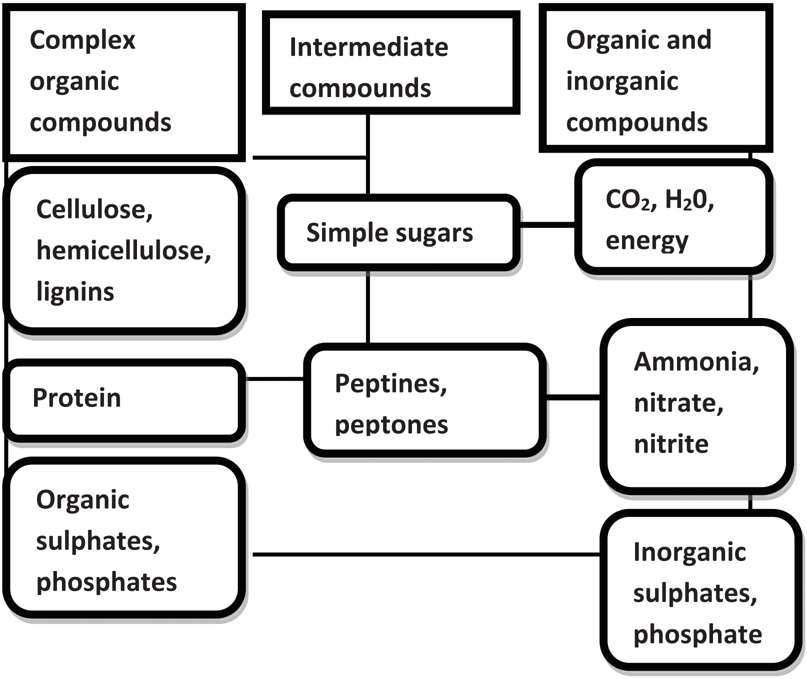

Figure 3.1: Conversion of organic wastes into simpler forms during the composting process

Several groups of micro, meso and macro biota are actively involved in the composting process (Table 3.1). Some organisms are involved in the physical modification of larger organic molecules, whereas the microbes release certain enzymes for chemical changes.

Groups of organisms	Types
Microflora	Fungi, algae, bacteria
Microfauna	Protozoans
Mesoflora	Mushrooms
Mesofauna	Mites, ants, termites
Macrofauna	Millipedes, centipedes, beetles and earthworms

Table 3.1: Diversity of organisms involved in composting process (Dash, 2012)

The composting procedure, which includes the earthworm as an active player, is known as vermicomposting. Vermicomposting is the combination of the biological process with a systematic arrangement of composting techniques, which includes potential earthworm species suitable for the production of a large number of manures. The worms in the compost pit are allowed to consume and digest various organic wastes. They digest the ingested materials through enzymes and microbes present in their gut. The naturally rich organic excreta with microbes come out in the form of casts. The faecal matter of earthworms has good water holding capacity, excellent porosity and high levels of nutrients, enzymes and microbes. Unlike normal compost, vermicompost holds the worm mucus, which protects it from washing away. In addition to that, vermicompost does not contain human pathogens as the materials containing pathogens are killed while passing through the gut of the worms. During eating, earthworms also maintain aerobic conditions for substrates and cover the substrate with their faecal material to avoid unwanted flies and bad odour.

Stages in vermicomposting

The production of vermicompost can be done in three basic phases (Dash, 2012).

Phase 1: This phase includes the collection of different organic wastes and the separation of unwanted materials like glass, stones, etc., from it. Some wastes like leaves and peels are air-dried before application.

Phase 2: This phase involves the maintenance of the earthworm culture in the pit. Suitable vermicompost species must be inoculated and mixed with soil and organic wastes. The farmyard manure layers are settled in between organic waste layers, and the pit is covered with jute/cotton bags to prevent evaporation and predation. The moisture content of the composting material should be maintained at 30–40% by periodically sprinkling water on it.

Phase 3: This phase involves the screening of worms from compost and undecomposed wastes. Earthworms are separated for reuse, and composts are ready for marketing. For the separation of earthworms, two methods are used. One is the pyramid method, which describes the arrangement of vermicompost in a pyramid shape so that worms will crawl out due to sunlight exposure. The second method is the hand scooping method, which includes the separation of worms using a hand-sorted procedure.

Bedding

Materials that provide the worms with a comfortable and relatively stable habitat are known as bedding. The bedding materials should have high absorbency, moisture-holding capacity and good bulking potential. Different types of materials have different impacts on porosity. The bedding materials should have low protein and nitrogen contents and high C/N ratios. High protein and nitrogen may create harmful conditions for worms. Some bedding materials are independently used, whereas certain others provide a better environment in combination with materials based on their features (Table 3.2).

Bedding Material	**Absorbency**	**Bulking Potential**	**C:N Ratio**
Horse Manure	Medium-Good	Good	22 - 56
Peat Moss	Good	Medium	58
Corn Silage	Medium-Good	Medium	38 - 43
Hay – general	Poor	Medium	15 - 32
Straw – general	Poor	Medium-Good	48 - 150
Straw – oat	Poor	Medium	48 - 98
Straw – wheat	Poor	Medium-Good	100 - 150
Paper from the municipal waste stream	Medium-Good	Medium	127 - 178
Newspaper	Good	Medium	170
Bark – hardwoods	Poor	Good	116 - 436
Bark -- softwoods	Poor	Good	131 - 1285
Corrugated cardboard	Good	Medium	563
Lumber mill waste -- chipped	Poor	Good	170
Paper fibre sludge	Medium-Good	Medium	250
Paper mill sludge	Good	Medium	54

Sawdust	Poor-Medium	Poor-Medium	142 - 750
Shrub trimmings	Poor	Good	53
Hardwood chips, shavings	Poor	Good	451 - 819
Softwood chips, shavings	Poor	Good	212 - 1313
Leaves (dry, loose)	Poor-Medium	Poor-Medium	40 - 80
Corn stalks	Poor	Good	60 - 73
Corn cobs	Poor-Medium	Good	56 - 123

Table 3.2: Common bedding materials and their features (Munroe, 2007)

Small scale vermicomposting

The small scale vermicomposting can be maintained using kitchen wastes. A small wooden box or plastic container is used with proper holes for aeration. Sieved soil and cow manure with kitchen wastes such as partly decomposed leaves, shredded waste paper and straws can be used as bedding material for composting. The size of the container is approximately 60 cm x 60 cm x 20 cm. The container, filled with 4 kg of pre-incubated kitchen wastes, has around 100 adult worms added (Fig 3.2). The kitchen wastes are added at ten-day intervals, and the moisture level is maintained at roughly 30-40% by adding water. Species like *Lampito mauritii, Drawida bolaui* and *Perionyx excavatus* can be used in kitchen level composting. After 6–8 weeks of composting, the manures are collected. The worms feed, grow and lay cocoons to hatch juveniles. Worms are photophobic and should be kept in a dark environment. If the worms are trying to escape, it is a signal that conditions are not ideal inside the bin. Too wet and dry conditions make the worms uncomfortable. Composting worms can tolerate a wide range of temperatures, but the optimum range is between 70 and 80 °F (21–26 °C) (Yadav et al., 2014). Surplus worms are good sources of food for chicken and fish. Common problems that may arise in small scale composting and their management are presented in Table 3.3.

Problems	Cause	Solution
Bad smell	Overfeeding / Bin too wet / Not enough air	Stop feeding for two weeks. Bury food completely. Mix in dry bedding; leave the lid off. Fluff bedding; clear drainage holes.
Flies attracted towards the bin	Food scraps exposed, rotten food Too much food; especially citrus	Bury food completely. Cover with clean bedding. Do not overfeed Reduce acidic foods.
Worms are dying or crawling away	Bin too wet or too dry Not enough air / food	Mix in dry bedding, leave the lid off. Add more bedding and food
Bedding drying out	Too much ventilation	Dampen bedding; keep a lid on

Table 3.3: Common problems, causes and solution in small scale vermicomposting (Adapted from Selden et al., 2005)

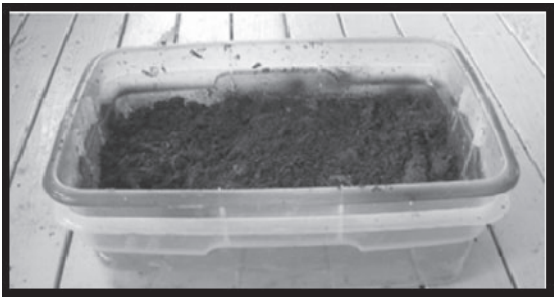

Figure 3.2: Small scale vermicomposting (adapted from Selden et al., 2005)

Large scale vermicomposting

The large scale vermicomposting systems are of different types such as windrows, beds or bins, and flow-through reactors. Each type has several variants. The composting process is operated in two ways. One is a batch system where bedding and food are mixed, and then worms are inoculated. Second is a continuous-flow system in which worms are placed on bedding, and both bedding materials with food are added at regular intervals.

a) Windrows

A windrow is a long row of material that is used in vermicompost bedding. There are three different types of windrow methods for large scale worm farming and compost production (Fig 3.3).

1. Static pile windrows

In this method, piles of different shapes like elongated squares or rectangles are used. Bedding materials and foods are layered on top with an inoculation of worms and allowed to stand until the processing is complete. The size should not exceed one metre in height before settling. The materials are mixed by turning with a tractor bucket and materials like poultry manure, shredded fibre, cattle manure are used.

2. Top-fed windrows

Top-fed windrows are similar to the static pile windrows except that they follow the continuous-flow operation. In this method, bedding is followed by the inoculation of worms. A layer of food is covered periodically. The consumed food by worms comes out in the form of casting near the bottom of the windrow. A layered windrow created over time contains the end product on the bottom, partially consumed in the middle, and the fresher food on top. The major drawback of this method is that it needs continuous feeding, and the windrow covers need to be replaced every time the worms are fed. The work requires extra effort for the operator. On the other hand, top-feeding provides ample opportunity for the operator to modify environmental conditions such as feeding rate, pH, moisture content, etc. as required. This procedure tends to result in higher worm growth and vermicompost production.

3. Wedges

The vermicomposting wedge is a variation to a top-fed windrow. This system also follows a continuous flow pattern of bedding. In this type, the stock of worms in the bedding is placed inside a corral-type structure. The sides of the corral can be concrete, wood, or even bales of hay or straw.

The bucket loader is used to add fresh organic material through the open side regularly. This process can be continued indefinitely, and worms do not need to be separated from the vermicompost pit.

Figure 3.3: Windrow method of vermicomposting (Adapted from Munroe, 2007)

b) Beds or bins

Unlike the windrow system, beds are covered with four walls and a floor. The bed can be insulated from the side or layered as an insulating "pillow" on top made up of bags or bales of straw. Beds are usually top-fed, but the only disadvantage is the small surface area (Fig 3.4). The bins can add a vertical dimension to address this issue.

Figure 3.4: Cinder-block worm beds on Scott farm (Adapted from Munroe, 2007)

c) Flow-Through Reactors

Dr Clive Edwards and colleagues demonstrated this concept in England in the 1980s and it has since been adopted by several vermicompost companies. A rectangular box is used and the worms are placed on top of the waste materials. The material is added to the top, and the product is removed through a grid at the bottom, usually using a hydraulically driven breaker bar. The materials are consumed by worms and released as casts to the bottom powered by "breaker bars". The cardboard is placed under the garbage to protect them from falling. Waste materials are thoroughly grounded and layered on the top regularly. The wall of the retractor is weatherproof, and the bottom is open for the collection of vermicompost. The harvesting of compost is done either manually or by an eliminator.

Above ground and commercial vermicomposting pit

The vermicomposting system can be prepared above the ground. One of the models is the cement ring. A ring of 90 cm in diameter and 30 cm in height can be made using cement. The commercial model of vermicomposting consists of four walls. The walls are made by normal bricks, hollow bricks, asbestos and locally available rocks. This model consists of a partition wall to allow the free movement of worms in between chambers. Each chamber is provided with a slight slope to drain excess water, which can be reused in crop fields. All the chambers are filled with organic materials, and earthworms are released in the first chamber. After completion of processing in the first chamber, worms move to the next chamber. This model reduces the cost and labour required for the process.

Different wastes and substrates in vermicomposting

Certain wastes are not independently eligible for potential vermicomposting. It has been observed that the industrial wastes are not proved as a good matrix, and the ability of worms is greatly reduced in industrial wastes. This drawback has been compensated for by adding organic substrates, which increase the efficiency of worms in vermicomposting. Organic materials like cow dung, biogas plant slurry and poultry manures add value to vermicomposting when mixed with industrial wastes (Ali et al., 2015). It also enhances the fertilizer value of the vermicomposting. The amendment of cow dung in different waste materials also provides a nutrient-rich environment for worms and facilitates the degradation rate. In contrast to this result, sometimes it is reported that distillery sludge mixed with cow dung decreases the potential of vermicomposting (Suthar, 2008). Significant improvement is also noted in the rate of vermicompost production when cow dung is added to 30% of industrial food sludge (Yadav & Garg, 2009). The solid paper mill sludge provides the best result when mixed with sewage sludge in 3:2 ratios. Still, it shows high mortality in worms when mixed with pig slurry because of the change in environmental conditions (Elvira et al., 1997). The vermicomposting potentials of some earthworms species for degrading different types of wastes amended with the combination of the suitable organic substrate are given in Table 3.4.

Organic amendment	**Wastes**	**Earthworm species**	**References**
Cow dung	Waste of citronella plant	*Eudrilus eugeniae*	Deka et al., 2011
Cattle dung	Tannery sludge	*Eisenia foetida*	Vig et al., 2011
Sawdust and cow dung	Guar gum	*Perionyx sansibaricus*	Suthar, 2007
Sewage sludge	Polycyclic aromatic hydrocarbons	*Eisenia foetida*	Contreras-Ramos et al., 2009
Poultry droppings	Textile mill sludge	*Eisenia foetida*	Garg & Kaushik, 2005
Biogas plant slurry	Textile mill wastewater sludge	*Eisenia foetida*	Garg et al., 2006
Cow dung and agricultural residues	Solid textile mill sludge	*Eisenia foetida*	Mata-Alvarez et al., 2014
Sugar mill filter cake	Horse dung	*Eisenia foetida*	Chen et al., 2010
Wheat straw and biogas slurry	Vegetable solid waste	*Eisenia foetida*	Suthar, 2009

Sheep manure	Cotton industrial waste	*Eisenia foetida*	Albanell et al., 1988
Cattle dung	Sugar industry waste	*Eisenia foetida, Perionyx excavatus,* and *Eudrilus eugeniae*	Meena & Renu, 2009
Cattle manure	Dairy industry sludge	*Eisenia andrei*	Elvira et al., 1998
Cow dung	Fly ash	*Eisenia foetida*	Gupta et al., 2005
Cow manure	Hospital wastes	*Eisenia foetida*	Singh et al., 2010
Cow dung and agriculture residues	Leather processing sludge	*Eisenia foetida*	Nogales et al., 2005
Cow dung	Solid textile mill sludge	*Eisenia foetida*	Kaushik & Garg, 2003

Table 3.4: Vermicomposting using a combination of different organic substrates and wastes (Ali et al., 2015**).**

Earthworm species in vermicomposting

The time taken by diverse earthworm species to produce vermicompost is different in different types of organic wastes. The selection of earthworm species for vermicompost depends upon minimal gut transit time, fast growth rate and high reproductive potential of worms. Maximum results can be achieved by the appropriate species and their capabilities in the degradation of organic wastes. The species *Eisenia foetida* is mostly popular in vermicomposting for the stabilization of organic wastes such as neem leaves, the dung of cow, buffalo, horse, donkey, sheep, goat, and camel, biogas slurry, vegetable market waste, kitchen waste, agro residues, and industrial wastes. However, *Dendrobaena veneta* and *Lumbricus rubellus* species are widely used in temperature regions and *Eudrilus eugeniae, Perionyx excavatus* and *Perionyx hawayana* are used in tropical regions. The decomposition efficiency of *Perionyx sansibaricus* is high in the decomposition of agriculture wastes and urban solid waste. The African species, *Eudrilus eugeniae* is widely used all over the globe as a potential vermicomposting species. In most cases, different species are used in a combination known as polyculture. On the other hand, *Lampito mauritii* is not as efficient as other worms in vermicomposting although its potential in the modification of soil structure is appreciable. Species like *Drawida sunderghensis* and *Lampito mauritii* are also useful in the conversion of canteen organic wastes into manures. The vermiculture characteristics and high reproductive fitness of endogeic-anecic earthworm, *Pontoscolex corethrurus* found in the rubber plantation of Tripura, India, makes it better vermicomposting species over other species. The anecic species *Lumbricus terrestris* is also a potential candidate for vermicomposting. The vermicomposting time scales of organic waste using a diverse range of earthworms is given in Table 3.5. 3.6.

Organic wastes	**Earthworms**	**Time (Days)**	**References**
Coffee pulp	*Eisenia foetida*	98	Reddy & Shantaram, 2005
Soybean straw, wheat straw, chickpea straw and city garbage	*Perionyx excavatus*	180	Kaushik & Garg, 2004
Coffee pulp	*Eudrilus eugeniae*	112	Gratelly et al., 1996
Pig manure	*Eisenia foetida*	252	Nogales et al., 1999
Cattle dung	*Perionyx excavatus*	75	Suthar, 2012
Cow and horse manure	*Drawidia nepalensis*	240	Kaushal & Bisht, 1992
Sewage sludge	*E. foetida*	126	Benitez et al., 2000
Spent mushroom compost	*E. foetida* and *E. andrei*	90	Hemalatha & Meenambal, 2005

Table 3.5: Vermicomposting using diverse earthworm species (Ali et al., 2015**)**

1 kg of each waste	Semi dry cattle dung (in days)	Kitchen wastes (in days)	Garden wastes (in days)
E. foetida	59	78	89
E. eugeniae	44	61	69
P. excavatus	62	83	91
Mixed species	45	70	80

Table 3.6: Comparison of vermicomposting time (days) taken by different species in different wastes (Rao, 1997; Sinha et al., 2002)

Factors affecting vermicomposting

Some important factors including feeding pattern, pH, moisture and temperature of the bedding materials affect the growth and reproduction of worms as well as the vermicompost production and quality.

Feeding and food

The feeding pattern plays an important role in the propagation and cocoon production of earthworms during vermicomposting. Toxic metals in organic materials create lethal conditions for worms. High organic matter consumption by worms decreases their activities and enhances anaerobic microbes, which may create a foul odour. Worms also consume anaerobic microbes. The thumb rule says worms should consume ½ of their body weight. The feedstock, like dairy and beef manures, is generally considered a natural food. In addition to poultry, horse manure, fresh food scraps and pre-composted manure, seaweed and legume hays are also used in vermicomposting procedures as the worm's food.

Moisture and aeration

In contrast to conventional composting systems, the vermicomposting process requires a high amount of moisture as the earthworms respire through the skin. A moisture content in bedding of less than 30% causes lethality. The optimum moisture content for ideal vermicomposting is 40–50%. The high moisture level in the compost bed enhances the biomass and growth rate of worms. The reduction in moisture content delays the sexual maturity in worms. Certain earthworms like *Lumbricus terrestris* can survive in less moist conditions, whereas species like *Allolobophora* *chlorotica, Allolobophora caliginosa,* and *Aporrectodea rosea* cannot tolerate dry conditions. Proper oxygen supply is essential for an efficient composting process. An anoxic condition not only kills the worm but also creates favourable conditions for certain microbes that produce toxic gases.

Temperature and pH

Vermicomposting worms can tolerate a wide range of temperature fluctuations. Species like *E. foetida* can survive under low-temperature ranges, but they neither consume food nor reproduce in this situation. The tolerance range of *E. foetida* varies between 15 and 20 °C. The earthworm *L. terrestris* increases biomass at an optimal temperature range of 15–17.5 °C. Higher temperatures (above 30 °C) induces chemical and microbial activity in the substrate, which leads to the reduction of oxygen level which affects the earthworms adversely. The temperature tolerance levels are different for different earthworms. For example, *E. foetida* shows a high growth rate at 25 °C with a 0–35 °C temperature tolerance range, whereas *Dendrobaena veneta* grows at lower temperatures and has less tolerance of extreme temperatures. *E. eugeniae* and *P. excavatus* show maximum growth at 25 °C.

On the other hand, these species show a higher hatching percentage at lower temperatures (20–24 °C) when compared to high temperatures (27–30 °C). The maximum temperature for both vermiculture and vermicomposting should not exceed 35 °C as worms either die or escape at extreme temperature conditions. Most of the vermicomposting worms can survive in a pH range of 5–9. The microbial activity in the decomposition process alters the physicochemical properties of wastes and affects the pH. During the vermicomposting process, the pH level shifts from alkaline trend to neutral or acidic. The pH is altered by the nature of different wastes used in vermicomposting. As the food and bedding materials affect the pH of the compost medium, these should be closely monitored.

Other toxic compounds

Earthworms are highly sensitive to salts, and these may cause mortality in populations. The leaching of salts from upper layers may mean that worms come in contact with salts. The salt contents should be removed from feed and bedding materials before use. Animal materials may contain urine content, and this can create toxic gases in bedding. Animal wastes must be processed to remove urine before applying to the compost pit. Toxic compounds like detergent cleaners, industrial chemicals, pesticides, etc. in sewage or septic sludge, paper-mill sludge, or some food processing wastes can be harmful to worms. The feed and waste materials should be safe and less toxic to avoid the mortality of earthworms.

Pests and disease

Although microbes are not problematic in vermicomposting, worms are prone to a common disease known as “sour crop” or protein poisoning caused by altered environmental conditions. This condition is caused by excess protein in the bedding due to overfeeding. The excess protein produces acids and gases and causes swollen clitellum in earthworms. This anomaly can be treated by using mycins (Munroe, 2007). In addition to that, worms in windrow methods are directly exposed to birds and other insects. Earthworms are easy targets for moles and centipedes. Although centipedes cannot grow in vermicompost bed, they can attack cocoons and decrease the worm population. Ants and mites cause problems by competing for the food that is meant for earthworms. White and brown mites consume earthworm’s food, whereas red mites act as parasites for worms. Precautions should be taken by using windrow covers and keeping the bedding site unfavourable for unwanted competitors.

Vermicompost: The organic fertilizer

The organic waste which comes out from the gut of earthworms is known as vermicast or vermicompost. Vermicomposting is a natural process of recycling of various wastes into valuable organic manures by earthworms. The worms release about 50% of the consumed waste in the form of cast each day. The chemical and biological property of vermicast depends upon the organic substrates, moisture, pH and the species used in vermicomposting (Amouei et al., 2010; Amouei et al., 2017). The nutrient properties of casts using various organic wastes and species are presented in Tables 3.7 and 3.8. The cast of worms contains a higher percentage of both macro and micronutrients than the conventional compost (Table 3.9). Major components like NO_3, PO_4, Ca, K, Mg, S and micronutrients present in vermicompost enhance plant growth like any chemical fertilizer. The microbial activity in the cast is 10–20 times higher than in soil, and several nitrogen-fixing microorganisms like actinomycetes, *Azotobacter, Rhizobium, Nitrobacter*, etc. are found in the vermicompost.

The worm compost contains approximately 54 x 10^6 CFU/g bacteria, 8 x 10^4 CFU/g fungi and 1 x 10^4 actinomycetes. The average pH of vermicompost is 7.0, and the moisture content of cast ranges from 32% to 66% (Sharma et al., 2009).

Nutrient elements	**Food waste vermicompost**	**Cattle manure vermicompost**	**Paper waste vermicompost**
N (%)	1.3	1.9	1.0
P (%)	2.7	4.7	1.4
K (%)	9.2	1.4	6.2
Ca (µg/g)	18614	23245	9214
Fe (µg/g)	23264	3454	17811
Mg (µg/g)	4364	5802	7661
Mn (µg/g)	610	160	447
Na (µg/g)	842	3360	613
S (µg/g)	2587	5524	1929
Zn (µg/g)	279	516	127

Table 3.7: Physical and chemical values of vermicompost produced by different earthworm species (Yadav et al., 2014**)**

	Lumbricus castaneus	***Aporrectodea caliginosa***	***Eisenia foetida***	***Aporrectodea longa***	**Mixed species**
Colour	Blackish	Deep Brown	Blackish	Deep Brown	Blackish
Odour	Odourless	Odourless	Odourless	Odourless	Odourless
Texture	Soft	Dense	Soft	Dense	Soft
Organic carbon (C)	13.82%	9.89%	15.52%	11.29%	12.74%
Nitrogen (N)	1.47%	0.82%	1.32%	1.09%	0.97%
Phosphorus (P)	0.12%	0.24%	0.28%	0.19%	0.16%
Potassium (K)	0.38%	0.24%	0.49%	0.31%	0.33%

Table 3.8: Nutrient values of vermicompost produced from different wastes (Arancon et al., 2005**)**

Elements	Vermicompost (%)	Garden compost (%)
Organic carbon	9.8 - 13.4	12.2
Nitrogen	0.51 - 1.61	0.8
Phosphorous	0.19 - 1.02	0.35
Potassium	0.15 - 0.73	0.48
Calcium	1.18 - 7.61	2.27
Magnesium	0.09 - 0.56	0.57
Sodium	0.05 - 0.15	<0.01
Zinc	0.004 - 0.11	0.0012
Copper	0.0026 - 0.0048	0.0017
Iron	0.2 - 1.33	1.16
Manganese	0.01- 0.2	0.04

Table 3.9: Comparison of nutrient values between vermicompost and garden compost (Nagavallemma et al., 2004)

Vermiwash: The liquid fertilizer

The activity of earthworms in burrows maintains the drilosphere, which is also rich in microbes. Water passing through these burrows collects the fluid of earthworms and certain nutrients which are then absorbed by the roots of plants. This phenomenon helps promote the growth of plants, and the concept is utilized to produce vermiwash. The vermiwash technique includes the washing of earthworms to collect their fluids and certain plant promoting factors. The excreta of earthworms also contains nitrogen-fixing bacteria and all macronutrients required for plants. The liquid yellow biofertilizer containing the mixture of excretory products and mucus of worms is known as vermiwash. The chemical characteristics of vermiwash are presented in Table 3.10.

Vermiwash can be prepared by filling a 250 L drum with a 25 cm layer of coarse sand at the bottom, 25 cm of gravel at the middle and 30-40 cm of loamy soil on the top. This drum is layered with pre-decomposed organic matter and dried animal manures. Earthworm species such as *Lampito mauritii* and *Perionyx excavatus* are inoculated for 15 days with the addition of water to maintain the moisture level. Five litres of water is poured into the vermiwash drum. The water percolates through the earthworms and compost and is collected through a tap at the bottom. The production cycle is repeated to collect vermiwash. The entire set up is emptied and restarted after prolonged use and overproduction of worms in that system (Fig 3.5)

Vermiwash can be used as potent fertilizer for plant growth. The liquid contains several micronutrients and enzymes like phosphatase, protease, amylase, urease and organic acids. It can be applied as a foliar spray, biopesticide and fertilizer. Certain plant growth hormones such as auxin, cytokinin and potent antibiotic compounds produced by microbes in the gut of worms make this liquid an ideal growth regulator for plants. Sometimes vermiwash is also mixed with cattle urine for better productivity. Shreds of evidence indicate that the application of vermiwash is effective in several plants like potato (*Solanum tuberosum*), onion (*Allium cepa*) and spinach (*Spinacia oleracea*). The nematicidal effect of vermiwash on plants is also documented.

Parameters	**Vermiwash**
pH	7.5
EC (dS/m)	0.25
OC (%)	0.008-0.010
N (%)	0.01-0.015
P (%)	1.7-1.75
K (ppm)	23-27
Na (ppm)	7-9
Ca (ppm)	3-4
Cu (ppm)	0.01-0.015
Fe (ppm)	0.05
Mg (ppm)	157-180
Zn (ppm)	0.02

Table 3.10: Chemical characteristics of vermiwash (Dash, 2012**)**

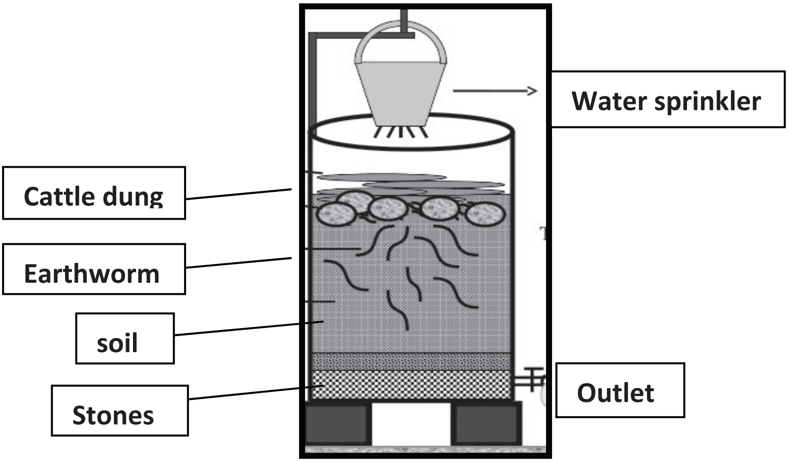

Figure 3.5: Vermiwash production and collection

Application of vermicompost

Vermicompost is found to be superior to conventional composts in many areas. The vermicompost provides better nutrient-holding capacity, enhanced ability to retain moisture, good soil structure and higher levels of microbial activity. Traditional compost contains high ammonium, while vermicompost tends to be higher in nitrates, which is a superior plant-available form of nitrogen. Several nutrients, including P, K, S and Mg, are higher in vermicompost in comparison to conventional composts, which are easily available to plants. According to Dr Clive Edwards, vermicompost is 1000 times more microbially active than traditional compost. These microbes facilitate nutrient availability to plants. Vermicompost stimulates additional plant growth independent of nutritional transformations and availability. It has a beneficial impact on seed germination, enhanced seedling growth and development in addition to plant productivity. The potentiality of vermicompost to protect plants against various diseases has also been observed. It has been reported that the high levels of beneficial microorganisms in vermicompost protect plants by outcompeting pathogens for available resources and also block the access of pathogens to plant roots by occupying all the available sites. The ability of vermicompost to repel pests has not been well documented. Nevertheless, some reports claim castings sometimes repel hard-bodied pests due to the production of the enzyme chitinase by worms, which breaks down the chitin in insects' exoskeletons (Munroe, 2007). Experiments have indicated that the application of vermicompost in fields may increase macropore space from 50 μm to 500 μm leading to improved air-water relationships and enhanced crop growth. Application of vermicompost not only alters the soil physicochemical properties but also reduces the soluble chemical species in soil that causes soil contamination (Mitchell & Edwards, 1997).

The vermicompost can be conveniently applied to agricultural, horticultural, and ornamental crops. The rates of application have been mentioned below.

1. General field crops: The application rate is 2–3 t/ha, mixed with seed either at the time of sowing or by row application.
2. Fruit trees: Around 5–10 kg compost per tree is applied by making a 15–18 cm deep ring and also in combination with dry cow dung and bone meal. The dose is applied as per the age of the tree.
3. Vegetables: 1 t/ha compost is applied in a nursery bed for the raising seedlings to be transplanted. Around 400–500 g of compost per plant is used for transplants at the time of planting and before irrigation.
4. Flowers: Around 750–1000 kg/ha compost is applied.

Earthworms are nature's ploughman for sustaining adequate aeration of the soil and maximal activity of aerobic microbes responsible for the decomposition of organics. Besides, they play a pivotal role in converting complex organic matter into simpler forms with the release of essential nutrients which could sustain microbial colonization of soil and plant growth. Vermicompost is a precious gift of nature. The high nutrient values of vermicompost can fulfil the nutritional needs of crops. The judicious application of vermicompost can provide economic stability to the farmers as well as environmental sustainability to agroecosystems.

Application of earthworms to increase crop productivity

Earthworms are soil-dwelling invertebrates, and their activities (both biotic and abiotic) influence soil properties, which in turn modulate crop growth. Apart from vermicompost production, the introduction of earthworms in fields also significantly contributes to the field crop productivity. The best performance in crop production is evidenced in fields treated with earthworm species in addition to organic manures. The addition of organic manure or high-quality crop residues provides excellent conditions for earthworm activity. In a project funded by the European Union, scientists from eight countries experimented on crop productivity through the inoculation of 13 earthworm species. The success rate of productivity depends upon the earthworm density, type of species and fertilization process. Earthworms release the nitrogen locked away in residue and soil organic matter and encourage plant growth. As per the report by Brown et al. (1999), shoot biomass increased by 57% and grain biomass by 36% in crops like rice, cowpea, tea, grass, beans, and wheat after inoculation of earthworms. Some studies concerning the successful improvement of crop productivity through earthworm inoculation in different regions are presented in Table 3.11.

Therefore, it is evident that besides their natural ecological role in maintaining soil fertility, earthworms could be utilized as potential agents for remediation of contaminated soils and reclamation of degraded land to a considerable extent, thus preventing desertification and facilitating re-vegetation and crop production.

Plants	**Per cent growth in crop production after earthworm inoculation**	**Earthworm species**	**Country**	**References**
Turves and grass pastures	58-98%, 31-110%	*A. caliginosa*	New Zealand	Nielson (1951)
Barley	240-280%	*A. caliginosa*	Lithuania	Atlavinyte (1971)
Oats	18-49% shoot	*L. terrestris*	Germany	Graff (1971)
Barley	Increased growth	L. rubellus	Sweden	Uhlen (1953)
Oak	26%	*A. caliginosa*	USSR	Zrazhevskii (1958)
Black spruce	17% top growth	*A. caliginosa*	USA	Marshall (1971)
Oat seedling	8.7% shoot	*Eisenia foetida*	Germany	Aldag and Graff (1975)
Rice	4-36% shoot	*Drawida willsi*	India	Senapati et al. (1985)

Tea	Shoo biomass (42-88%), root biomass (20-108%)	*P. corethrurus*	India	Giri (2006)
Wheat	Higher grain	*A. trapezoides*	Australia	Stephens and Davoren (1996)
Wood barley seedlings	15% shoot	*O. lacteum*	Germany	Klebsch et al. (1995)
Maize	4-52% shoot, 55-120% root, 2-9% total yield	*P. corethrurus*	Peru	Pashanasi et al. (1994)
Wheat, barley, faba beans	Increase in only wheat and barley, no growth in faba beans	*A. trapezoids, A. rosea*	Australia	Doube et al. (1997)
Hordelymus europaeus	29% root growth, no effect on shoot	*A. caligniosa*	Germany	Alphei et al. (1996)
Mimosa scabrella	43-69% shoot	*Amynthas sp.*	Brazil	Kobiyama (1994)
Patures	0-17 shoot after 15 months	*A. longa*	Tasmania	Garnsey (1994)
Wheat	39%	*A. trapezoids and A. rosea*	Australia	Williams and Baker (1993)
Maize	24-34% shoot, 17-65% root	*P. corethrurus, H. africanus*	Ivory coast	Spain et al. (1992)
Ryegrass	30% shoot after 350 days	Lumbricids	Ireland	Curry and Boyle (1987)
Beech seedlings	Higher stem biomass	*Octolasion lacteum*	Germany	Wolters and Stichan (1991)
Mustard, wheat	64%	Lumbricids	U.K	Russel (1910)
Hay/clover	Average 150%	Lumbricids/ *Diplocardia* spp.	USA	Hoop and Slater (1949)
Winter wheat	Increased yield	Lumbricids	Europe	Dreidax (1931)

Bean	8-150% shoot, 36% root, 3-88% total yield	*P. elongate, P. corethrurus*	Mexico	-
Brachiaria decumbens	9-50% shoot, 20-88% root, 1-53% total yield	*P. corethrurus*	Mexico	Ibarra et al. (1998)

Table 3.11: The effects of various earthworm species on crop productivity (Dash, 2012)

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CHAPTER FOUR

NUTRACEUTICAL VALUES OF EARTHWORMS

Nutritional value of earthworms

Food production is increasing continuously to sustain the incessant human demand. The International Feed Industry Federation predicts that livestock production will be doubled by the year 2050. Meat production mainly comes from cattle, pigs and poultry. The latter represents one of the most consumed animal foods, and consumption is predicted to rise over 90% in the next 50 years. In Europe, 14.6 M tons of poultry meats were consumed in 2017. The per capita consumption of poultry meat increased from 21 kg/yr to 24 kg/yr between the year 2007 to 2017 (AVEC, 2018). In addition to livestock production, the global demand for edible fish products has increased from 9.9 kg to 20.0 kg per capita per year and reached 171 M tons in 2016. Since the cost of a conventional feed comprising of fish meal, prawn meal and soybean meal has been rising over time, it has become necessary to look for an alternative cost-effective source that could partially or completely replace these feed sources.

Earthworms as a source of animal and human nutrition

Earthworms effectively convert the waste materials to nutrient-rich assimilable forms, which could be easily absorbed and utilized by the plants. Waste management and earthworm culture can go hand in hand, and surplus worms could be used as a potential non-conventional animal diet source for formulating fish and poultry feed as well as a source of human nutrition. Earthworms are excellent sources of protein with a number of essential amino acids. These animals have variable lipid contents naturally high in ω-3 fatty acids. Extensive analysis of the lipid fraction of earthworms has revealed a high proportion of polyunsaturated fatty acids (linolenic; ω-3 fatty acids), which is essential for formulating fish feed for many species. The protein and lipid contents of earthworms vary from 50 to 70% and 5 to 10%, respectively. The quantity of sodium, calcium, and potassium available in the earthworms is sufficient in meeting the required levels for catfish and all tropical fish (NRC, 1993). Earthworms are used broadly as nutritional protein sources either alone or in mixture with different feed substances in formulating the fish diets.

Earthworms as substitute diet in feed formulation for aquaculture

Every responsible aquaculture sector focuses on reducing production costs, improving the efficiency of the production systems and promoting environmental sustainability. Given that fish feeds are among the most expensive inputs in aquaculture production it is, therefore, necessary to provide cost-effective, eco-friendly and nutritionally complete fish feeds. Fishmeal is the popular protein source in fish feeds due to its higher protein levels, essential amino acid profile, minerals, vitamins, attractants, palatability and digestibility (FAO, 2017). However, the fish meal has progressively become more scarce and expensive due to diminishing capture fisheries, high competition between the human and animal industry, the global increase in the cost of energy and uncertain year-round supply (FAO, 2013). Therefore, the consistent use of fish meal in aquaculture has not only threatened the sustainability of fisheries ecosystems but has also increased fish demand, thus affecting profit margins of the fish farmers. Several attempts to produce cheap, reliable and eco-friendly alternative fish feeds are still debatable in their success. The sources of protein for fish feed are limited by the insufficiency of amino acids such as methionine, lysine and isoleucine. Moreover, the sustainability of the use of animal-based protein sources is faced with the challenges of microbial contamination and potential transfer of diseases (i.e., from livestock to fish and humans such as bovine spongiform encephalopathy, or mad cow disease). It is also difficult to quantify the amount produced, the water pollution potential and fatty acid rancidity attributed to animal protein sources. Furthermore, there are religious and cultural restrictions that limit the use of animal protein sources in some communities, especially in developing countries. On the other hand, the plant-based protein derivatives such as soybean are limited by low levels of methionine and inconsistency in availability and cost ineffectiveness. Similarly, sunflower has inadequate lysine, phenylalanine, methionine, phosphorus, high fibre levels, low energy and poor palatability. Generally, plant-based protein derivatives are limited by mismatching essential amino acids, the presence of endogenous anti-nutritional factors that reduce their efficiency of utilization in fish. Moreover, they have low palatability, high ash and fibre contents which, if included at high levels, reduce digestibility and protein conversion by fish as well as pellet quality of the feed. All these attributes are known to reduce the bioavailability of nutrients to the fish and decrease the efficiency of utilization, thereby increasing the feed conversion ratio and thus reducing economic success. Indeed, the challenges associated with the sustainability of protein feed ingredient sources in view of cost, nutritive value and resources have necessitated further research on viable animal protein replacers in fish diets (FAO, 2016). Therefore, non-conventional protein sources, such as earthworms, have gained interest in providing an alternative protein source thanks to its nutritional values that are close to that of fish meal.

Several earthworm species have been tested for fish feed production. For example, the blue worm (*Perionyx excavatus*) and African nightcrawler (*Eudrilus eugeniae*) have comparable nutritional content with fishmeal that is within the recommended nutritional requirements of most fish (Pucher et al., 2014). However, these two earthworm species are not adaptable to a wide range of climates and excess handling. *Perionyx excavatus* indicated similar or higher growth rates, protein efficiency, and energy retention for the common carp (*Cyprinus carpio*) compared to fishmeal in their diet (Ngoc et al., 2016). However, *P. excavatus* meal, if not thermally treated, might depress fish growth rates due to the presence of anti-nutritional factors (Pucher et al., 2014). Other species such as *Lumbricus rubellus* and *Lumbricus terrestris* have also been studied with limited success to replace fishmeal due to incomparable nutritional contents. *Lumbricus rubellus* has a low amino acid index. In contrast, *L. terrestris* has crude protein levels as low as 32.6%, which is below the dietary requirements of most carnivorous fish in intensive culture. Other commonly tested earthworm species for fish feed production include *Libyodrilus violaceus*, *Allobophora longa*, *Hyperiodrilus africanus*, *Libyodrilius vilaceous* and *Alma mansoi*. Studies have shown that *Eisenia foetida* has recommendable levels of protein, essential amino acids and lipids, similar to those found in fishmeal and are in line with the nutritional requirements of many fish species (Vodounnou, et al., 2016). Other studies have recommended that *E. foetida* can be utilized to replace conventional fish feed protein sources without compromising growth performance and reproduction (Stafford & Tacon, 1985). *Eisenia foetida* has superior growth rate thanks to its high feeding rate of up to 50% of its half body size. It is adaptable to different organic materials with the ability to convert biodegradable matter up to five-fold. Compared to most earthworm species, *E. foetida* has a relatively high reproduction rate (i.e., three hatchlings per egg) that are quick to mature. Moreover, *E. foetida* has low mortality compared to most earthworm species since it can balance and model its energy expenditure priorities. This attribute enables it to survive in extreme conditions such as low temperatures, toxic and saline environments. Besides, unlike *Lumbricus terrestris*, which is an anecic (deep burrowing) earthworm, *E. foetida* is epigeic (a surface dweller). This phenomenon facilitates harvesting and lowers its production cost as it requires less human labour to feed and continuously turn its substrate to promote aeration.

Earthworms are conventionally cultured using organic wastes such as livestock manure, household remains and agro-industrial residues that are not often used for most farm activities. These attributes lower its production cost as it can be cultured in backyard bins or cheaply constructed holding units using locally available materials such as offcuts and stones. For this reason, rural fish farmers with low income or urban dwellers with limited resources are able to produce earthworms with ease.

Methods of utilizing earthworms in fish feed

Whole earthworm diet

Whole earthworms (after 48 h of fasting) are boiled (with a pinch of common salt) in water, thoroughly washed with clean water and chopped into pieces and stored in a refrigerator at 4 °C until use.

Earthworm custard

For the preparation of earthworm custard, first the earthworms are boiled for 45 min in water, adding a pinch of common salt, washed thoroughly in clean water, and then ground using a mixer grinder. The required quantities of skimmed milk powder and hen's egg (yolk + albumin) can be added to the ground earthworm and mixed thoroughly. A desired quantity of water is heated in a beaker to 80 °C, and the required amount of gelatin is dissolved into it with slow stirring. After the gelatin is dissolved properly, it is added to the feed mix. Then the mineral and vitamin mix is added, and the whole feed mix is thoroughly mixed. The required quantity of lukewarm water is added to the feed mix, blended properly, and dough of feed mix is prepared. The dough is placed in a container and steam cooked for 20 min using a pressure cooker to obtain the earthworm custard. The custard is then cooled at room temperature and stored in a refrigerator at 4 °C until use. For daily feeding of the custard, it is cut into small pieces (0.5 mm) and fed to the fish.

Ingredient	Whole earthworm diet	Earthworm custard	Pelleted earthworm diet
Earthworm	1000	600	400
Fishmeal	_	_	100
Ground nut oil cake	_	_	300
Prawn meal	_	_	100
Skimmed milk powder	_	100	_
Egg	_	220	_
Gelatin	_	60	60
Vitamin and mineral mixture	_	20	20
Vegetable oil	_	_	20

Table 4.1 Ingredient composition (g/kg) of various types of earthworm fish diets (Adapted from Mohanta et al., 2016)

Pelleted earthworm diet

For the preparation of the pelleted earthworm diet, the earthworms are boiled in water for 45 minutes, adding a pinch of common salt and washed thoroughly in clear water. These are then oven-dried for 24 h and powdered with a grinder, and the earthworm meal is prepared. The required quantities of dried earthworm meal, fish meal, groundnut oil cake, prawn meal, mineral and vitamin mix and vegetable oil are mixed thoroughly in a mixer. A desired quantity of water is heated in a container to 80 °C, and the required amount of gelatin is dissolved into it with slow stirring. After the gelatin is dissolved properly, it is added to the feed mix. The required quantity of lukewarm water is added to the feed mix, blended properly, and dough of feed mix is prepared. The dough is passed through a hand pelletizer to obtain feed pellets. The pellets are dried at 60 °C and stored in a refrigerator at 4 °C until use. The comparative account of the ingredients and chemical composition of the different fish diets are presented in Tables 4.1 and 4.2, respectively

Parameters	**Whole earthworm diet**	**Earthworm custard**	**Pelleted earthworm diet**
Dry matter	182	450	923
Crude protein	520	508	501
Ether extract	160	150	180
Crude fibre	35	18	85
Ash	124	79	126
Gross energy (MJ/kg)	17.56	17.97	17.14

Table 4.2 Proximate composition (g/kg dry matter) of the fish diets (Adapted from Mohanta et al., 2016)

Advantages of an earthworm-based diet over conventional fish diets

Fish meal is considered the best protein source for formulating diets in pisciculture due to its balanced amino acids, vitamin content, palatability, growth factor, and attractant properties, but fish meal represents a finite fishery resource. It will not be able to supply the aquaculture industry with a continuous source of cheap protein indefinitely. Moreover, the increasing cost of high-quality fish meal required for aquafeed, the declining stocks of fish from capture fishery and the competition for feed in animal husbandry have compelled fish nutritionists around the globe to investigate and identify novel and renewable alternative non-conventional protein sources for the continued expansion and sustainability of aquaculture. The utilization of non-conventional feedstuff of plant origin had been restricted as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, peptidase inhibitors, heamatoglutinins, saponegin, mimosine, cyanoglycosides, and linamarin, despite their nutrient values and low-value implications. Thus, the animal protein supply is more wellliked over the plant protein in formulating the fish feed. Many researchers have evaluated earthworms as a substitute protein supply in formulating fish meals for various species.

It has been reported that there was no adverse effect on the growth performance or feed utilization efficiency of fish rainbow trout *Salmo gairdneri* fed diets containing low levels of earthworm meal (Stafford and Tacon, 1985). Better growth and nutrient utilization were observed in carp (*Cirrhinus mrigala*) fed a diet containing *E. foetida* worm meal compared to the fish meal-based diet (Ganesh et al., 2003). Experimental results (Keshavappa et al., 1989) showed no difference in weight gains in carp fry (*Catla catla*) fed feed with 30% earthworm meal compared to 30% fish meal. Further, survival was higher in the former (75.75%) than the latter (66.66%). Aquarium fish *Poecilia reticulata* fed earthworm (*E. foetida*) biomass resulted in significantly increased brood numbers and also produced twice the off-spring than the fish fed a diet without earthworms present (Kostecka and Pączka, 2006). Worm meal obtained from *E. foetida*, when used to replace 25 and 50% of the fish meal component in the diets for rainbow trout, gave higher growth rates in fish fed these diets compared to the control diet without any worm meal (Velasquez et al., 1991). Nandeesha et al. (1988) replaced the fish meal with dried worm meal in a culture of common carp. They found that the diet where the fish meal was partially replaced by a worm meal as a protein source gave the best result in comparison to the diets where the fish meal was completely replaced by a worm meal or a diet prepared solely from fish meal. On the other hand, Tacon et al. (1983) reported marginally lower growth responses in rainbow trout when worm meal (*E. foetida*) was used to replace 50% dietary herring meal. The growth and feed utilization efficiency of rainbow trout is not affected when the herring meal protein is replaced by earthworm (*Dendrodrilus subrubicundus*) meal at 10%. Still, there was a decline in fish performance at higher replacement levels of 50 and 100% (Stafford and Tacon, 1984). Akiyama et al. (1984) reported that salmon (*Oncorhynchus keta*) fry fed with fish meal diets supplemented by earthworm powder (5%) shows superior growth performance and feed efficiency in comparison to the fish fed with fish meal diets supplemented with silkworm pupa powder (5%), dried beef liver (5%), and krill meal (5%).

Case studies with earthworm as a substitute source of nutrient for fish

Gbai et al. (2018) conducted an experiment to test the earthworm *E. eugeniae* as a substitute source of protein in place of conventional sources for Nile tilapia *Oreochromis niloticus*. The experiment was conducted in the Ivory Coast to evaluate the efficacy of dietary protein from maggot meal and earthworm meal to replace fish meal protein in juvenile feed. The fish were fed with the formulated diets fish diet (FD), maggot diet (MD) and earthworm diet (ED) content 40% crude protein and the commercial diet (CD) content 34.5% crude protein. At the end of this larval phase, the average weights recorded were 0.75 ± 1.93, 0.71 ± 3.55, 0.55 ± 2.52 and 0.62 ± 2.52 g for FD, MD, ED and CD, respectively. It was concluded that FD had maximum growth-promoting effects. However, MD and ED promoted growth too, indicating promising results as substitute protein sources.

Sobana and Jagadeesan (2016) used *E. eugeniae* as a substitute protein, carbohydrate and lipid source for the *Labeo catla* (formerly *Catla catla*) in India. They found significantly enhanced protein and carbohydrate levels in these fish relative to fish fed with a conventional diet.

Pucher et al. (2014) evaluated whether earthworm meal can fully replace fishmeal in supplemental feeds for common carp (*Cyprinus carpio*) that also feed on natural food resources in semi-intensive aquaculture. A net cage trial (32 nets) was carried out in Vietnam using three iso-nitrogenous feeds fed to common carp either at a level of 10 $g/kg^{0.8}$ metabolic body mass (5 fish per cage) or 20 $g/kg^{0.8}$ metabolic body mass (10 fish per cage). The fishmeal protein was replaced by 0, 50 or 100% of protein from sun-dried earthworms of species *Perionyx excavatus*. At both stocking densities, control groups were fed only on natural food resources. The growth rate of fish increased with the rising replacement of fishmeal by earthworm meal at both feeding rates. Large zooplankton were the predominant natural food resource. With the increasing availability of large zooplankton, sun-dried earthworm meal in plant-based supplemental feeds seemed better able to meet the nutritional requirements of common carp than fishmeal.

Mohanta et al. (2016) used the earthworm *E. foetida* in three forms, (i) whole earthworm, (ii) earthworm custard, and (iii) pelleted earthworm diet to prepare three iso-nitrogenous (500 g protein kg^{-1} diet) and iso-caloric (17.0 MJ kg^{-1} diet) experimental diets (Tables 4.1 & 4.2). The formulated diets were fed ad libitum twice daily to the freshwater fish *Labeo rohita* advanced fry (0.71 ± 0.04 g) in triplicates for 35 days. In each replicate, ten fish were stocked. The 300 L tanks containing 100 L of water with the provision of continuous aeration were used for rearing the fish. At the end of the experiment, the weight gain (g), food conversion ratio, specific growth rate, protein efficiency ratio, protein retention efficiency (%), and energy retention efficiency (%) of fish fed pelleted earthworm diet (2.19, 1.58, 4.21, 1.26, 23.0, and 18.6, respectively) was significantly higher than the corresponding values of whole earthworm (1.53, 2.30, 3.38, 0.84, 14.34, and 11.93, respectively) and earthworm custard (0.94, 3.18, 2.42, 0.62, 10.50, and 8.21, respectively) fed diets.

Beg et al. (2016) evaluated the potential of earthworm meal (*E. foetida*) as a replacement of fish meal for the culture of three Indian major carps, Catla (*Labeo catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) with stocking ratios of 1:1:1 in 12 cement tanks with the size of 2.5 m ×1.5 m ×1.5 m for 90 days. Four experimental iso-nitrogenous (35% crude protein) and iso-caloric (15 MJ kg^{-1}) diets were prepared with (i) no replacement of fish meal (Diet A), (ii) 20% replacement of fish meal with earthworm meal (Diet B), (iii) 50% replacement of fish meal with earthworm meal (Diet C) and 100% replacement of fish meal with earthworm meal (Diet D). The fishes were fed with the diets two time s daily at 3% body weight. Diet C feeding resulted in higher growth of fish relative to other diets. The total production of fish in diet C was 63% higher than Diet A, 45% higher than Diet B and 16% higher than Diet D.

The earthworm *E foetida* was tried as a partial replacement for commercial pellets for the rainbow trout, *Oncorhynchus mykiss* (Pereira and Gomes, 1995). Earthworms were blanched and treated with 10% sodium chloride. Fish were submitted to four treatments: control fish were fed with a commercial diet, and three groups of fish were fed with diets partially supplemented with earthworms. All fish showed the same appetite for the earthworm-supplemented diets as for the control diet during the experiment (8 weeks). No significant differences were detected in the mean final body weights of all groups of fish.

Boaru et al. (2016) experimented with supplementation of different proportions of the granulated feed of *Xiphophorus hellerii* juveniles with meal obtained from earthworm biomass (*E. foetida*). For this purpose, the fry from the *X. hellerii* female were distributed in three aquaria and were fed differently. The control group fish were fed with a commercial feed, while the experimental groups were fed with two feed mixtures, supplemented with 10% and 20% worm meal. The fish were weighed after 120 days to evaluate the impact of the worm meal supplementation. Results indicated significantly higher growth and weight gain in the experimental fish fed with an earthworm-supplemented diet.

Hasanuzzaman et al. (2010) analysed the nutritional composition of a wild earthworm (*Perionyx excavatus*) and fishmeal used by the local fish-feed industry in Bangladesh to evaluate the nutritional replacement potentiality of the earthworm for fishmeal used in aquaculture feeds and as a supplement in feeds for other animals. Leaving aside moisture content, the relative chemical values in *P. excavatus* did not differ significantly from that in fishmeal. On average, the earthworm feed had lower protein contents (46.57 ± 0.97%) than fishmeal (54.97 ± 7.49 %). On the other hand, the mean lipid content (8.03 ± 0.44%) in this earthworm was found to be higher compared to fishmeal (7.97 ± 1.60%). The average ash contents in the earthworm and fishmeal feeds were 24.26 ± 0.68% and 24.13 ± 8.44%, respectively. The results of the study indicated that this worm species had an almost similar nutritional value to fishmeal, and thus would be a potential source of animal protein

Eutyphoeus gammiei (Beddard) is a large native earthworm in northeast India, which is commonly used as fish bait and chicken feed in the state of Tripura. The tissue of this worm contains 63.98% protein, 15.79% carbohydrate, 7.78% fat, 1.90% crude fibre and total ash 10.55%. Important amino acids present in the body tissue are lysine (1.45 g/100 g), methionine (1.56 g/100 g), valine (2.78 g/100 g) and leucine (5.52 g/100 g). The non-essential amino acids of earthworm are dominated by glutamic acid (1.37 g/100 g) and ornithine (3.68 g/100 g). Interestingly, the proportions of unsaturated fatty acids are relatively high (polyunsaturated fatty acids 26%, monounsaturated fatty acids 27.4%). In polyunsaturated fatty acids, arachidonic acid (20: 4 ω -6) and linoleic acid (18: 2 ω -6) represent 36.39% and 29.50%, respectively. Several nutritionally important minerals such as iron, calcium, potassium, magnesium, zinc and copper are also present in the tissue of the worm. All these nutritional components make this worm suitable as an ingredient in formulating a diet for aquaculture.

The earthworm as a suitable component for poultry feed

Eggs and chicken meat, which are among the animal protein sources, are inevitable products for human nutrition. Egg contains nearly all of the energy, fatty acids, protein, vitamin, and minerals needed by the human body at suitable amounts and rates. Chicken meat, on the other hand, is preferred more than red meat because of its ease in production and consumption, low cholesterol, calorie and fat amounts, high protein and calcium contents, and because of its low price. When compared to other nutrients, egg protein ranks first in biological availability with nearly 95%

digestibility. Chicken meat, on the other hand, contains the amino acids that are not synthesized by the human body in a sufficient amount and rate and has proteins with high biological availability (Öztürk, 2016). Due to superior food conversion ratios and short production time, poultry husbandry gives better returns than other farm animals. Nearly 70–75% of the total poultry husbandry activities consist of feeding costs, and 15% of this rate consists of supplying proteins (Özen et al., 2005). Soybean meal and fish meal, which are used as protein sources in poultry rations, increase the cost of farming. For this reason, it has been reported that earthworms may be used as protein sources to ensure that poultry animals are fed in a balanced and sufficient manner, and the practice of poultry farming becomes sustainable.

Earthworms are conventionally an important nutrient source for poultry birds. For example, these animals can pick up earthworms and their juveniles in and on the soil surface. When the natural role of worms in some farm animals as nutrients is considered, they may be re-evaluated to be used as nutrients for certain poultry (Van Huis et al., 2013). Earthworms pass the soil with complex organics through their stomach at a rate of 60% of their live weights and make the soil become enriched with simple organic compounds, thus contributing to sustainable agriculture as well as generating a resource for their alternative utility as a nutrient source.

The major limiting factors in feeding poultry are the protein and energy contents of the rations. Fish and soybean meals are commonly used as sources of protein in poultry feeds. However, the cost-effectiveness of these supplements has constantly been debated. Therefore animal nutritionists looked for alternatives protein sources. Worms are among the alternatives that may be used as protein sources for this purpose. When the compositions of the nutrients are analysed, it is observed that worms contain amino acids, lipids, carbohydrates and minerals that are necessary for poultry animals at high levels (Paoletti et al., 2003; Dedeke et al., 2010).

Case studies on earthworm as a substitute component in poultry diet

Fisher (1988) reported that the cost of utilizing earthworms in the poultry diet as a substitute protein source may be higher than fish and soybean meals. It was also reported that the essential amino acid structure of the earthworms that will be added to the rations of poultry was suitable and earthworms could be added to the ration at a rate of 15% (Taboga, 1980). Zhenjun et al. (1996) had concluded from a study that earthworms could be used as an animal feed with the protein levels they contained. They proposed that they had an adequate nutritional value that is greater than fish and soybean meals in terms of protein rate and amino acid composition. Ali (2002) conducted a study and reported that *Perionyx excavatus*, an epigeic worm in Bangladesh among the many earthworms, had the potential to be used in feeding poultry because it was available throughout the year in adequate numbers. Hatti (2013) reported that protein, lipid and glycogen contents of the earthworms *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui* and the increase in their biomass were highest in Summer, medium during monsoon, and the lowest in Winter. It has been proposed that these worms could be used as nutrients in feeds for fish and poultry animals and pigs due to the high protein, lipid and glycogen contents. Ton et al. (2009) determined that adding 2% worms to broiler rations caused increases in the live weight of broilers in the 10th week, and this did not have any negative effects on meat quality. A study was conducted to determine the rate of replacement of fish meal with worm meal in quails (Prayogi, 2011). It was found that 10% of worm meal can be used in the mixture instead of the fish meal without any detrimental effect on the birds; however, when the rate was increased to 15%, there was a decrease in consumption. It has also been reported that adding 0.4% of earthworm meal to the rations improved the consumption rate and weight with the digestibility of the nutrients (Son and Jo, 2013).

Nutritional value of earthworm for humans

Earthworms have been conventionally used as food for centuries in China. Once upon a time, the people in Fujian and Guangdong provinces had the habit of consuming earthworms. Even now, in Taiwan and Henan and Guangdong provinces, some local people prepare special dishes featuring the earthworm as a basic ingredient. Records from the ancient Chinese book “On Guo Yi Gong” say that the people who lived in Fujian, who were considered different from other people, considered earthworms to be a delicacy. They cut the earthworms into small pieces and mixed them with meat filling to make their food tastier. Even now, earthworm soup, a traditional delicacy, is still offered in some restaurants of Guangdong province. Worms continue to be preferred delicacies for Ye’kuana Amerindians of the Alto Orinoco in Venezuela. Western European nations and the South-East Asian countries produce several worm products such as canned worms, mushroom-worms and worm biscuits and bread. People in certain African and South American countries commonly consume earthworms. The nutrient values of the earthworm *E. foetida* relative to other sources are mentioned in Tables 4.3 & 4.4. Owing to the high content of good quality protein and high content of vitamin B and other bioactive substances, it is very likely that earthworms could become an important source of animal protein in human nutrition if other sources become limited. With the fast development of biochemical science in recent times, significant progress has been made in isolating active compounds, including small molecular proteins, peptides and amino acids, to be used in human diets.

An earthworm protein powder as a functional food

Various research results have indicated that earthworms contain certain nutrients, which have important physiological functions and may be useful for human health. In China, the earthworm protein has been declared as a new food resource. Earthworm protein powder has been produced through hydrolysis extraction, ultrafiltration, nanofiltration and spray drying processes. The protein powder is light yellow and without impurities. In the powder, there are lots of water-soluble small molecular proteins. It tastes sweet with a special flavour. According to the provisions of the GB/T5009.5-2003 detection method, the minimum protein content is greater than or equal to 75%.

Feed material	DM	CP	Fat	Ash	Ca	P	ME (KCal/g)
Fresh earthworm	15.7	11.2	1.89	1.4	0.22	0.65	-
Earthworm meal	90.6	54.6	7.34	21.2	1.55	2.75	2.99
Earthworm cast	82.2	7.9	1.1	34.2	1.42	0.28	0.95
Fish meal	90.8	62.0	9.7	14.4	3.91	2.9	2.9
Cow milk	12.7	3.5	3.5	0.7	0.12	0.09	0.65
Egg	26.3	12.9	11.5	1.0	0.05	0.21	1.63
Soybean meal	88.1	43.0	5.4	5.9	0.32	0.50	2.64
Corn meal	86.5	8.6	3.5	1.4	0.04	0.21	0.32
Wheat bran	82.2	14.2	2.0	4.4	0.14	1.06	1.78

Table 4.3 Nutrient composition of the earthworm *E. fetida* and other feed materials. Data are presented as % (weight/weight on dry matter

basis). DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus and ME, metabolic energy(Modified from Sun and Jiang, 2017)

Aminoacid	Earthworm meal	Earthworm cast	Fish meal	Egg	Cow milk	Wheat bran
Thrionine*	2.72	0.46	2.88	2.42	1.20	0.45
Serine	2.71	0.46	2.63	3.64	1.57	0.74
Glycine	3.12	0.49	4.26	1.58	0.54	0.84
Cystine	0.42	0.09	0.56	1.16	0.22	0.33
Valine*	2.39	0.44	2.80	3.26	1.57	0.67
Methionine*	1.01	0.19	1.65	1.60	0.68	0.15
Isoleucine*	2.40	0.38	2.42	2.99	1.28	0.37
Leucine*	3.94	0.78	4.28	4.20	2.58	0.80
Tyrosine	1.73	0.24	2.12	1.98	1.28	0.52
Phenyl* alanine	2.12	0.31	2.68	2.73	1.46	0.48
Lysine*	4.26	0.68	4.35	1.32	2.11	0.47
Histidine*	1.36	0.12	1.68	1.16	0.72	0.35
Arginine	3.27	0.64	3.87	2.90	0.89	0.95

Table 4.4 Amino acid composition of the earthworm *E. fetida* meal and other feed materials (weight/weight on a dry matter basis). *Essential aminoacid for humans (Modified from Sun and Jiang, 2017)

The actual nutritional value of earthworm protein

The nutritional value of protein depends upon its specific amino acid composition. Comparing the amino acid contents of *E. foetida* reported in with those in other earthworms and with those in *Eudrilus eugeniae. Lumbricus rubellus*, *A. caliginosa* and *P. guillemi*, there is considerable variability in amino acid contents among the species and even within the same species. Nevertheless, some research reports suggest that the contents of individual amino acids differ between species by no more than 17% and usually by considerably less. It has been observed that valine, leucine and isoleucine were higher in earthworm meal than in fishmeal, but lower than in meat meal. The content of methionine in earthworm meal was closer to that of meat meal, but 200% of that in fish meal. Arginine, histidine and phenylalanine contents in earthworm meal were closer to those in meat meal and marginally higher than in fishmeal. Threonine, cysteine and tryptophan in the earthworm meal were significantly higher relative to fishmeal and meat meal. The results thus indicated that earthworm protein was higher in essential amino acids, including the sulfur-containing amino acids and therefore should be very suitable for animal feed. Biological value and net protein utilization are the two most important parameters used conventionally to evaluate the protein quality of feed materials. Some scientists reported a biological value of 84% and net protein utilization of 79% in a rat growth assay with *E. foetida* protein. These results were verified in fish and chicken tissues subsequently. Earthworm protein was easily dissolved by enzymes into free amino acids. This fact suggests that animals easily metabolize earthworm protein. Thus, earthworms seem to be an excellent source of protein supplement not only for animal feed but also for humans.

Earthworms risk assessment in animal feeding

Even though earthworms have already been incorporated as feed supplements for poultry, fish and pigs, they remain a nutritional source at risk for animals. Among the most important sources of contamination that enter animals through earthworms, notable are heavy metals, pesticides, bacteria and fungi. They can end up in humans through the food chain. Some nematodes for which earthworms are intermediate hosts are a particular case in the contamination of animals such as poultry.

Nonbiological risks

Heavy metals

Heavy metals refer to naturally occurring metals having an atomic number greater than 20 and an elemental density of greater than 5 g/cm^3. Many studies have shown that earthworms are important bio-accumulators and bioindicators of heavy metals in soil. Sharma et al. (2005) had cautioned that worms could receive heavy metals and other pollutants and that these might be transferred to the poultry that consumed these worms. Son (2009) reported that there were the heavy metals As (4.41 ppm), Cd (1.23 ppm), Cr (1.18 ppm), Hg (0.00 ppm) and Pb (3.39 ppm) in worm meal. However, he has reported that these metals were not transferred to the meat or egg, and did not affect meat or egg quality.

The earthworms can accumulate metals that are more likely to be transferred to other animals through the food chain. The metals identified in the earthworm body are aluminium, beryllium, cadmium, chromium, copper, iron, manganese, nickel, lead, mercury, strontium, uranium and zinc. Metals can be categorised into two types, essential metals (copper, nickel, zinc and iron) beneficial only at low concentrations and heavy metals (lead, mercury and cadmium), which are toxic even at very low concentrations. The most harmful metals are mercury, lead, aluminium, cadmium and arsenic. It has been found that aluminium can have a severe toxic impact on earthworms (Annapoorani, 2014). It causes serious damage to the earthworm's reproductive system and cycle. Metals get mainly deposited in the earthworm tissue. According to the type of substrate on which earthworms grow, the concentration of metals in the body differs.

The fact that earthworms can act as a vector of toxic substances shows that the use of earthworms containing that kind of toxic substance is a risk for farming animals such as poultry. The deposition of metals in the tissue of poultry birds could significantly enhance the health risk for humans. Earthworms can accumulate a variable amount of heavy metals depending on the type of substrate. After the vermicomposting process, Soobhany et al. (2015) obtained metal concentrations in the order of 6,900 mg/kg for cadmium, 2,068 mg/kg for copper and 621 mg/kg for zinc, in the tissue of earthworms. From poultry manure, Arroyo et al. (2014) obtained different mean concentrations at 1.2 mg/kg for cadmium, 15 mg/kg for copper and 140 mg/kg for zinc. Wang et al. (2018) reported a range from 99.9 to 646 mg/kg for zinc and 8.60 to 157 mg/kg for copper. They concluded that tissue metal concentration increases with increasing concentrations in the soil, which means that heavy metal concentration in soil is positively correlated to their contents in earthworm tissue. Schlich et al. (2013) observed a correlation between Ag concentration in earthworm tissue and soil. As per the European Commission recommendation, the maximum acceptable concentrations of heavy metals in animal feed should be 1,100 and 500 mg/kg for cadmium, copper and zinc, respectively (Okoye et al., 2011). Cadmium is considered highly toxic, which can easily contaminate omnivorous feed through earthworms. On the contrary, zinc has biological benefit effects and can be used as an efficient dietary supplement for enhancing the physiological state in animals. Another benefit of dietary zinc demonstrated by Yang et al. (2017) is that at 40 mg/kg, it improves carcass and muscle yields, enhances the fat content in thigh muscle and depletes the accumulation of toxic metals in breast muscles.

Pesticides

When pesticides occur in the environment, earthworms usually accumulate the less toxic ones preferentially. Earthworms accumulate pesticides by ingestion or epidermal contact. All pesticides do not have the same impact on soil, earthworms and animals, and it has been observed that organophosphorus pesticides are the most toxic relative to other pesticides.

Although the ways of contamination are different, earthworms may play a role in this process. Cox (1991) reported cases of disruption of thyroidal hormone activity and genetic damages in quails that eat earthworms exposed to pesticides such as malathion. In Canada, cases of death occurred as a result of contamination of wild birds by insecticides such as carbofuran, diazinon and fensulfothion and earthworms were identified as vectors of toxics in the body of birds. The use of neonicotinoid insecticides such as imidacloprid affects insects and earthworms, which are a part of poultry feed. This involved the decline of the population of 15 species of birds of 3.5% per year between 2003 and 2010 in the Netherlands, where the amount of pesticides used in agricultural practices has been multiplied by 10 in 10 years. Because of its harmful effects on the environment, imidacloprid has been banned in Europe since December 2013 (Hallmann et al., 2014).

Pesticides accumulated along the food chain, especially endocrine disruptors, pose a long-term risk to animals such as poultry. It is known that diet influences the composition of tissues and poultry eggs. Therefore, toxic substances in feed could enter the body of poultry or eggs and therefore end up in humans.

Biological risks

Microbes

Soil is an appropriate environment for the development of eukaryotes (algae, fungi, protozoa) and prokaryotes (bacteria, archaea). A part of the diet of the earthworms consists of microbes, including fungi and bacteria that appear during the process of composting. Unfortunately, some microorganisms produce mycotoxins that induce food intoxication when the animal feed contains contaminated earthworms. Bacteria are mainly located in the posterior intestinal region of earthworms because these portions have optimal conditions for their development. *Staphylococcus aureus* and *Bacillus cereus* belong to a group of bacteria that are responsible for various food toxicities. Other bacteria from the soil are also involved in food intoxication such as *Bacillus licheniformis*, *Bacillus mycoides*, *Bacillus thuringiensis* and *Brevibacillus laterosporus*. *B. cereus* is present in the digestive tract of earthworms and is responsible for several cases of animal food contamination. Bacteria of the genus *Clostridium* are also responsible for food intoxication. *Clostridium* *botulinum* is responsible for botulism, which is caused by a neurotoxin which affects poultry and many other animal species. The botulinum toxin has been identified in many invertebrates, including earthworms, operating in contaminated soils. This means that earthworms could be an important way of transmission of this toxin to other animal species such as poultry or fish.

Nematodiasis

Apart from metals, chemicals and microbes, the earthworm can be a vector of parasites that can lead to specific animal concerns, especially poultry. The earthworm is an intermediate host of some nematodes that do not produce toxins but whose amounts in animals, especially birds bred on their carrier hosts, cause disease. Thus, the use of feed containing contaminated earthworms should be the starting point for parasite infestation in poultry. Syngamosis, which is caused by *Syngamus trachea* and *Syngamus merulae* which are ingested at their larval forms by earthworms. Histomoniasis is caused by the flagellated protozoan *Histomonas meleagridis* contamination, especially in turkeys, through earthworms.

Measures to avoid contamination of earthworms and animal diet

It is always advisable to utilize earthworms cultured with a non-contaminated substrate to ensure the safety of the feeds prepared. Vermicomposting is probably the best way to limit contamination of earthworms by metals, even though it was shown in a few cases that some vermicompost can contain heavy metals according to the origin of the substrate used. Earthworm production based on adapted rearing techniques should be developed to reduce the risk of contamination for further animal production. For microorganisms in general, heat treatment used in earthworm transformation processes into meal should be sufficient to destroy microorganisms in the food produced. Nevertheless, some bacteria can develop resistance to conventional heat treatments. This is the case of *C. botulinum*, the staphylococcal enterotoxins and the emetic toxin of *B. cereus*. Stronger temperature and heating duration should then be adapted to manage earthworms as safety material to include in animal feed. Staphylococcal enterotoxin withstands temperatures of 100 °C for 30 min; the emetic toxin of *B. cereus* resists for 90 min at 126 °C. The botulinum toxin is destroyed at 85 °C for 5 min, and after sporulation, it should keep on for at least 3 min at 121 °C.

Therapeutic uses of earthworms

Fibrinolytic enzymes for cardiovascular ailments

In traditional Chinese medicine, earthworms have been used to improve blood circulation, to treat apoplectic stroke and as antipyretic and diuretic agents. The earthworm fibrinolytic enzyme (EFE) is a complex protein. Due to their ability to dissolve blood clots, they could be used in cardiac and cerebrovascular diseases. The fibrinolytic enzymes could find a place in the pharmaceutical industry as an agent for the treatment of deregulated hemostasis for the prevention of blood clots and the balance of fibrinolysis. The only hurdle in commercial production of the enzyme is low yield. However, with the advancement of genetic engineering, this problem could be tackled conveniently.

Antitumor activity of earthworm extracts

There has been increased interest in the antitumor activity of EFE. The EFE isolated from the earthworm *E. foetida* has been evaluated on human hepatoma cells. EFE showed antitumor activity *in vitro* and *in vivo,* perhaps through induction of apoptosis. Macromolecular mixtures (G-90) from earthworm homogenate have shown promising results against many human tumor cell lines.

Antimicrobial properties

In recent years the interest in antimicrobial peptide has increased. They serve as a first-line defence against microbial invasion, supplementing the host's humoral and immune system. Earthworms do not produce specific antibodies, and they rely on the innate mechanisms for protection against microbial attacks. Such defences are present in the coelomic fluid of these animals. This activity is attributed to proteins, including lysozyme like molecules and factors with haemolytic activity as well as a pattern recognition protein named coelomic cytolytic factor (CCF). It has been shown further that glycolipoprotein mixture (G-90) from earthworms shows strong antibacterial property against facultative pathogenic bacteria. Six antimicrobial peptides from earthworm tissues and coelomic fluid have been isolated and purified. The peptides contained 5-50 amino acid residues with the same or similar sequence of Ala- Met-Val-Ser-Gly named antibacterial vermipeptide family (AVPF). AVPF has been found to have strong antibacterial properties against Gram +ve and Gram –ve strains along with fungi.

Earthworms in wound healing

Mitogenic, antibacterial, haemostatic and antioxidative properties determined in earthworms have a major influence on wound healing and epithelization. The earthworm paste prepared from the tropical species *Lampito mauritii* has anti-inflammatory and anti-oxidative properties influencing haematological parameters important for the wound healing process (Prakash et al., 2007) and preparations from *L. rubellus* and *E. foetida* promote wound healing. Both preparations shorten the healing time by increasing epithelization, granulation and synthesis of collagen.

Antipyretic and antioxidative properties

The antipyretic activity has been detected in the earthworm extracts from *Lumbricus* sp. and *L mauritii.* These extracts show promising results in the treatment of peptic ulcers in rats—the extracts from *L mauritii* show hepatoprotective potential. Research on evaluating the potentialities of earthworm as a source of animal and human nutrition and active molecules for manufacturing drugs against various diseases is in progress in a number of countries across the globe. Advancement in genetic engineering and biotechnological tools and techniques will help scientists to use earthworms as a model animal for isolating useful biomolecules with high nutraceutical values.

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CHAPTER FIVE

THE EARTHWORM AS AN INDICATOR OF SOIL CONTAMINATION AND ITS POTENTIALITY IN BIOREMEDIATION

Soil contamination: The emerging environmental risk

These days soil contamination has become a societal concern in every corner of the world that needs to be addressed on a war footing. The current situation demands the protection of an extremely stressed soil to sustain the biosphere. The hidden danger of the drastic reduction in soil quality is closely linked to the nutrition and health status of all living organisms, including humans. The filtering, buffering, and attenuation capacities of soil are facing a challenge due to the accumulation of toxic xenobiotics. The deterioration in soil quality in most cases is the outcome of anthropogenic activities. Rapid population growth, coupled with increasing industrialization, has culminated in the generation of huge quantities of wastes, which contribute significantly to soil contamination. Further, chemical agricultural practice is responsible for soil quality deterioration. Waste products from both industries and agricultural fields accumulate on a large scale, which may alter the natural physicochemical and biological properties of soil. Modern agricultural practices embrace several toxic pollutants in crop fields (Samal & Mishra, 2017). The major soil contaminants may be grouped into various categories:

1. **Domestic and sewage sludge**: The sewage sludge released into the soil from both domestic and industrial sources contains various organic and inorganic toxic compounds. The toxicity of sewage sludge has been confirmed on millipedes and it can affect the digestive system of these animals. Vinasse, a product of the alcohol industry, has proved to be a major health hazard for several organisms and can cause genetic alterations. Municipal waste disposal and incineration may deposit pollutants such as heavy metals, polyaromatic hydrocarbons and pharmaceutical compounds on the soil surface.

2. **Industrial and radioactive pollutants**: Industries liberate several toxic compounds, including polycyclic aromatic hydrocarbons and dioxins, into the soil. These are potential carcinogens and mutagens. The major source of origin of these chemicals in industrial processes is the combustion of organic matter and the burning of coal. Studies have shown the adverse impact of these compounds on the midgut tissues of diplopod *R. padbergi* exposed to contaminated soil (Da Silva Souza et al., 2011). Toxic radionuclides such as Sr-90, Cs-137, I-129, which are generated from nuclear reactors or during mining, have proved to be highly deleterious to biota. Gaseous pollutants and radionuclides from industries and nuclear plants can enter the soil directly through acid rain or atmospheric deposition. Industrial wastes such as phosphogypsum and paper mill sludge pose a high environmental risk.
3. **Agrochemicals**: The applications of pesticides and chemical fertilizers in agricultural fields have increased significantly over the decades to achieve high crop productivity. The residues of these potentially toxic chemicals contaminate soil and affect beneficial, non-target organisms. The major agrochemicals used in fields are the chlorinated hydrocarbons, Lindane, BHC, Aldrin, etc., organophosphates including Malathion, Parathion, Ethion and and trace metals like As, Pb, Fe, Cd from fertilizer sources. Although fertilizers are the sources of nutrients for plants, their unregulated use causes damage to the upper and underground organisms (Fig 5.1).

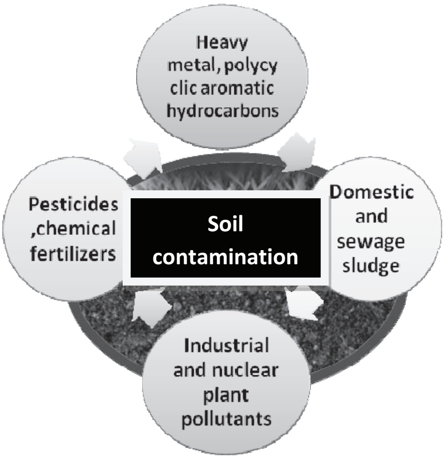

Figure 5.1: Major sources of soil contamination

Bioindicator: The natural monitoring system

Monitoring different types of toxic substances and their effect on the environment is a herculean task. Although there are some traditional chemical methods by which the level of toxicity can be evaluated. Still, it does not give a full impression of the degree of impact on the biota. Besides, chemical methods are time-consuming and not cost-effective. Environmental stress can be determined in terms of several physical and chemical parameters, but these parameters are less sensitive to contaminants. Hence, living organisms that are sensitive to environmental perturbations have been used as bioindicators, which have immensely helped in monitoring the stability of ecosystems. In addition to providing clues about toxicity, this biomonitoring process provides information on the affected organisms in an ecosystem.

Applied ecology includes a number of important concepts, including biomonitoring or bioindication. Certain biological agents could be useful tools to indicate the status of the environment. Organisms that have minimal ecological tolerance to environmental alterations and reflect specific changes—that may be physiological, morphological or behavioural—are referred to as bioindicators.

According to the level of biomonitoring, the bioindicators may be divided into the following categories.

1. **Environmental indicators** – These organisms respond to environmental disturbance or to a change in the environmental state in ways that can be readily observed and quantified. Organisms are used as early-warning devices or to delimit the results of a disturbance. They can also be used as accumulators of chemicals that are used to quantify the concentration of contaminants.
2. **Ecological indicators** – Organisms that indicate the effects of habitat disturbance, fragmentation or climate change are called ecological indicators. These disturbances could embody a decline in the size of the population, an alteration in the spatial distribution of life history deviations.
3. **Biodiversity indicators** – These are groups of organisms whose diversity reflects a major change in the species composition in the population.

Alterations in certain sensitive biochemical, cellular or physiological parameters of an organism could be used to predict the environmental quality. These parameters are called biomarkers. In other words, a biomarker can be defined as “biochemical, cellular, physiological or behavioural alterations that can be assessed in tissue or body fluid samples, or at the level of the whole organism, to provide signs of exposure and/or effects from one or more contaminants” (Depledge, 1994). The effects of contaminants at lower levels of biological organization (e.g., biochemical, cellular, physiological) in general occur more rapidly than those at higher levels (e.g., ecological effects). The former may, therefore, provide a more sensitive early warning of deleterious effects within populations. Any noticeable change in the molecular, cellular, biochemical, and physiological processes within an organism due to environmental perturbations on exposure to contaminants could be used as a biomarker. Biomarkers may be categorised into the following kinds.

1. **Biomarkers of exposure**: Certain biomarkers in an organism after exposure to a pollutant. These are called biomarkers of exposure. These biomarkers could provide qualitative and quantitative estimates of various biomolecules in response to the degree of exposure. However, the alteration in these markers may not be predictive of an adverse impact on the organism or the population. Both the biomarker of exposure and biomarker of effect could contribute significantly to evaluating the quality of the environment. Besides, the former might have the potential as an alternative to chemical analysis methods or to measure the effects of short-lived chemicals. It could also provide a biologically relevant indication of exposure (Hagger et al., 2006).
2. **Biomarkers of effect**: These are linked to the specific mechanism of action of contaminants. Further, this includes the degree of modification of the biomarker in response to the environmental stressors. These markers could be useful in obtaining information on certain qualitative aspects of the environmental hazard (Chambers et al., 2002).

The earthworm as an indicator of ecosystem perturbation

Earthworms, being the dominant soil animals, are frequently used in toxicity tests. These invertebrates come into contact with a great variety of pollutants by their movement and ingestion of contaminated soil or leaf litter. Several factors like permeability of skin to water and bioaccumulation potentialities make the earthworms excellent indicators for the evaluation of toxicity arising out of chemical-residual contamination of soil. Since earthworms are the most abundant animals in soil occupying the lowest position in the terrestrial food webs, uptake of a pollutant by their body tissue could reflect environmental toxicity. The species *Eisenia foetida* and *E. andrei* have mainly been chosen for several toxicity tests as indicators of agrochemical contamination. Other species such as *Lumbricus terrestris* and *L. rubellus* have been used in studies of bioaccumulation of metals. Earthworms are able to bioaccumulate a number of heavy metals such as Cd, Cu, Zn and Pb from contaminated soils. These animals could tolerate high metal concentrations using a variety of sequestration mechanisms (Andre et al., 2009).

Markers in earthworms for contamination diagnosis

Biomarkers conventionally provide information about the biological effects of contaminants or environmental stressors and their mechanism of action on the organisms. In response to the contaminants/stressors, molecular, biochemical and physiological compensatory processes are activated in organisms, which may result in the inhibition or facilitation of one or more physiological processes or functional and structural changes in biomolecules. The level of indication in earthworms can be measured either in infra-organismal or at the community level. The relationship between pollutants and response of stress in different sub-organismal levels is restricted to the infra-organismal level. In contrast, the impact on growth, reproduction and cocoon production may be linked to the population indicators (Fig 5.2). The biomarker system can indicate the different states of a particular species in terms of behavioural, morphological and histopathological alterations. According to their applications, biomarkers are categorised into the following categories.

1. Morphological and histological markers
2. Cellular and genetic markers
3. Biochemical markers
4. Behavioural markers

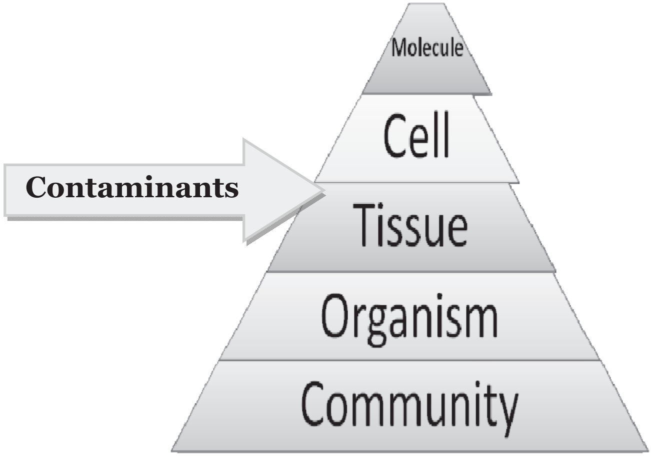

Figure 5.2: Biomarkers at different levels of biomonitoring

Morphological and histological markers

The detection of damage at the tissue or cellular levels due to contamination is possible by the use of morphological markers. Changes in certain morphological features in earthworms may provide qualitative evidence of a functional adaptation to the external environment. Further, the qualitative evaluation of such alterations in the organism may provide early indications of toxicity. Different types of morphological alterations such as clitellar swellings, pigment loss, discoloration, dermal rupture and skin undulations have been noticed in different earthworm species in response to pesticides and chemical fertilizers. Changes may also occur in the setal structure of worms, and anomalies appear as a reduction in setal length or its deformation (Fig 5.3, 5.4). Chemical fertilizers and pesticides may induce the folding of setae in earthworms (Samal et al., 2019). Histology and ultrastructure are used to diagnose cellular, and sub-cellular symptoms resulted from intoxication. Insecticides and herbicides might cause damage in skin and muscle layers of earthworms and indicate the toxicity in the form of the damaged cuticle, epidermis and muscles (Fig 5.5). Histological anomalies such as vacuolation, necrosis and displacement of both circular and longitudinal muscles are seen in organisms exposed to different industrial wastes (Samal et al., 2017). Metals are often concentrated in a few organs or specific regions of the tissues in most of the soil invertebrates. Epithelial tissue damage of the earthworm *Eisenia foetida* due to the herbicide Butachlor and in *Nsukkadrilus mbae* by atrazine have been observed (Gobi et al., 2009).

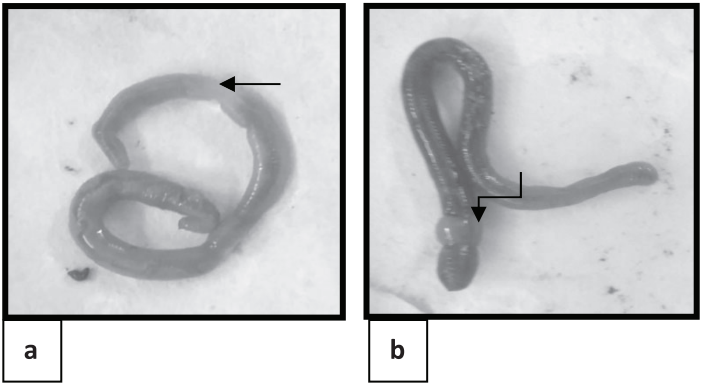

Figure 5.3: Morphological alterations in earthworms in response to agrochemicals. a) Discolouration, b) Clitellar swelling in *Drawida willsi* exposed to 15 g/kg paper mill sludge

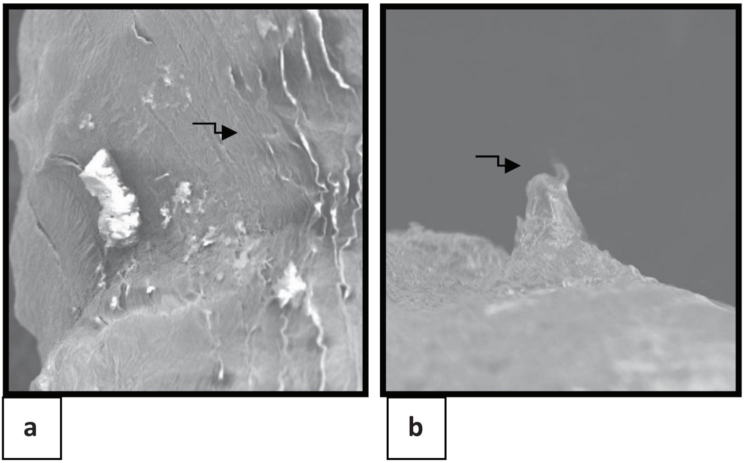

Figure 5.4: Scanning electron micrograph of the dermis in *Eudrilus eugeniae* after exposure to 3.0 g/kg of monocrotophos. a) Skin undulation b) Folding of setae

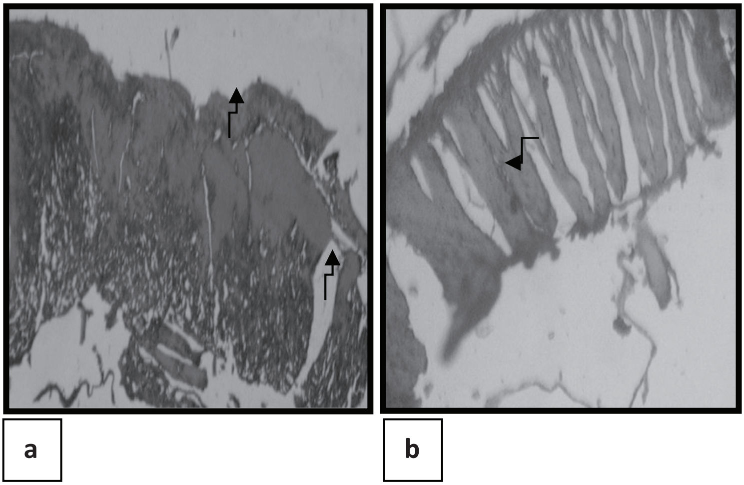

Figure 5.5: Histological anomalies in earthworms in response to high concentration of agrochemicals. a) *E. eugeniae* at 1.5 g/kg of urea, b) *L.mauritii* at 0.2 g/kg of glyphosate.

Cellular and genetic markers

Coelomic fluid in earthworms is particularly important and interesting from a toxicological perspective in the development of novel cellular biomarkers. Pollutants can be transported throughout an exposed organism, and its cells (coelomocytes) might be involved in the internal defence system. The most researched coelomocyte alteration in earthworms is represented by lysosomal membrane stability, which has been used as an indicator of chemical exposure and associated biological effects (Fig 5.6). The alteration in the membrane of granulocytes and lysosomal enzymes stipulates the level of oxidative stress induced by pollutants.

Xenobiotics could impact organisms, inducing gene mutations after releasing into terrestrial ecosystems. Due to the highly conserved structure of the genetic material, it is possible to use a wide variety of species in genotoxicity tests. The induction of aberrant metaphases, anaphases and telophases, such as bridges, loss and chromosome stickiness, polyploidy, irregular nuclei and nuclear buds are useful parameters for the genotoxicity analysis. In contrast, the micronuclei and chromosome breaks allow the mutagenicity analysis. DNA damage and chromosomal aberration could serve as good quality biomarkers. Besides, comet assay and micronucleus tests have emerged as the most powerful methods for assessing chromosome damage (both chromosome loss and chromosome breakage) accumulated during the lifespan of the cell in animals, including earthworms.

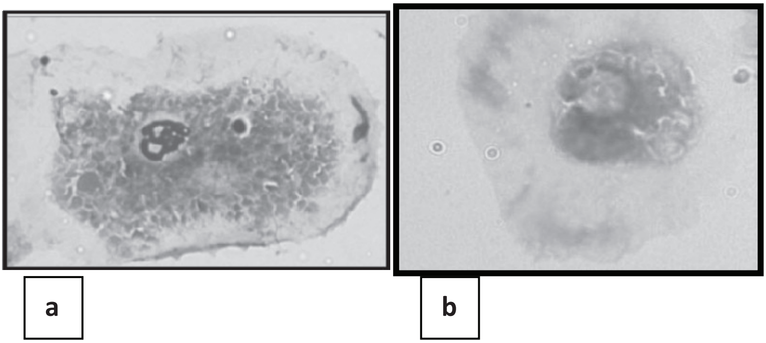

Figure 5.6: Granulocyte alterations in earthworms in response to copper sulfate. a) *L. terrestris* b) *E. fetida* (Calisi et al., 2009, 2011)

Biochemical markers

Enzymes have been used widely as sensitive biomarkers because toxic compounds have a high affinity for electron pairs found in the amino acids that form the enzymes. Heavy metal exposure could accelerate the generation of reactive oxygen species (ROS). ROS are normally produced in cells during metabolism, but their excess production could lead to oxidative stress and induce cellular damage. Enzymatic and non-enzymatic antioxidants are produced to counter oxidative damage in cells. Superoxide dismutase, catalase, glutathione reductase, and glutathione-S-transferase are some important enzyme antioxidants.

Metallothioneins (MT) are low-molecular-weight cysteine-rich metal-binding proteins that are involved in the homeostasis of essential metals like Cu and Zn and detoxification of nonessential metals such as Ag, Cd and Hg. The proteins could be useful in the evaluation of metal contamination. When earthworm species like *Lumbricus rubellus*, *Eisenia foetida*, *Eisenia andrei* when exposed to cadmium, *Lampito mauritii* to Pb and Zn and *Lumbriucus terrestris* to cadmium, copper and mercury, significant induction of MT proteins were observed. Acetylcholinesterase (AChE) is an important enzyme in the nervous system, terminating nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It is the target site of inhibition by organophosphorus and carbamate pesticides. It has been observed that Chlorpyrifos and Azodrin, two organophosphate pesticides, caused time-dependent AChE inhibition in *Eisenia foetida*. Various stress enzymes like Lactate dehydrogenase (LDH) and catalase (CAT) show activity variations in different earthworms, *D. willsi, L.mauritii, E. eugeniae*, in soil treated with pesticides and chemical fertilizers. Nayak et al. (2018) have reported the toxicity related changes in the enzyme activities in *Glyphidrillus tuberosus*, a tropical earthworm exposed to a high concentration of phosphogypsum. Earthworms have also been used to monitor the reclamation of organically amended mine spoil by monitoring the activities of metabolic and antioxidant enzymes (Nayak et al., 2020 a&b).

All soil fauna, in response to environmental stress, synthesize highly conserved proteins known as heat shock proteins (HSPs). The presence of toxicants in soil could be detected using HSP70 as a marker in the earthworm *L. terrestris*. The total protein contents and lipid peroxidation levels in earthworms are sensitive enough to indicate changes in soil quality. Apart from the toxicity of chemical compounds, biochemical markers of earthworms are useful to predict the impact of environmental stressors such as light, moisture and temperature on soil (Mishra et al., 2018, 2019, 2020).

Behavioural markers

Studying earthworm's behaviour is not an easy task as they live inside the soil, but the avoidance behaviour of worms in response to different agrochemicals has been observed and indicates that invariably all species prefer to avoid contaminated soils. A reduced earthworm population in contaminated soil is an early indication of soil toxicity (Mishra et al., 2017). Certain species also adopt coiling to overcome stress-induced by unfavourable environmental conditions such as soil moisture deprivation and toxicity due to xenobiotic compounds. This behaviour of worms is meant to ensure minimal exposure of the body to stressors. The behavioural pattern linked to an organism's fitness is also influenced by different toxic compounds. The locomotion, feeding and cast production of worms are affected by pesticides. The diameter of earthworm burrows has been analysed in species like *Aporrectodea icterica* and *Aporrectodea nocturna* using X-ray tomography, which shows a reduction in length and depth in the pesticide-contaminated area. The cast production by different species also gets affected in contaminated soils and may be considered as a novel biomarker to sense toxicity. The behavioural repertoire of earthworms has not been explored in comparison to mammals, birds or insects, but it may address some important soil functions that are affected by their activity (Pelosi et al., 2014).

Earthworm assisted remediation: The solution to soil pollution

Compensation of high-cost technologies is possible by replacing the physical and chemical remediation techniques with bioremediation. The utilization of biota to remediate contamination from a medium is a superior and eco-friendly concept. The soil organisms not only indicate the toxicity but also eliminate those contaminants from trophic spheres.

Discrimination between natural biodegradation and bioremediation is a difficult task. Bioremediation facilitates the rate of microbial degradation of pollutants by providing the microorganisms with essential nutrients, carbon sources or electron donors. The process demands the addition of indigenous microorganisms having characteristics to degrade the desired contaminant at a faster rate. Production of H_2O and CO_2 without producing the toxic intermediates is the unique feature of bioremediation (Frazar, 2000).

Bioremediation technologies can be broadly classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex situ* bioremediation deals with the removal of the contaminated material to be treated outside the site. The later include bioreactors, biofilters, and land farming, whereas the former includes bioventing, biosparging, biostimulation and liquid delivery systems. *In situ,* technology is more popular due to its less equipment requirement, lower cost and eco-friendly nature. However, this treatment has limited practical applications. Bioremediation processes may be either aerobic or anaerobic based on the contaminated site and types of contamination.

Animal remediation or zoo remediation is applied in contaminated fields according to the characterization of some soil invertebrates like earthworms based on their contaminant adsorbing, degrading and removing ability. The field of animal remediation is usually limited to invertebrates owing to ethical concerns. Among different invertebrates, earthworms are the most widely known animals used for both bioindication and bioremediation. These animals have been widely used for land recovery, reclamation and rehabilitation to rectify sub-optimal soils such as poor mineral and open cast mining sites (Fig 5.7). Exotic species such as *Eisenia foetida* and *Eudrillus eugenia*e are highly suitable for the bioremediation of different pollutants like heavy metals, polycyclic aromatic hydrocarbons and pesticides. Besides the direct application of earthworms in the polluted sites, digested material (vermicast) of these animals could be used to facilitate the remediation process. Several authors have observed the positive influence of soil animals like earthworms on the utilization of organic compounds in their metabolism and enhancing the metabolic activity of soil microbes (Prakash et al., 2017). The part of the remediation process using animals has not been thoroughly investigated.

Bioremediation with earthworm mediation may involve

- Inoculation of earthworms directly to contaminated soils.
- Application of earthworms to contaminated soils with an organic media.
- Providing the contaminated media to earthworms as part of a feeding regime.
- Alternate use of earthworms via application of materials pre-digested by the worms. These substrates are expected to be rich in promoted degraders with high catabolic potential.

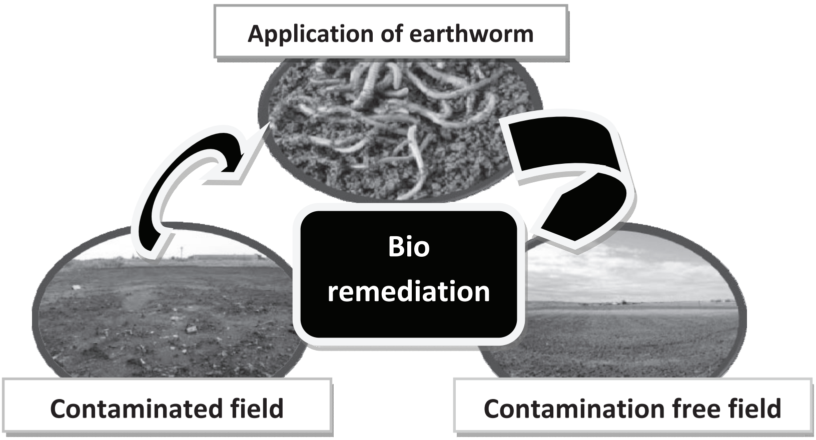

Figure 5.7: Bioremediation using earthworms

Research results have established that earthworm-assisted bioremediation is likely to increase hydrocarbon availability and remobilise dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) bound residues. Further findings indicate that earthworm (*L.*

terrestris and *Aporrectodea calignosa*) activity would promote atrazine mineralisation by altering the size and diversity of microbial communities.

In contrast to the agrochemical studies, many of the subsequent studies are descriptive investigations as opposed to mechanistic ones. However, to highlight the increased interest of earthworm inclusion in bioremediation for hydrocarbons, one study demonstrated the co-application of compost and earthworms for bioremediation. An earlier study also concluded that increased microbial catabolic activity due to *E. foetida* presence is responsible for the loss of 91% of crude oil contamination. However, this is not the universal rule applicable to all types of remediation processes (Hickman & Reid, 2008).

Problems identifying markers and bioremediation using earthworms

Although several biomarkers have been successfully used, there are some limitations to relying on them solely. In certain cases, it is not easy to differentiate natural variability from changes due to human impacts, limiting the applicability of the indicators in heterogeneous environments. Accordingly, populations of indicator species may be influenced by factors other than the disturbance or stress. It is also true that the soil ecosystem is very complex and that terrestrial fauna are less sensitive to contamination than aquatic biota.

Unlike phytoremediation, the earthworms method should be implemented at specific times. Organisms included in the process of bioremediation need a congenial environment to degrade the pollutants. Other limitations, like the results of cost/benefit ratios, i.e., the cost versus overall environmental impact, need to be considered carefully. The bioremediation process does not degrade all kinds of pollutants and may not give a full proof results with all chemical contaminants. The molecules and enzymes involved in this process are partially compound-specific, and the mechanisms vary from one chemical to the other. Hence, the application of this technology should be case and site-specific.

The earthworm's role in wasteland reclamation

The reclamation process involves the conversion of derelict industrial wasteland into fertile soil for agricultural purposes. The fertilizing potential of the earthworm makes it a natural reclamation agent to restore the quality of the degraded land. Hence, research has been conducted to introduce the earthworms in the wasteland for the reclamation process.

The establishment of an earthworm species (*Aporrectodea caliginosa*) in a pasture of New Zealand was accompanied by an increase in the proportion of higher fertility plants such as ryegrass and clover but it did not show the result in hill pasture. The introduction of earthworm activities facilitates the conversion of compact infertile soil into to fertile soil. Significant improvement in soil structure was seen in an irrigated pasture on sandy loam soil of Australia after the establishment of *A. caliginosa.* Inoculation of earthworms in pastures reduces the soil bulk density and enhances the N content with C/N ratios. An experimental study on wasteland in Uzbekistan demonstrated that *Aporrectodea trapezoides* and *A. prashadi* were suitable species to advance soil formation. Several reports established that the reintroduction of earthworm species such as *Aporrectodea longa, A. caliginosa* and *Lumbricus rubellus* in reclaimed polder soils of the Netherlands accelerates the maturation of soils (Barley and Kleinig, 1964; Van Rhee, 1969; Noble et al., 1970; Stockdill, 1982). Earthworms in improved hill pasture showed a higher density over those in unimproved acid peaty soil. The increase in the number of typical pasture species such as *A. caliginosa, A. Chlorotica, L. terrestris, A. longa* and *A. rosea* in improved pastures indicates the high fertility of that land (Guild, 1948). Species such as *A. caliginosa, A. rosea, D. octaedra, D. rubidus* and *L. rubellus,* from fen peat in Sweden, contributed towards the reclamation of degraded soils for over 60 years (Rosswall et al., 1977). Moreover, species like *A. caliginosa, A. chlorotica* and *L. rubellus* have proved to be the most successful fertility restorer due to their high reproductive rate, rapid disseminates ability, early colonizing potential in diverse situations. *Pontoscolex corethrurus* has been proposed to be used to restore the degraded land of central Himalayas. The identification of keystone earthworm species could be used as a tool to restore the wastelands. The relation between spatio-temporal variations in earthworm population and land uses provide clues about the reclamation of land by use of earthworms. The application of paper mill sludge and earthworms to acid soils might be able to revive infertile land to a greater extent. The nitrogenous waste excreted by the nephridia of earthworms, which is rich in urea and ammonia, is converted into nitrates and the worm cast containing enzymes like amylase, lipase, cellulase and chitinase, break down organic matter in the soil to release the nutrients to the plants. The impact of epigeic species like *Lumbricus rubellus*, anecic, *L. terrestris,* and endogeic *Aporrectodea calliginosa* on bacterial community and nitrogen mineralization states the importance of epigeic and endogeic species on enhanced mineralization of organics and favours the fertility which could be used as land reclamation tools.

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