



ISSN : 0973-7057

Int. Database Index: 663 www.mjl.clarivate.com

## Role of earthworms in heavy metal accumulation

Keshav Singh\* & Nishat Fatima

Vermibiotechnology Laboratory, Department of Zoology, D. D. U. Gorakhpur University, Gorakhpur U.P. India.

Received : 16<sup>th</sup> April, 2019 ; Revised : 28<sup>th</sup> June, 2019

**Abstract:-** Due to rapid increase in urbanization in the whole world, there arises the problem of management for organic wastes. The process of vermicomposting is the method by which such waste materials can be remediated easily and the compost which is released can be used in growing food crops for human consumption. By using earthworms, the management of hazardous wastes with high metal contents can be done easily, because earthworms soak up or bioaccumulate these toxic heavy metals into their body tissues and make the soil and surrounding pollution free. The importance of earthworms for bioaccumulation and monitoring of metal pollution was widely recognized in terrestrial ecosystem. They have a great power of accumulation of these hazardous metals as a natural ecological indicator of soil contamination. The contamination which was done by heavy metals can cause severe impact on the functioning of soil ecosystem by affecting the activities of soil fauna as well. Therefore, vermibiotechnology is the best technique for management of biological waste and accumulation of heavy metals from soil by in-situ application of earthworms. Vermicompost is a good alternative of chemical fertilizers for production of crops and it also enhances the soil fertility. This is eco-friendly management process and protects our soil and ecosystem free from heavy metal contaminations by the vermiremediation process of earthworms.

**Key words:** Earthworms, Heavy metals, Bioaccumulation, Soil, Organic wastes, Vermiremediation.

### INTRODUCTION

Environmental quality of urban area is vital to be investigated as large number of human population's lives in the cities. However, due to rapid increase in urbanization and industrialization in urban areas cause a severe adverse effect on environment as well as on humans. Due to this factor there is an enhancement of pollution levels in our environment, especially due to the heavy metals which are serious source of contamination throughout the world. The advances of humans need towards industrialization caused the production and entrance of hazardous pollutants

such as carcinogenic compounds, toxins and heavy metals into the environment. The main pollutants present in the environment are heavy metals which cause big problem due to their toxicity and accumulation in the environment. Due to the rapid increase in population, there is requirement also enhanced for food, agricultural land per unit area needs to achieve maximum efficiency. Agricultural productivity and its quality depend on nutrition of the plant. There is only one way to achieve this target is abundant use of fertilizers to supply these nutrients. Excess fertilizers may cause impact of heavy metal accumulation. The presence of heavy metal affects the plant growth.<sup>1</sup> There are several heavy metals like Nickel, Cadmium, Boron and Iron, they have tendency to accumulate in human bodies. Due to these properties they are called important pollutants which

\*Corresponding author :

Phone : 9450433313

E-mail : keshav26singh@gmail.com

cause chronic effects.<sup>2-6</sup> When these heavy metals inters into soil cause adverse environmental issues and due to their contamination level there is serious issue arise regarding to human health and other organisms.<sup>7-8</sup> Since, all the agricultural products are linked directly to the soil and because of the widespread human use of these products and the high potential, of these soils for contamination with heavy metals. In the decontamination of soils contaminated with heavy metals, stability and high resistance to degradation of the metals is considered to be one of the most challenging issues.

Heavy metal contamination in soil is a most common problem in terrestrial ecosystems which are affected by atmospheric deposition and industrial activities.<sup>9-10</sup> Heavy metal which is present in environment up-taken by crop plants, due to the process of food chain.<sup>11</sup> Left-over materials which are released from urban systems are termed as urban waste, which usually consist of household garbage such as kitchen waste, green waste, street sweepings, sanitation residues, etc.<sup>12</sup> There is a rapid increase in these urban wastes nowadays and their disposal is of great concern as it causes serious management problems particularly in developing countries like India, where the management of waste are mostly unsystematic and unscientific.<sup>13-14</sup> These urban wastes usually consist of hazardous persistent pollutants such as heavy metals, which cause severe threat to ecosystem as well as on human beings.<sup>15-16</sup> Heavy metals are persistent in the environment, contaminate the food chains, and various health issues are also occurred due to their toxicity. Metal concentrations above threshold levels affect the microbiological balance of soil and can reduce their fertility.<sup>17</sup>

Heavy metals are persistent in the environment; they can easily accumulate in living organisms and are transferred from one trophic level to another trophic level into the food chain. Heavy metals are transferred from soil-to-plant and it is very important step in the trophic transfer of such metals in food chains. Plants uptake these metals from polluted soil and easily transferred to higher level of environment.<sup>18</sup> Human beings are considerably exposed to toxic heavy metals in the environment through different pathway including ingestion, inhalation and dermal absorption. Most of the people are exposed to heavy metals in developing countries.<sup>19</sup> Earthworms play a vital role in

metal pollution monitoring and widely recognized in terrestrial ecosystems. Earthworms have a great tendency for bioaccumulation of heavy metals in their tissues and can be used as an ecological indicator of soil contamination. According to their virtue habitation earthworms participate in the formation of soil structure and regulate dynamics of soil organic matter, usually earthworms directly or indirectly modulate the transfer of organic and inorganic chemicals (toxicants) in soils. Due to this reason, earthworms are considered as useful biological indicator of several metals in soils.<sup>20</sup> Earthworms are considered as important bioindicators for risk assessment and they are used in ecotoxicological studies to assess the power of bioaccumulation of heavy metals in food chain.<sup>20-23</sup> Earthworm bodies are externally and internally always came in contact with soil, due to this factor they readily take up and bioaccumulate many heavy metals including Cd, Zn, Cu and Pb.<sup>24-25</sup> Different species of earthworm show different tolerance level to contaminated soils and presence of heavy metals consistency.<sup>26-31</sup>

### **Heavy Metal**

When heavy metal are found at above threshold concentration they are considered as one of the most major pollution which act as threat to man and other organisms by the route of food chain. Pollutants such as heavy metals, pesticide, polychlorobiophenys (PCBs), etc. are occur most dangerous, due to this factor they show severe impact on the human race and environment.<sup>32</sup> The term 'heavy metals' mostly refers to those metals having specific weights more than 5 g/cm<sup>3</sup>. The densities of these metallic elements are relatively high however; being a heavy metal has more to do with their chemical properties. These metals are usually found dispersed in rock formations and about 40 different metals have been come under this category. In the environment due to rapid rise in industrialization and urbanization, heavy metals levels are also increased. Heavy metals cause serious health issues to humans and perturbing the environment, because these metals are spread broadly over the globe. Urbanization, land use changes, and industrialization, are major root cause for this heavy metals pollution, especially in developing countries with extremely high populations, such as India and China.<sup>33</sup> Non-essential to metabolic and other biological functions are classification for several hazardous heavy

metals and metalloids (e.g., As, Pb, Cd, and Hg). Those metals are deleterious in various respects.<sup>34</sup> and they metals have therefore been included in the top 20 list of dangerous substances by the United States Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry.<sup>35-39</sup> Due to the result of human caused activities these heavy metals become concentrated, but naturally they are found in the earth. Some common sources are as mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilizers, paints and treated woods.

#### **Entry routes**

Due to the process of air inhalation, diet and manual handling heavy metals enter plant, animal and human tissues. Motor vehicle emissions are considered as a major source of airborne contaminants including arsenic, cadmium, cobalt, nickel, lead, antimony, vanadium, zinc, platinum, palladium and rhodium. Another potential source of heavy metal contamination is also caused due to absorption through skin contact with soil. Heavy metals are hard to metabolize, that's why they are accumulated in organisms.

#### **Detrimental effects**

Heavy metals can easily bind to the vital cellular components, such as structural proteins, enzymes, and nucleic acids, and therefore do interfere with their functioning." Long-term exposure in the presence of heavy metals can cause carcinogenic, central and peripheral nervous system and circulatory effects. From thousands of years these heavy metals have been used by humans. When heavy metals are up taken by any means of food consumption will show confirmative negative impact towards the human health.<sup>40</sup>

#### **Earthworms in vermicomposting**

Vermicomposting is a type of process which utilizes earthworms; it is an eco-bio-technological process that transforms energy rich and complex organic substances into stabilized humus like product<sup>41</sup>. Vermicomposting is a type of kindred process to composting, by the addition of different species of earthworms used to enhance the process of waste conversion and produce a better end product. All the urban waste which consists of organic component can be bio-converted and recycled into a useful end-product called compost and Vermicompost when

processed by earthworms. Not only are the organic fractions of urban wastes converted into available nutrients with the help of earthworms.<sup>14</sup> But also consequently earthworms remediate the persistent heavy metals from the wastes by bioaccumulation into their bodies via Vermicomposting process.

The transformation of organic waste into Vermicompost is a bio-oxidative mesophilic process in which detritivorous earthworm species interact with micro-organisms strongly affecting decomposition processes, and also accelerating the stabilization of organic matter and greatly modifying its physical, chemical and biological properties. Earthworms are ecological engineers that through remediation process involved in the indirect stimulation of microbial populations through fragmentation and ingestion of contaminated soil and organic matter. The potential of metal accumulation is maintained by earthworms and make the ecosystem in a balanced state. Moreover, earthworms ingest soil and various forms of biomass to produce vermicasts. Thus adopting the vermicomposting process not only provides availability of plant mineral nutrients but also act as a best method for agricultural benefits and bioaccumulation of various heavy metals present in the soil.

#### **Earthworms**

Earthworms are macroscopic clitellate oligochaete annelid that usually lives in soil. They are segmented worms, bilaterally symmetrical, with an external gland for production of egg case (cocoon), with present of sensory lobe in front of the mouth and an anus at the end side of the body, along with small number of setae on each segment. The distribution of earthworms in soil are influenced by several factors such as soil texture, aeration, temperature, moisture, pH, organic matter, dung and reproductive potentials.<sup>42-43</sup> For the earthworm population external biotic parameter and nutritive resources of the soil are primary controlling factors.<sup>44-45</sup> During the process of feeding and digestion earthworms are act as an aerator, chopper, crusher, chemical degrader and a biological stimulator.<sup>46</sup> Aristotle mentioned earthworms 'territory intestines' in 330 B.C, and he confront that soil is an organic entity and very important role played by an earthworms for maintaining the life of soil. Earthworms are burrowing animals which are grouped into 13 families. Although about

5,000 species have been described, most of the species are still unknown, mainly in the tropics.<sup>47</sup> These worms are found in soil ecosystem of many parts of the world, but these earthworms are abundantly found in temperate and tropical regions, due to their large fraction of living biomass, providing agro-ecosystem sustainability.<sup>48</sup>

The earthworms are members of large, ubiquitous group with evolutionary history. About 6000 species of earthworms are found on all continents except Antarctica. Most of the species of the earthworm dwell in soil, whereas some live in leaf litter, decaying log and river banks, as well as lives in trees and even along the seashore. Earthworms are major terrestrial ecosystem engineers and hence their economic impact is immense. Although earthworms are the members of most familiar and economically important groups of large invertebrates, their evolutionary history is not well understood. Earthworms leave an impressive record for trace of fossils, but it is not easy to determine which species made a particular set of fossilized burrows, since body fossils of these earthworms are extremely rare. Some analyses reveal that the ancestor of all the living earthworms probably lived over 209 million years ago, this quality makes earthworms about as old as mammals and dinosaurs.

The reputation of earthworms was rehabilitated when Darwin published his book in-titled “The Formation of Vegetable mould through the action of worms with observations on their habits” in 1881. Due to their antiquity and relative stability in soil, those that persist today, especially in primitive families, are more akin to “living fossils”. The terms “alien”, “exotic” and “invasive” are often described earthworms that are dispersed over a wide range and in geographically remote localities, they are widely distributed in many parts of the world or merely present in more than one country, but with the ecological implication of passive or unintentional transportation.<sup>49</sup>

Although Darwin mainly commented on their intimate ecological relationship with soils and plant growth, his contributions to earthworm studies extended into the realms of anthropology and archaeology. Earthworms usually have remarkably higher rate of biodiversity. The natural spread of earthworms can either be a slow or negligible, the dispersion of exotics appears to closely follow cultural and technological trade and exchanges gradually in pre-agricultural societies. Thus, earthworm transportation is

considered to be mainly human mediated with exotic species, origins often corresponding with provenances of traditional agricultural and horticultural crops. There are about 3,320 species of earthworms are distributed all over the world.<sup>50</sup> Whereas, in India approximately there are about 590 species of earthworms present.<sup>51</sup> But their functional role of the majority of the species and their influence on habitat are lacking. India is a very diverse country and thus showed a very rich diversity of earthworms, mostly present in Western Ghats and Eastern Himalayas. The scientific exploration of earthworm’s diversity in India dates back to the 19<sup>th</sup> century.

The naming of first earthworm species in the Indian subcontinent credit goes to Templeton (1844) when he discovered *Megascolex caeruleus* from Sri Lanka.<sup>52</sup> Whereas, Perrier (1872) was the first to describe earthworm species from the Indian mainland.<sup>53</sup> Earthworms not only play important role for agricultural maintenance but also they act as a nutritive content for the growth of the plants as well. They are the most attention seeker organisms on this earth due to their activities.

The digestive systems of the worms are apparently capable of detaching heavy metal ions from the complex aggregates and humic substances in the waste as it rots. Various enzyme driven processes seem to lead for assimilation of the metal ions by all earthworms so that these metals lock up in the tissues of the organism rather than being released into the compost as worm casts. Soils are contaminated with metals and their accumulations are reported in earthworms many times.<sup>54</sup> Aristotle mentioned earthworm as “intestine of the earth”. Heavy metals are usually remaining in the soil immobilized by the addition of various chemicals (solidification/stabilization).

When earthworm treatment was introduced into the soil, the distribution of heavy metals gradually dropped due to action of earthworm activity. The earthworms are considered as ecosystemivorous feeding on entire soil microbial ecosystems.<sup>55</sup> Metals are up taken by earthworms into their gut are bounded by a protein called ‘metallothioneins’. The chloragogen cells which are present in earthworms appear to accumulate heavy metals absorbed by the gut and cause metals immobilize in small spheroidal chloragosomes.<sup>56</sup> Excreted material of earthworms such as mucus and urine into the soil environment which caused increase in microbial activity into the soil ecosystem. And

as well as also release other stimulating substances in addition to the mucus and urine.<sup>57</sup> This statement is very clear and understood that earthworms are very important soil fauna which act as an indicator organism for recycling of organic wastes which are present in soil environment and as well as accumulates heavy metals into their body and protect our various food products from the contamination of these hazardous heavy metals and make our environment clean and safe from these toxic metals because heavy metals are poisonous for consumption of human beings when they enter our food chain.

Heavy metals can enter the soil ecosystem from different sources. Fertilizers, pesticides, organic and inorganic amendants, wastes and sludge residues can contain variable amount of these metals. Accumulation of these metals by earthworm species occurs through two ways.<sup>25</sup> Which include absorption directly into their body tissues or either by ingestion of organic matter.<sup>58-59</sup>

The direct measurement of heavy metal concentrations in earthworm tissue could provide a means of assessing environmental pollution levels, given the demonstrated correlation between soil contamination and earthworm metal bioaccumulation.<sup>60</sup> Cadmium, Copper, Lead and Zinc are some heavy metals which are generally accumulated by the earthworms.

Various earthworms' species are crucial drivers of the remediation process as they are involved in the indirect stimulation of microbial population through ingestion and fragmentation of contaminated soil and organic matter. And most importantly, the potential rates of earthworms for bioaccumulation of heavy metals have maintained our ecosystem in a well-mannered and balanced state.

By accumulation of these heavy metals into their tissues, they maintained nutrient enriching abilities of the soil ecosystem. The vermicasts of most earthworm species are known to contain hormones and enzymes which stimulate plant growth and discourage pathogens. Earthworms have the potential for safety management of all municipal and industrial organic wastes and convert them from ending up in the landfills. Thus vermicomposting technology acts as a best method for bioaccumulation of heavy metals from the soil ecosystem by the activities of earthworms.

Earthworms are known to cause contribution to soil formation through consumption process of dead plant and

animal matter. Earthworms are also known to cause recycling of carbon and nitrogen into the ecosystem. The cast of the worms have higher available nitrogen, potassium, calcium and phosphorous content than the surrounding soils as well as higher cation exchange capacity. Soil passed through gut of earthworm has a neutral pH.

Earthworms excrete material that has concentration of beneficial microbes that help decompose crop residue. Earthworms play a major role in monitoring soil structure and fertility because it increase mineralization and humification of organic matter by food consumption, respiration and may indirectly stimulate microbial mass and activity as well as mobilization of nutrients.

The accumulation was occurred in the yellow cells of body tissues of earthworms. Depending upon the concentration of heavy metals, the body of earthworm gets affected. Hg, Co, Pb and Zn are some toxic metals which cause severe impact on the growth of earthworms. Whereas, Hg is more toxic for earthworms species because it effects cocoon production, coelomocytes, body weight and their length as well.

The earthworm digestive system is apparently capable of detaching heavy metal ions from the complex aggregates between these ions and humic substances in the waste as it rots. The separation of dead worms from compost is a relatively straight process allowing the heavy metal to be removed from the organic waste.

Heavy metals may cause damage to an organ, inhibition of an enzymatic activities and significant alteration in their various metabolic activities. On the basis of morphological characteristics earthworms have been classified into three categories.<sup>61</sup> These categories are- Epigeic, Endogeic, and Anecic.

### **Epigeic**

Epigeic earthworm species are non-burrowing litter and dung-feeding earthworm's habitat is in organic horizons and near the surface litter. By commuting the litter, they modify its physico-chemical characteristics, usually reducing its C/N ratio, making it suitable for further microbial breakdown. These species are known as "litter transformers". Their body size is about small-to-medium, uniformly pigmented species, they have dorsoventrally flattened body, tolerance to disturbance, high metabolic

rate, high fecundity, short life cycle and they also have high power of regeneration. They are r-selected species earthworms. Epigeic earthworms form the forest floor community of temperate and tropical countries. When the environmental conditions are not favorable or their food is limited, Epigeic species are difficult to find. Examples include *Eisenia fetida*, *Perionyx excavatus*, *Eudrilus eugeniae*, *Dendrobdena rubida* (Savigny) etc. Epigeic earthworms are of often bright red or reddy-brown in colour, but they are not stripy. These earthworms usually do not form permanent burrows.

#### **Anecic**

Anecic earthworm's species live in permanent burrows as deep as 3m below the soil surface. These earthworms species are called the 'ecosystem engineers' because they play a great role in soil turn over, and improve water holding capacity, aerobicity and as well as exerting regulatory force in soil functioning. These earthworm species lives in vertical burrow system that usually open up to the soil surface. Their casts are formed on the surface of soil. They gradually feed on decaying plant litter with little amount of soil, so they are called as geophytophagous. Anecic earthworms are large in size, moderate to heavily pigmented and intolerant to disturbances. These worm species are found in temperate part of the world. Their reproductive rates are relatively low. Examples of anecic earthworms are *Lampito mauritii*, *Metaphire houlleti*, *Lumbricus terrestris*, *Drawida grandis* etc. Anecic species have dark coloured at the head end (red or brown) and have paler tails. They have long life cycle with the limited power of regeneration.

#### **Endogeic**

Endogeic species of earthworms are geophagous because they live deeper in the soil profile and feed primarily on both soil and related organic matter. They form both sub-surface and surface casts. These species of worms are non-pigmented or lightly pigmented and form horizontal burrows in top and sub-soils. These species have symbiotic relationship with the gut micro-flora. Endogeic earthworms are found in tropical parts of the world. They have long life cycle with the limited power of regeneration and also have diverse effects on soil properties. They increase the proportion of large aggregates along with bulk density of soil. These species are apparently of no major importance

in litter incorporation and decomposition since they feed on subsurface material, and also play a vital role in other soil formation process. These species are concerned with formation of humus in the surface soil. These endogeic species also increase overall soil porosity and enhance water infiltration. Species such as *Polypheretima elongata*, *Metaphire posthuma*, *Allobophora caliginosa*, *Octolasion nycaneum* (Savigny) are included in this group. Endogeic earthworms are often pale in colors, grey, pale pink, green or blue.

These three different categories of earthworms thus contribute differently to soil fertility process and structure maintenance. They may be manipulated in a given area in a specific agricultural situation.

#### ***Metaphire posthuma* (Vaillant, 1869)**

*Metaphire posthuma* is an endogeic species mostly common to tarai region, which lives deeper in the soil profile and feed primarily on both soil and associated organic matter i.e. geophagous and they form both sub-surface and surface casts. They are generally found near anthropogenic habitats like paddy fields, ridges of paddy fields, and in wastewater saturated soil from households. In India, *Metaphire posthuma* are distributed in Jharkhand, Rajasthan, Uttar Pradesh, Pakistan.<sup>62-64</sup> They are dark brown in colour. Length 110-158mm, clitellum width 3-8mm, segment number 91-124. Prostomium epilobous or tanylobous, setae 111-130 in 8, 71-83 in 20, 18-19 between male pores. Clitellum 14-16, annular, setae absent, dorsal pore absent. Their cast is thick pellets like. These worms are very active and twist their body away when touched. Among the physico-chemical parameters, only moisture was observed to affect the density of *Metaphire posthuma*. It is widely distributed in Southeast Asian countries. Coelomocytes, the chief immune effector cells of earthworms perform diverse physiological functions under the challenge of toxic heavy metals. These worms are highly resistance to heavy metals so that it is called as heavy metals bioindicators, capable of being a waste processor or Vermicomposting.<sup>65-68</sup>

#### ***Eisenia fetida* (Savigny, 1826)**

*Eisenia fetida* corresponds to the striped or banded morph; they are reddish-purple in colour. The original range where this species is supposed to be from the Caucasus to the forest-steppe zone of Russia where these species

are occurring under bark of fallen trunks and in decaying organic material.<sup>69</sup> In India, they are distributed in Himanchal Pradesh, Punjab, Pondicherry, Uttarakhand, Jharkhand and in Guana, Iran, Spain as well. *Eisenia fetida* are generally found in paddy field, grassland and sugarcane field. Their casts are very small pellets like. Length is about 60-90mm; diameter 3-4mm; with red purple or brown segmented bands over dorsum separated by paler intervals. Prostomium epibolous ½. Dorsal pore from 4/5. Setae slender, ornamental, closely paired.

Clitellum from xxiv, xxv or xxvi to xxxii (=7-9). Male pore with fairly large raised areas which do not transgress the limits of xv. Spermathecal pores two pairs, in 9/10 and 10/11, near the middorsal line. The growth and reproduction of this earthworm species is affected by several factors such as food quality, moisture, temperature and population density. *Eisenia fetida* are very affecting earthworm for management of waste via vermicomposting process and bioaccumulation of heavy metals are also often done from contaminated field and soils.<sup>68</sup>

#### ***Lampito mauritii* (Kinberg, 1867)**

*Lampito mauritii* was found in all habitats such as groundnut field, millet field, garden, grassland, cultivated field, paddy field, sugarcane field and vegetable field. In India, this species are most widely distributed in different agro-systems of Punjab, Rajasthan, Tamil Nadu, Pondicherry, Uttar Pradesh.<sup>70-73,62</sup> This is one of the most common earthworm species in the loamy soils of Gorakhpur, Deoria districts as well. This species is abundantly distributed throughout the country *Lampito mauritii* are dark yellow in colour. Length 92-168mm, diameter 5.0-5.3mm, total segments 126-170. Colour dorsally is greyish, brownish or yellowish with purplish tinge at anterior end. Prostomium is epilobous with tongue 1/2, closed. First dorsal pore start on 10/11 or 11/12. Setal arrangement is perichaetine, 40-50 setae per segment in the preclitellar region. No genital markings are there. Their casts are granular like.

#### ***Perionyx excavatus* (Perrier, 1872)**

*Perionyx excavatus* are cosmopolitan species. In India they are found in Himanchal Pradesh, Uttarakhand, Mizoram, Tripura, Pondicherry, Tamil Nadu, Punjab, and Uttar Pradesh. As well as also in Sri Lanka, Southeast Asia, Taiwan, Hawaii, West Indies, Madagascar, Samoa,

Fiji, U.K, U.S.A, Australia and in New Zealand. They are widely distributed in Paddy field, sugarcane field, garden millet field, groundnut field, cultivated field, grassland and vegetable field. Their casts are loose pellets like. This species are commonly found in compost heaps. They are reddish brown in colour. Length 65-150 mm, diameter 3-4.5mm, total segments 90-155. Colour deep purple to reddish brown dorsally but pale ventrally.

*Prostornium epilobous*. Setae small, perichaetine 42-52 per segment. Clitellum thin often indistinct. Body dorso-ventrally flattened and the posterior portion more tapering than the anterior. No genital marking. Gizzard is not prominent. This is an Epigeic species that lives solely in organic wastes, and high-moisture contents and adequate amounts of suitable organic material are required for populations to become fully established and to process organic wastes efficiently. This tropical earthworm is extremely prolific, and it is easy to handle and very easy to harvest.

Its main drawback is its inability to withstand low-temperature conditions, but for tropical conditions it seems an ideal species. They have shorter maturation and incubation time period. *Perionyx excavatus* does not grow much at low temperatures although it can survive those 4°C (39.2 °F), but it is less susceptible to high temperatures over 30 (86°F). Even in tropical areas, *Perionyx excavatus* does not survive the high summer temperatures. The life cycle of *Perionyx excavatus* takes 40-50 days. Sexually maturity is attained within 20-28 days.<sup>68</sup>

#### ***Eutyphoeus waltoni* (Michaelsen, 1907)**

*Eutyphoeus waltoni* are distributed throughout in India such as Punjab, Sikkim, Jharkhand, Himanchal Pradesh and Uttar Pradesh. Their casts are tower-like. This species are usually found in cultivated field, grassland, groundnut field, millet field, paddy field, sugarcane field and vegetable field. They are brownish in colour. Length is about 100-210mm, diameter 4.5-6.5mm, and body segments 190-210, body colour brownish to violet-grey dorsally. Dorsal pores from 12/13 or 11/12. Setae rather smaller, paired but not closely. Clitellum ring shaped, but thinner ventrally, 1/2 xiii-xvii. Septum-7 is the first and being thickened. Septa, calciferous glands, and vascular system as usual in mentandric species. From the eighth to the twelfth segment also the internal and external segmentation do not

correspond; and septum-9 is actually situated in segment x as delimited externally by the furrows, if not on a level with groove; Gizzard large. Intestine are begins from xiv.<sup>68</sup>

Earthworms are called as hermaphrodite, they have both male and female sex organs but they need to the same species to produce off springs due to different maturation period of sperms and ova. Earthworms can breed throughout the year under ideal conditions, but cocoon output is known to decrease rapidly after a period of prolific production.

#### **Therapeutic properties of earthworms**

Since, from centuries medicinal values of earthworms are known. The earthworms are usually acted as a source of proteins, peptides, enzymes and physiologically active substances. The pastes which are prepared from earthworm tissue are used in the treatment of numerous diseases. Earthworms are also been used as a drug for the improvement of blood circulation for centuries. Worms have wide variety of biologically active properties such as Nitrogen, Antibacterial, Antioxidative, and Antipyretic as well as wound healing abilities. Earthworms are also used in pharmaceutical industries as well. Ashes of earthworm have been used as an stimulant for hair growth in head and as tooth powder.<sup>74</sup> And earthworm pastes are also used for the treatment of piles, fever, small pox, jaundice and removal of stones in bladder.<sup>75</sup> The 'leucocytes' of earthworm can recognized human cancerous cells as 'foreign' and can kill them.<sup>76</sup> Paste or extract which are obtained from earthworms are used to cure thrombotic diseases, arthritis, diabetes mellitus, pulmonary heart disease, mumps, exzema, anemia, and paralysis of limbs.<sup>77</sup> On the basis of above these accounts, the main aim of the present investigation is to study about the earthworms and their role in heavy metal accumulations.

#### **Influence of Environmental factors on survival and growth of earthworms**

Different conditions of earthworms such as cocoon production, rates of development and as well as growth are critically affected by environmental conditions. Epigeic earthworms are said to be relatively tolerant to the environmental conditions of the organic wastes. However, these earthworms are tolerance to other environmental parameters, such as moisture and temperature. If these factors are greatly exceeded, earthworms may move to

more suitable zones in the waste, leave it or die. The most important abiotic factors which include combination of different organic wastes, aeration, pH, temperature, moisture, C: N ratio etc.

#### **A-Temperature:**

Earthworms have complex responses to changes in temperature. The suitable temperature range which is best for earthworms during vermicomposting process is about 12-28°C). Their activities are significantly influence by variation in temperature. During, winter time period their temperature are maintained above 10°C and in summer the temperature are maintained below 35°C.<sup>78</sup> When earthworms are affected by temperature below 10 (50°F), then generally reduced or little feeding activity occurs and below 4 (39.2°F) cocoon production and development of young earthworm ceases completely. When temperature was extreme, earthworms tend to undergo hibernation and migrate to deeper layers for protection. *Eisenia fetida* can survive properly in very harsh environmental condition especially temperatures (5-43°C).<sup>79</sup>

#### **B-Moisture:**

The growth rate and maturation of earthworms was said to be best at 20°C and 85% moisture content. During the vermicomposting process, the optimum range of moisture for most of the species has been concluded to be between 50% and 90%. Whereas, the optimum moisture range up to 50% and 90% are suitable for *Eisenia fetida* and *Eisenia andrei* for their survival but they grow more rapidly between 80% and 90% in organic wastes.<sup>80-81</sup> During vermicomposting process, poor aeration may arise due to high moisture greasy and oily wastes in the vermicombed.<sup>82</sup>

#### **C-pH:**

Most of the species of the earthworms are relatively tolerant to variation in pH range and can tolerate the pH levels of 5-9, but they prefer mostly pH range of 5.0, they move toward the more acid material. The pH is usually a parameter which greatly influences the influences the process of vermicomposting and life cycle of earthworms. Most majorities of species prefer slightly alkaline soils with pH ranging between 7.4 and 7.8. *Eisenia fetida* and *Perionyx excavatus* prefer pH range of 8.1 in dung heap for better growth rate.

#### D-Aeration:

The earthworms are usually aerobic organisms, and they need oxygen for their vital activity which is very important for their growth and reproduction process. Because earthworms lack specialized respiratory organs, and oxygen and carbon dioxide diffuse through their body wall. Earthworms are said to be very sensitive to anaerobic conditions. For better aeration conditions, vermibed should be properly aerated either mechanically or manually and cause better growth of earthworms.<sup>78,82</sup>

#### Effect of heavy metals on earthworms

Heavy metals according to their non-biodegradable nature is said to be remain attached to soil for a very long time, and these metals can easily bioaccumulate into soil biota, they can also leached into the underground water and caused a severe threat to the environment biodiversity and public health.<sup>83</sup> Whereas, earthworms are very important components of soil zone. In various, food chain they are abundantly distributed and act as a key organism for determining soil function and health.<sup>84</sup>

Earthworms have highly tendency of bioaccumulation of heavy metals such as cadmium, mercury, lead, copper, manganese, calcium and zinc into their tissues without affecting their physiology and this particularly when the metals are mostly non-bioavailable.<sup>85</sup> The presence of excess heavy metals in the soil is cause lead to increased mortality of the worms.<sup>86-88</sup>

If the organic material are present in soil is low, so that earthworms was unable to digest the soil and, as a result, the consistency of toxicity of cadmium increases in their body, and the mortality rate as well as disorder in reproduction system rised.<sup>89</sup> Toxic metal-induced oxidative stress including lysosomal membrane stability and also altered their genetic expressions.<sup>90</sup>

Heavy metal ions-induced DNA damage, DNA repair system impairment and as well as alteration in coelomocytes frequency in earthworms *Eisenia Andrei*.<sup>91</sup> *Metaphire posthuma*, an endogeic earthworm, cause encounters a substantial range of physiological stress due to exposure of metals like cadmium, chromium, lead and mercury which are abundantly present in the soil of industrial and agricultural regions of India. The bioaccumulation of heavy metals was occurred in the yellow cells of body tissues of earthworms. Mercury is

more toxic for earthworm species because it effects cocoon production, coelomocytes, body weight and their length as well. Heavy metals may cause damage to an organ, inhibition of an enzymatic activities and significant alteration in their various metabolic activities.

The effects of sub-lethal concentrations of lead nitrate are shown on reproduction and growth rate of *Perionyx excavates*.<sup>92</sup> Earthworm populations was having tendency of mechanism by which they are able to tolerate or resist the negative effect of metal induced stress. Such tolerance levels of earthworms are acquired by through their variation in their genetic structure or changes in their physiology patterns. The bioaccumulation of methyl-mercury in the *Eisenia fetida* and its effect on regeneration after excision of the caudal end.<sup>93</sup>

#### Effect of heavy metals on other organisms

The presence of very high poisoning level of heavy metals causes negative impact on the environment as well as aspects of life. <sup>94-95</sup>. There are about 13 heavy metals which are listed as a hazardous environmental pollution such as As, Be, Cd, Cr, Sb, Cu, Hg, Ni, Pb, Se, Ag and Zn according to the U.S. Environmental Agency (USEPA). These heavy metals are very dangerous for human health and can show continuity throughout life in the neighborhood. The individual those who are exposed to contaminants in their environment and show responses towards that in a measurable and predictable way, and these responses are observed at different level of biological organization.<sup>96</sup>

Among these short lists of heavy metals, some are carcinogenic in nature such as Cd, Ni and Cr. Accumulation of heavy metals had a wide range of toxicity, neurotoxicity and mutagenic or teratogenic.<sup>97</sup> Bioaccumulations of heavy metals are also entered in food crops and cause severe impact on human health and this problem is a great concern across worldwide.

The main reason behind is due to rapid rise in population growth and industrialization which cause excessive changes in land-use pattern and addition of sustained efforts are enhanced the agricultural productivity of limited areas for production of adequate quantities of food. Along with fertilizers and other sources these heavy metals enter into irrigation zone because they act as low-cost sources for production of food crops but caused

negative impact on health of organisms those who consume these food crops.

In India, due to use of long term wastewater irrigation shown contamination of food crops with heavy metals and causes severe health hazards.<sup>98-102</sup> Heavy metals are present in soil and transfer to crops via accumulation and these metal can further transferred to other media through the food chain. Contamination of Lead can cause adverse effect on mental growth, neurological and cardiovascular disease also occurs in humans as well as in children.<sup>103-105</sup> Excessive higher concentration of Arsenic in soil, food crops can cause cancer, dermal problem, respiratory complications, and many other diseases.<sup>104,106</sup> Contamination with Cd heavy metals in food crops and their impact on human health are also reported extensively.<sup>107</sup> When Zn concentration will occur higher in human body, it can disturb the immune system.<sup>104</sup> When humans consume vegetables which are contaminated with heavy metals, are more deleterious for health.<sup>108</sup> Heavy metals are usually entered the cells of food crops via metal transporters such as phyto-siderophores.<sup>109,39</sup> Toxicity level of metal and behavior in soil are influenced by soil pH, carbonate contents, and their level of contaminations.<sup>110</sup>

## CONCLUSION

From the findings of the present work and that of others, it can be clearly concluded that It is very well vermicomposting is the best way for remediation of soil from heavy metals like nikle, cadmium, boron and iron. It can also protect environmental damage through the implementation of earthworms. This vermiremediation process is an eco-friendly way. By accumulation of heavy metals into the body tissues of earthworms, they make soil free from metal contamination and improve soil quality. If earthworms are applied in in-situ application, they minimized the impact of heavy metals hazards and make the soil pollution free. But while application of earthworm, the problem will be reduced. An earthworm produces vermicompost from organic materials and improves soil fertility level. All different species of earthworm play very important role for bioaccumulation of heavy metals, but while accumulation process the earthworms are also affected in term of their survival growth and rate of reproduction. The present study will be helpful and told about the role of earthworms for accumulation of heavy

metals by biological means. Thus, earthworms can safely manage all municipal and industrial organic wastes and divert them from ending up in the landfills and makes the soil pollution free.

## ACKNOWLEDGEMENT

Authors are thankful to Prof. Ajay Singh, Head, Department of Zoology, DDU, Gorakhpur, U.P, India.

## REFERENCES

1. **Schwartzkopf, C. 1972.** Potassium, calcium, magnesium-how they relate to plant growth. *Usga Green Section Record.* 1-2
2. **Bhalerao, S.A., Sharma, A.S., Poojari, A.C. 2015.** Toxicity of nickel in plants. *Int. J. Pure App. Biosci.* **3(2):** 345-355.
3. **Brittenham, G.M. 2004.** Safety of flour fortification with iron.
4. **Silva, J.A., Evensen, C.I., Bowen, R.L., Kirby, R., Tsuji, G.Y., Yost, R.S. 2000.** Managing fertilizer nutrients to protect the environment and human health. *Plant Nutrient Management in Hawaii's Soils. Approaches for Tropical and Subtropical Agriculture.* 7-23.
5. **Tomovi-Petrovi S., Markovi, V., Slavicazec. 2002.** The Effect of boron on the amount and type of carbides in chromium white irons. *J. Serb. Chem. Soc.,* **67(10):** 697-707.
6. **Bakirdere, S., Örenay, S., Korkmaz, M. 2010.** Effect of boron on human health. *The Open Mineral Processing Journal.* **3:** 54-59.
7. **Chen, T.-B., Zheng, Y.-M., Lei, M., Huang, Z.-C., Wu, H.-T., Chen, H., Fan, K.-K., Yu, K., Wu, X., Tian, Q.-Z. 2005.** Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. *Chemosphere.* **60:** 542-551
8. **Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D., Raskin, I. 1997.** Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology.* **31:** 860-865

9. **Wei, B.G., Yang, L.S., 2010.** A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchem. J.* **94**: 99-107.
10. **Xu, Y., Wu, Y., Han, J., Li, P., 2017.** The current status of heavy metal in Lake Sediments from China: pollution and ecological risk assessment. *Ecol. Evol.* **7**:5454–5466.
11. **Zhao, F., Ma, Y., Zhu, Y., Tang, Z., McGrath, S.P., 2015.** Soil contamination in China: current status and mitigation strategies. *Environ. Sci. Technol.* **49**:750-759.
12. **CPCB. 2000.** Management of Municipal Solid Waste, Delhi: Central Pollution Control Board.
13. **Chattopadhyay S, Dutta A, and Ray S. 2009.** Municipal solid waste management in Kolkata, India -A review. *Waste Manage.* **29**:1449–1458.
14. **Pattnaik, S. and Reddy, M. V. 2010.** Assessment of Municipal solid waste management in Puducherry (Pondicherry), India. *Resour Conserv. Recy.* **54**: 512-520
15. **Smith R. 2009.** A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* **35**:142-156.
16. **Reddy MV, Pattnaik S. 2009.** Vermi-composting of Municipal (Organic) Solid Waste and its implications. In: Singh, S.M. (Ed.), *Earthworm ecology and environment*. International Book Distributing Co., Lucknow, India, pp. 119-113.
17. **M. Barbieri. 2016.** “The importance of enrichment factor (EF) and geo accumulation index (Igeo) to evaluate the soil contamination,” *Journal of Geology & Geophysics.* **5(1)**: 237.
18. **D. V. Nica, M. Bura, I. Gergen, M. Harmanescu, and D.-M. Bordean, 2012.** “Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain,” *Chemistry Central Journal.* **6(1)**: 55.
19. **S. A. M. A. S. Eqani, R. Khalid, N. Bostan et al. 2016.** “Human lead (Pb) exposure via dust from different land use settings of Pakistan: a case study from two urban mountainous cities,” *Chemosphere.* **155**: 259–265.
20. **Suthar S, Singh S, Dhawan S. 2008.** Earthworm as bioindicators of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological categories. *Ecol. Eng.* **32**: 99-107.
21. **Brown, G.G, Edwards, C.A., Brussaard, L. 2004.** How earthworms affect plant growth: burrowing into the mechanisms. *Earthworm Ecol.* **2(1)**:13-49.
22. **Vijver, M.G., Wolterbeek, H.T., Vink, J.P.M., van Gestel, C.A.M. 2005.** Surface adsorption of metals onto the earthworm *Lumbricus rubellus* and the isopod *Porcellio scaber* is negligible compared to absorption in the body. *Sci. Total Environ.* **340**:271-280.
23. **Pérès, G., Vandenbulcke, F., Guernion, M., Hedde, M., Beguiristain, T., Douay, F., Houot, S., Piron, D., Richard, A., Bispo, A., Grand, C., Galsomies, L., Cluzeau, D., 2011.** Earthworm indicators as tools for soil monitoring, characterization and risk assessment. An example from the national Bioindicator programme (France). *Pedobiologia.* **54**:S77-S87.
24. **Li L, Xu, Z., Wu, J. and Tian, G. 2010.** Bioaccumulation of heavy metals in the earthworm *Eisenia fetida* in relation to bioavailable metal concentrations in pig manure. *Biores. Technol.* **101**:330-3436.
25. **Nannoni, F., Rossi, S., Protano, G. 2014.** Soil properties and metal accumulation by earthworms in the Siena urban area (Italy). *Appl. Soil Ecol.* **77**:9-17.
26. **Nahmani, J., Hodson, M.E., Black, S. 2007.** A review of studies performed to assess metal uptake by earthworms. *Environ. Pollut.* **145**: 402-424.
27. **Holmstrup, M., Sørensen, J.G., Overgaard, J., Bayley, M., Bindsbøl, A., Slotsbo, S., Fisker, K.V., Maraldo, K., Waagner, D., Labouriau, R., and Asmund, G. 2011.** Body metal concentrations and glycogen reserves in earthworms (*Dendrobaena octaedra*)

- from contaminated and uncontaminated forest soil. *Environ. Pollut.* **159**: 190–197.
28. Luo, W., Verweij, R.A., van Gestel, C.A.M. 2014. Determining the bioavailability and toxicity of lead contamination to earthworms requires using a combination of physicochemical and biological methods. *Environ. Pollut.* **185**:1–9.
29. Demuyneck, S., Succiu, I.R., Grumiaux, F., Douay, F., and Leprêtre, A. 2014. Effects of field metal-contaminated soils submitted to phytostabilisation and fly ash-aided phytostabilisation on the avoidance behaviour of the earthworm *Eisenia fetida*. *Ecotoxicol. Environ. Saf.* **107**: 170–177.
30. Sivakumar, S. 2015. Effects of metals on earthworm life cycles: a review. *Environ. Monit. Assess.* **187**:530.
31. Nirola, R., Megharaj, M., Saint, C., Aryal, R., Thavamani, P., Venkateswarlu, K., Naidu, R., Beecham, S. 2016. Metal bioavailability to *Eisenia fetida* through copper mine dwelling animal and plant litter, a new challenge on contaminated environment remediation. *Intern. Biodeterior. Biodegrad.* **113**: 208–216.
32. Bhuvaneshwari, R, A.Paneer, S S. Srimurali, K. Padmanaban, R. B. Rajendran. 2016. Human and Ecological Risk evaluation of Toxic Metals in the Water and Sediment of River Cauvery. *International Journal of Scientific and Research Publications.* **6**(3).
33. UN-HABITAT. 2004. The State of the World's Cities: Globalization and Urban Culture. UN-HABITAT, Human Settlements Programme, Nairobi.
34. Gall, J.E., Boyd, R.S., Rajakaruna, N. 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environ. Monit. Assess.* **187**:201.
35. ATSDR. 2007. Toxicological Profile for Barium. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.
36. Xiong, T., Austruy, A., Pierart, A., Shahid, M. 2016a. Kinetic study of phytotoxicity induced by foliar lead uptake for vegetables exposed to fine particles and implications for sustainable urban agriculture. *J. Environ. Sci.* 1–12.
37. Xiong, T., Dumat, C., Pierart, A., Shahid, M., Kang, Y., Li, N., Bertoni, G., Laplanche, C. 2016b. Measurement of metal bioaccessibility in vegetables to improve human exposure assessments: field study of soil–plant–atmosphere transfers in urban areas, South China. *Environ. Geochem. Health.* **38**(6): 1283–1301.
38. Khalid, Sana, Shahid, Muhammad, Niazi, Nabeel Khan, Murtaza, Behzad, Bibi, Irshad, Dumat, Camille. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Explor.* **182** (B):247–268.
39. Rai, P.K. 2018a. Phytoremediation of Emerging Contaminants in Wetlands. CRC Press, Taylor & Francis, Boca Raton, Florida, USA, pp. 248.
40. Pan XD, Tang J, Chen Q, Wu PG, Han JL. 2013. Evaluation of direct sampling method for trace elements analysis in Chinese rice wine by ICP-OES. *Eur Food Res Technol.* **236**:531-535
41. Benitez, E., M. Romero, M. Gomez, F. Gallardo-Lara and R. Nogales. 2001. Biosolid and biosolid ash as sources of heavy metals in plant-soil system. *Water Air Soil Pollut.* **132**:75-87.
42. Garg, A., Funke, S., Janisch, D. 1988. One-handed dynamic pulling strength with special application to lawn mowers. *Ergonomics* **31**:1139-1153.
43. Suthar S. 2006. Potential utilization of guar gum industrial waste in vermicompost production. *Bioresource Technology.* **97**:2474-2477
44. Albanell E, Plaixats J, Cabrero T.1988. Chemical change during vermicomposting (*Eisenia foetida*) of sheep manure mixed with cotton industrial waste. *Biology and Fertility of Soils.* **6**:266-269
45. Edwards CA, Bohlen PJ.1996. Biology and Ecology of Earthworm. 3rd ed. New York, London: Chapman and Hall. Dash, M.C. and Senapati, B.K. (1980). Cocoons morphology, hatching and emergence pattern in tropical earthworms. *Pedobiologia*, **20**: 317-324.

46. **Sinha, R.K., Sunil, H., Agarwal, S., Asadi, R. and Carretero, E. 2002.** Vermiculture technology for environmental management: study of action of earthworms *Eisenia fetida*, *Eudriluseuginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia. *The Environmentalist*. U.K., **22(2)**: 261-268.
47. **Brown, G. G., Callaham, M. A., Niva, C. C., Feijoo, A., Sautter, K. D., James, S. W., Schmelz, R. M. 2013.** Terrestrial oligochaete research in Latin America: The importance of the Latin American meetings on oligochaete ecology and taxonomy. *Applied Soil Ecology*. **69**:2–12.
48. **Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., & Vandenbulcke, F. 2014.** Pesticides and earthworms. A review. *Agronomy for Sustainable Development*. **34(1)**:199–228.
49. **Michaelsen, W. 1903.** Die geographische Verbreitung der Oligochaeten. Berlin.
50. **Bhatnagar, R.K. and Palta, R. 1996.** *Earthworm Vermiculture and Vermicomposting*. Kalyani Publishers, Ludhiana.
51. **Julka JM, Paliwal R, Kathireswari P. 2009.** Biodiversity of Indian earthworms-an overview. In: Edwards CA, Jayaraaj R, Jayaraaj IA, Achagam R(eds) Proceedings of Indo-US workshop on vermiculture technology in human welfare. Coimbatore, India, pp 36-56.
52. **Templeton, R. 1844.** Description of *Megascolex caeruleus*. Proceedings of Zoological Society, London. **12**:89-91.
53. **Perrier E. 1872.** Recherches pour Servir al historie des Lombriciensterrestres. Nouvelles annals du Museum d historienaturelle, Paris. **8**:5-198
54. **Morgan JE, Morgan AJ. 1999.** The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two ecologically contrasting earthworm species (*Lumbricus rubellus* and *Aporrectodea caliginosa*): implications foreco toxicological testing. *Appl. Soil Ecol*. **13**: 9-20.
55. **Pokarzhevskii, A.D., Zaboyev, G.N. Ganin and S.A. Gordienko. 1997.** Amino acids in earthworms: Are earthworm's ecosystemivorous? *Soil Boil. Biochem*. **29**:559-567.
56. **Sinha, R.K., G. Bharambe and U. Chaudhari. 2008.** Sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms: A low-cost sustainable technology over conventional systems with potential for decentralization. *Environmentalist*, **28**:409-428.
57. **Binet, F., L. Fayolle, M. Pussard, J.J. Crawford, S.J. Traina and O.H. Tuovinen. 1998.** Significance of earthworms in stimulating soil microbial activity. *Biol. Fertil. Soils*. **27**:79-84.
58. **Hobbelen, P.H.F., Koolhaas, J.E., Van Gestel, C.A.M. 2006.** Bioaccumulation of heavymetals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. *Environ. Pollut*. **144(2)**: 639–646
59. **Morgan, A.J., Stürzenbaum, S.R., Winters, C., Grime, G.W., Aziz, N.A.A., Kille, P. 2004.** Differential metallothionein expression in earthworm (*Lumbricus rubellus*) tissues. *Ecotoxicol. Environ. Safe* **57**:11–19.
60. **Motalib, A., Rida, M.A., Bouché, M.B. 1997.** Heavy metallinkages with mineral, organic, organic and living soil compartments. *Soil Biol. Biochem*. **29(3,4)**: 649–655.
61. **Bouché, M.B. 1977.** Strategies Lombriciennes. *Ecol. Bull*. **25**:122–132.
62. **Tripathi G, Bhardwaj P. 2004.** Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). *Bioresource Technology*. **92**:275-278
63. **Paliwal, R. and Julka, J.M. 2005.** Checklist of earthworms of Western Himalayas, India. *Zoos. Print. J*. **20(9)**: 1972-1976.
64. **Najar, I.A. and Khan, A.B. 2011.** New record of the earthworm *Eisenia fetida* (Savigny, 1826) from Kashmir valley, Jammu and Kashmir, India. *The BioScan*. **6(1)**:143-145.

65. **R. Panday, P. S. Bhatt, T. Bhattarai, K. Shakya and Lakshmaiah. 2016.** “Aldehyde dehydrogenase expression”. *BMC Res Notes*. **9**:491.
66. **S. Singh, J. Singha, P. V. Adarsh. 2016.** “Earthworm as ecological engineers to change the physico-chemical properties of soil: Soil vs vermicast”. *Ecological Eng.* **90**: 1–5.
67. **W. Budijastuti, S. Hariyanto, and A. Soegianto. 2017.** The specific species pattern of earthworms in contaminated area with heavy metals. *Am. Pub. Scie.* Vol. **2**.
68. **Kumar, Y. and Singh, K. 2013.** Distribution of earthworm in different block of Gorakhpur district in eastern Uttar Pradesh. *World Applied Science Journal*. **21(9)**: 1379-1385.
69. **Perel, T.S. 1997.** The Earthworms of the Fauna of Russia. Nauka, Moscow. pp.97.
70. **Dash, A.K. and Patra, U.C. 1977.** Density, biomass and energy budget of a tropical earthworm population from a grassland site in Orissa, India. *Rev. Eco. Bio. Soil*, **14**: 461-471.
71. **Kale, R.D. and Krishnamoorthy, R.V. 1982.** Cyclic fluctuations and distribution of three species of tropical earthworms in a farmyard garden in Bangalore. *Rev. d Ecologie et de Biol. Sol.*, **19**: 67-71.
72. **Reddy, M.V., Kumar, V.P.K. and Balashouri, P. 1997.** Responses of earthworm abundance and production of surface casts and their physico-chemical properties to soil management in relation to those of an undisturbed area on a semi-arid tropical alfisol. *Soil Bio. Biochem.* **29**: 617-620.
73. **Karmegam, N. and T. Daniel., 2007.** Effect of physicochemical parameters on earthworm abundance: A quantitative approach. *J. App. Sci. Res.*, **3**: 1369-1376.
74. **Stephenson, J. 1930.** *The Oligochaeta*. Clarendon Press, Oxford, UK. Pp. 978.
75. **Ranganathan, L.S. 2006.** Vermicomposting from soil health to human health. *Agrobios (India)*, pp. 49.
76. **Cooper, Edwin 2009.** New Enzymes Isolated from Earthworms is Potent Fibrinolytic; ACAM Integrative Medicine Blog; *Oxford University Press Journal (UK)*.
77. **Wang, Y., Buermann, W., Stenberg, P., Smolander, H., Ha me, T., Tian, Y., Hu, J., Knyazikhin, Y. and Myneni, R.B. 2003.** Hyperspectral remote sensing of vegetation canopy: Leaf area index and foliage optical properties. *Remote Sensing Environ.* **85**: 304-315.
78. **Ismail, S.A. 1997.** *Vermicology: The Biology of Earthworms*. Orient Longman, Hyderabad, pp. 92.
79. **Reinecke A J & Venter J M.1987.** Moisture preferences, growth and reproduction of the compost worm *Eisenia foetida* (Oligochaeta), *Biol. Fertil. Soils*, **3**: 135-141.
80. **Edwards, C.A. 1988.** Breakdown of animal, vegetable and industrial organic wastes by earthworms; In C.A. Edward, E.F. Neuhauser (ed). ‘Earthworms in Waste and Environmental Management’; pp. 21- 32; SPB Academic Publishing, The Hague, The Netherlands; ISBN 90-5103-017-7
81. **Dominguez, J. and Edwards, C.A. 1997.** Effects of stocking rate and moisture content on the growth and maturation of *Eisenia andrei* (Oligochaeta) in pig manure. *Soil Bio. Bioch.* **29**: 743-746.
82. **Yadav, A. and Garg, V.K. 2011.** Industrial wastes and sludges management by vermicomposting. *Rev. Environ. Sci. Biotechnol.*, **10**: 243-276.
83. **Ekperusi, O.A. and Aigbodion, I.F. 2015.** Bioremediation of heavy metals and petroleum hydrocarbons in diesel contaminated soil with the earthworm: *Eudrilus eugeniae*, *Springer Plus*, **4**: 540
84. **Edwards, C.A. and Arancon, N.Q. 2006.** The science of vermiculture: the use of earthworms in organic waste management, Soil Ecology, Laboratory, The Ohio State University, Columbus, Ohio, U.S.A. 1-25pp
85. **Hartenstein, R., Neuhauser, E.F., Collier, J. 1980.** Accumulation of heavy metals in the earthworm *Eisenia foetida*. *J. Environ. Qual.* **9**: 23-26

86. Spurgeon, D.J., Hopkin, S.P. 1995. Extrapolation of the laboratory-based OECD earthworm toxicity test to metal-contaminated field sites. *Ecotoxicology*. **4**: 190–205.
87. Haghparast, R. J., Golchin, A., Kahneh, E. 2013. Effect of different cadmium concentrations on growth of *Eisenia fetida* in a calcareous soil. *Journal of Water and Soil*. **27**: 24-35
88. Jamshidi, Z., Golchin, A. 2013. The effect of different levels of chromium and exposure time on growth parameters of earthworms. *KAUMS Journal (FEYZ)*. **16**: 625-626.
89. Irizar, A., Rodríguez, M., Izquierdo, A., Cancio, I., Marigómez, I., Soto, M. 2015. Effects of soil organic matter content on cadmium toxicity in *Eisenia fetida*: Implications for the use of biomarkers and standard toxicity tests. *Archives of Environmental Contamination and Toxicology*. **68**: 181-192
90. VanGestel CA, Koolhaas JE, Hamers T, van Hoppe M, van Roover M, Korsman C, Reinecke SA. 2008. Effects of metal pollution on earthworm communities in a contaminated flood plain area: Linking biomarker, community and functional responses. *Environmental Pollution*. **157**: 895-903.
91. Lourenco J, Pereira R, Goncalves F, Mendo S. 2013. Metals bioaccumulation, genotoxicity and gene expression in the European wood mouse (*Apodemus sylvaticus*) inhabiting an abandoned uranium mining area. *Sci Total Environ*. **443**:673-680
92. Maboeta MS, Reinecke AJ, Reinecke SA. 1999. Effects of low levels of lead on growth and reproduction of the Asian earthworm *Perionyx excavatus* (Oligochaeta). *Ecotoxicology and Environmental Safety*. **44**:236-240
93. Beyer WN, Cromartie E, Moment GB. 1985. Accumulation of Methylmercury in the earthworm, *Eisenia fetida*, and its effect on regeneration. *Bulletin of Environmental Contamination and Toxicology*. **35**: 157-162
94. Kavehei A, Hose GC and DB. 2017. *Environmental Pollution* **3**:1-7
95. Novotny V. 1995. *Diffuse Sources of Pollution by Toxic Metals and Impact on Receiving Waters* ed R Allan, U Forstner and W Salmons (Springer) 34-64
96. Bickham J, Sandhu S, Hebert P, Chikhi L, Athwal R. 2000. Effects of chemical contaminants on genetic diversity in natural populations: Implications for biomonitoring and ecotoxicology. *Mutation Research*. **463**: 33-51.
97. Duruibe, J.O., Ogwuegbu, M.O.C., Ekwurugwu, J.N. 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*. **2**: 112-118.
98. Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. *Agric. Ecosyst. Environ*. **109**: 310–322.
99. Ghosh, A.K., Bhatt, M.A., Agrawal, H.P. 2012. Effect of long-term application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. *Environ. Monit. Assess*. **184**: 1025–1036.
100. Garg, V.K., et al. 2014. Heavy metals bioconcentration from soil to vegetables and assessment of health risk caused by their ingestion. *Biol. Trace Elem. Res*. **157(3)**: 256–265.
101. Saha, S., et al. 2015. Assessment of heavy metals contamination in different crops grown in long-term sewage-irrigated areas of Kolkata, West Bengal, India. *Environ. Monit. Assess*. **187**: 4087.
102. Chabukdhara, M., Munjal, A., Nema, A.K., Gupta, S.K., Kaushal, R.K., 2016. Heavy metal contamination in vegetables grown around peri-urban and urban-industrial clusters in Ghaziabad, India. *Hum. Ecol. Risk Assess*. **22(3)**: 736–752.
103. Navas-Acien, A., Guallar, E., Silbergeld, E.K., Rothenberg, S.J. 2007. Lead exposure and cardiovascular disease: a systematic review. *Environ. Health Perspect*. **115**:472–482.
104. Zhou, H., et al. 2016. Accumulation of heavy metals in vegetable species planted in contaminated soils

- and the health risk assessment. *Int. J. Environ. Res. Public Health* **13(3)**: 289.
- 105. Al-Saleh, I., Al-Rouqi, R., Elkhatib, R., Abduljabbar, M., Al-Rajudi, T. 2017.** Risk assessment of environmental exposure to heavy metals in mothers and their respective infants. *Int. J. Hyg. Environ. Health.* **220**: 1252–1278.
- 106. Islam, S., Rahman, M.M., Rahman, M.A., Naidu, R. 2017.** Inorganic arsenic in rice and rice-based diets: health risk assessment. *Food Control.* **82**: 196–202.
- 107. Yang, Y., et al. 2018.** Assessing cadmium exposure risks of vegetables with plant uptake factor and soil property. *Environ. Pollut.* **238**: 263–269.
- 108. Shaheen, N., Irfan, N., Khan, I.N., Islam, S., Islam, M., Ahmed, M. 2016.** Presence of heavy metals in fruits and vegetables: health risk implications in Bangladesh. *Chemosphere.* **152**: 431–438.
- 109. Babula, P., Adam, V., et al. 2008.** Uncommon heavy metals, metalloids and their plant toxicity: a review. *Environ. Chem. Lett.* **6**: 189–213.
- 110. Waterlot, C., et al. 2017.** Impact of a phosphate amendment on the environmental availability and phyto availability of Cd and Pb in moderately and highly carbonated kitchen garden soils. *Pedosphere.* **27(3)**: 588–605.

\*\*\*