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# Earthworms: 'The Unheralded Soldiers' Standing Steadfast Against Metal Contamination

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## ABSTRACT
The increase in urbanization has led to an enhancement of pollution levels especially due to heavy metals which are a serious source of contamination throughout the world. Metals and metalloids are resultant of diffuse atmospheric fallouts of process particles. Contamination due to heavy metals can have a great impact on the functioning of soil ecosystem qualitatively and quantitatively by hampering the activities of soil fauna. Thus the importance of earthworms in metal pollution monitoring is widely recognized in terrestrial ecosystems. Earthworms have great potential for bioaccumulation of metals in their tissues and can be used as an ecological indicator of soil contamination. Species-specific metal accumulation pattern was observed in the study. In this review, the importance of earthworms as "ecosystem engineers" was portrayed through their different types of waste degrading abilities. The type of earthworm species, varieties of soil and different types of metals for which metal uptake and accumulation have been studied have been considered. A review of studies have been performed to assess the uptake of metals such as cadmium, copper, lead, zinc, etc by different species of earthworms. A detailed description of the mechanism involving the accumulation of earthworms were highlighted as per the studies done. A brief discussion of kinetics of metal accumulation was also laid importance. Thus the review brings these studies together in order to highlight the impact earthworms might have on metal mobility and availability in various contaminated sites.

**Key words:** Bioaccumulation, earthworms, kinetics, mechanism, metal uptake

## INTRODUCTION
Earthworms, the "Earth annelids", having super-streamlined and stripped down body are fairly highly evolved critters. Charles Darwin accentuated the role of earthworms in history of the world and also referred earthworms as "Nature ploughs" because of mixing of soil and organic matter. Earthworms (phylum Annelida, class Oligochaeta) are also called megadriles (or big worms), as opposed to the microdriles (or small worms) in the families *Tubificidae*, *Lumbriculidae* and *Enchytraeidae* and among others.

The importance of earthworms has been highlighted by several workers in the fields of waste management, environmental conservation, organic farming and sustainable agriculture (Bhawalkar, 1993; Ghatnekar *et al*., 1998; Senapati, 1992; Talashikar and Powar, 1998).

Earthworms promises to provide cheaper solutions to several social, economic and environmental problems plaguing the human society. Earthworms have proved themselves in decomposition of various waste substrates thereby reducing the problems of waste processing and management to a large extent. Application of earthworms in the breakdown of a wide range of organic residues including sewage sludge, animal wastes, crop residues and industrial refuse to produce vermicompost has been suggested (Dominguez and Edwards, 1997; Edwards and Lofty, 1972; Hartenstein and Beisesi, 1988; Van Gestel *et al.*, 1992).

Earthworms are one of the foremost components of soil communities and have ecological relevance in the formation and maintenance of soil structure. Earthworms play a significant role in soil ecosystem by affecting physical, chemical and biological properties of soil. Earthworms function as 'ecosystem engineers', directly and indirectly modifying the chemical, physical and biological properties of the soil and controlling ecosystem structure and functioning (Butenschoen *et al.*, 2009; Jones *et al.*, 1998; Lavelle, 1997). Earthworms are an essential part of soil fauna in most global soils. Their mixing of soil and organic matter improves the fertility of the soil by allowing the organic matter to be dispersed through the soil and nutrient to be held into becomes available to bacteria, fungi and plants. Earthworms restore and improve soil fertility by their secretions (growth hormones) and excreta (vermicast with beneficial soil microbes) and boost 'crop productivity'.

Total heavy element contents in soil are frequently used as a criterion for defining soil contamination. Metals have been shown to cause mortality (Fitzpatrick *et al.*, 1996; Neuhauser *et al.*, 1985; Spurgeon and Hopkin, 1995, 1996; Spurgeon *et al.*, 1994) and reduce fertility (Cikutovic *et al.*, 1993; Siekierska and Urbanska-Jasik, 2002), cocoon production (Ma, 1988; Spurgeon and Hopkin, 1996; Van Gestel *et al.*, 1992) and growth (Khalil *et al.*, 1996) of earthworms (Nahmani *et al.*, 2007). One of the most peculiar and special behavior of earthworms is to accumulate heavy metals in their tissues and gut. Earthworms can bio-accumulate and bio-transform many chemical contaminants including heavy metals and organic pollutants in soil and clean-up the contaminated lands for re-development. Earthworms are one of the best bioindicators of trace metals amongst soil invertebrates because they are able to accumulate metal ions in their body tissues (Elliott, 1997; Nahmani and Lavelle, 2002; Terhivuo *et al.*, 1994). These soil organisms can provide important information for assessing environmental risks and serve as useful biological indicators of contamination because of the fairly consistent correlation between the concentration of some contaminants in their tissues and those in soil. Mostly Earthworms are also often the subject of inoculation programmes during the restoration of degraded lands (Butt, 1999) and inoculation of earthworms to metal-contaminated soils has been suggested (Dickinson, 2000) largely due to the role earthworms are known to play in soil formation at such sites (Frouz *et al.*, 2006). Earthworms have great potential in risk assessment of contaminated land and acts as an indicator for ecosystem health (Nahmani *et al.*, 2007). Thus, earthworms being the dominant and dynamic macrofauna can do wonderful jobs for man and biosphere.

The purpose of this review is to bring together studies which focuses on earthworms inherent ability to accumulate heavy metals in their bodies and also on their nutrient enriching properties in soil by their composting abilities. This review emphasizes on: (1) Importance of earthworms in ecosystem (2) Their biology and ecology (3) Species associated with metal uptake, (4) Mechanism by which earthworms accumulate heavy metals and (5) A brief discussion about the kinetics of accumulation pattern.

## BIOLOGY AND ECOLOGY OF EARTHWORMS

Earthworms (Annelida, Oligochaeta) are relatively large detritivores (Sims and Gerard, 1985) as well as are soft-bodied, cylindrical, long, narrow, segmented, symmetrical organisms. Their body is dark brown, glistening, covered with soft cuticle. They generally ranges in weight from 1400-1500 mg after 8-10 weeks. The life-span of earthworms varies from 3-7 years depending upon the type of species and the ecological conditions prevailing there. Earthworm body contains 65% protein (70-80% high quality 'lysine-rich protein' on a dry weight basis), 14% fats, 14% carbohydrates and 3% ash (Gerard, 1960). They grow throughout their life and the number of segments continuously proliferates from a growing zone just in front of the anus (Hand, 1988; Sinha *et al.*, 2008).

Earthworms are burrowing in nature and forms tunnels by literally eating their way through the soil. Earthworms distribution in soil depends on factors like soil moisture, pH, availability of organic matter. They prefer to live in dark and moist places. Cattle dung, humus, kitchen waste and other organic materials are highly attractive sites for some species. Earthworms are very sensitive to touch, light and dryness. Worms can tolerate a temperature range between 5 and 29°C. Optimum temperature of 20-25°C and moisture content of 50-60% is optimum for earthworm function (Edwards and Bohlen, 1996; Hand, 1988; Sinha *et al.*, 2008).

## ROLE OF EARTHWORMS AS ECOSYSTEM ENGINEER'S

**Earthworms in vermicomposting:** Vermicomposting, utilizing earthworms, is an eco-biotechnological process that transforms energy rich and complex organic substances into a stabilized humus-like product (Benitez *et al.*, 2001). Vermicomposting (Latin vermes-worm) is a kindred process to composting, featuring the addition of certain species of earthworms used to enhance the process of waste conversion and produce a better end-product. Commonly used earthworms for vermicomposting are *Eisenia fetida*, *Perionyx excavatus*, *Lampito mauritii*, *Lumbricus rubellus*, *Lumbricus terrestris*, *Aporrectodea* and *Allolobophora*. Several ecological groups of earthworms are present: (1) Epigeic, (2) Endogeic, (3) Anecic (Bouche, 1977; Nei *et al.*, 2009):

- Epigeic earthworms (*Dendrobaena octaedra*; *Lumbricus rubellus*) feed on soil surface
- Anecic earthworms (*Lumbricus terrestris*) feed on plant material on the surface but they live in deep burrows in the soil
- Endogeic earthworms (*Aporrectodea caliginosa*; *Aporrectodea rosea*) digests the organic matter with soil microorganisms in the upper 30 cm mineral soil layer

The vermicomposting process includes two different phases involving the activity of earthworms (a) An active phase during which earthworms process wastes, thereby modifying their physical state and microbial composition (Lores *et al.*, 2006) and (b) A maturation-like phase marked by the displacement of the earthworms toward fresher layers of undigested waste, during which the microbes take over the decomposition of the earthworm-processed waste (Dominguez, 2004; Lazcano *et al.*, 2008) as shown in Fig. 1.

**Chemical and metal resistance in earthworms:** Earthworms are highly resistant to many chemical contaminants such as inorganic, organic pollutants in soil. Earthworms were even able

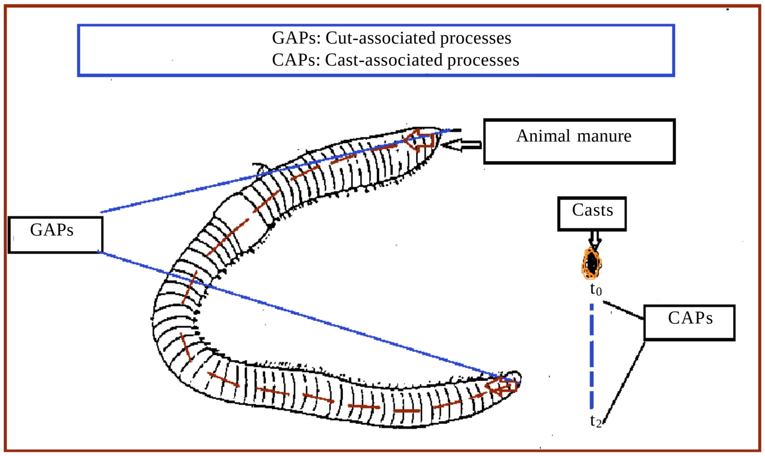


Fig. 1: Earthworms affect the decomposition of the animal manure during vermicomposting through ingestion, digestion and assimilation in the gut and then casting (gut-associated processes) and cast-associated processes which are more closely related with ageing processes (Source: Gomez-Brandon *et al*., 2013)

to survive the Seveso chemical plant explosion in 1976 in Italy, in which a large area inhabited by humans was contaminated with certain chemicals including the extremely toxic TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) due to which several fauna perished. Earthworm species which ingested TCDD contaminated soils were shown to accumulate dioxin in their tissues and concentrate it on an average of 14.5-fold (Satchell, 1983). They have the potential to replace the environmentally destructive chemical fertilizers from farm production thereby playing an important role in chemical element transformations (Lee, 1985).

Earthworms can degrade 'fly-ash' from the coal power plants which is considered as a 'hazardous waste' and poses serious disposal problem due to heavy metals content. Earthworms ingest the heavy metals from the contaminated site or fly-ash while converting them into vermicompost.

**Vermiremediation (Uptake of metals from contaminated sites by earthworms and immobilization):** Earthworms uptake metals from contaminated soil, fly-ash, slag, etc. through gut uptake. Earthworms accumulate heavy metals and other cations. Among the heavy metals Cd, Cu, Ni, Pb and Zn have been identified to be the most toxic to a wide range of earthworm species (Abdul Rida and Bouche, 1995; Morgan and Morgan, 1999; Spurgeon and Hopkin, 2000). Thus earthworms are known to be potential bioaccumulators of heavy metals and therefore they have been successfully demonstrated in mitigating the toxicity of industrial and municipal waste by vermicomposting technology.

A number of mechanisms are followed by the earthworms for uptake, immobilization and excretion of other metals (Sinha *et al*., 2008). They either biotransform or biodegrade the chemical contaminants turning them harmless in their bodies. The biotransformations and biodegradation takes place in the gut before entering of the metals in their tissues. All the contaminants do not follow the path of the gastro-intestinal tract. Some of the contaminants are excreted directly as casts via gut. Those entering the gut can be metabolized, immobilized and excreted or sequestered in tissues or vacuoles. Vermiremediation may prove a very cost-effective and environmentally sustainable way to treat polluted soils and sites contaminated with hydrocarbons.

**Earthworms as metal accumulators:** Earthworms are numerous large bodied individuals, resistant enough and sensitive enough to contaminants which make them good bioindicators. They are important micro-organisms in terms of soil functionality (Brown *et al*., 2000; Lavelle and Spain, 2001) and consequently play a key role in terrestrial ecotoxicological risk assessment (Sheppard *et al*., 1997; Weeks *et al*., 2004). They are exposed by direct dermal contact with heavy metals in the soil solution or by ingestion of pore water, polluted food and/or soil particles (Lanno *et al*., 2004). Soluble metal concentrations are the best descriptors of bioaccumulation in earthworms (Peijnenburg *et al*., 1999; Spurgeon and Hopkin, 1996).

Earthworms are soft-bodied, soil-dwelling organisms exposed to metals either through direct dermal contact with metals in soil solution or by ingestion of bulk soil or specific soil fractions (Lanno *et al*., 2004; Nei *et al*., 2009). According to Lanno *et al*. (2004) earthworms accumulate metals from soil either through direct dermal contact with chemicals in the soil solution or soil atmosphere, or else by ingestion of bulk soil or specific soil fractions. There studies suggest an important role of the gut uptake route (Morgan and Morgan, 1992). The main part is voided in casts containing particulate organic material and nutrients excreted, such as urine and mucopolysaccharides. These casts serve as habitat for microorganisms (Tiunov and Scheu, 2000) which mineralize the organic matter therein and release nutrients that contribute to plant nutrition. Various species of earthworms can tolerate and bio-accumulate high concentrations of heavy metals like cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn) in their tissues as shown in Table 1-4 without affecting their physiology and this particularly occurs when the metals are mostly non-bioavailable. Earthworms accumulate higher concentrations of (CF>10) of Zn (II) and Cd (II) ions and lower concentrations of Pb (II) and Cu (II) ions in their bodies. In earthworms, lead is accumulated in muscles, nerve cord, cerebral ganglion, seminal vesicles and chloragocytes. During the time of cocoon production, the lead of clitellar muscles may pass to cocoon or bio-available lead may enter the cocoon and disturbs the development of the embryo (Gupta *et al*., 2005).

**Mechanism of metal accumulation:** Earthworms can bio-accumulate and bio-transform many chemical contaminants including heavy metals and organic pollutants in soil and clean-up the contaminated lands for re-development. Their body work as a 'biofilter' and they can 'purify' and also 'disinfect' and 'detoxify' municipal and several industrial wastewater. The influence of metal-contaminated soils on earthworm activity and metal bioaccumulation has been reported many times (Morgan and Morgan, 1999). It has been shown that earthworms can rapidly invade remediated soil (Langdon *et al*., 2001; Spurgeon and Hopkin, 1999). They ingest soil particles and egest them as surface or subsurface casts. Aristotle called earthworms the "intestines of the earth". By ingesting organic debris, earthworms have been shown to enhance the bioavailability of soil nutrients such as Carbon (C), Nitrogen (N) and Phosphorous (P) (Devliegher and Verstraete, 1996). Morgan and Morgan (1990) and Morgan *et al*. (1989) have shown that the posterior alimentary canal of earthworms is a major site of metal accumulation, with the chloragogenous tissue separating the absorptive epithelium from the coelom being a major metal depository (Ireland and Richards, 1977; Morgan and Morgan, 1989a, b; Morgan and Morris, 1982; Richards and Ireland, 1978).

Table 1: Studies depicting metal uptake by *Eisenia fetida*

| Type of exposure | Time of exposure | Metals | Soil total content (mg kg$^{-1}$) | Measure in *Eisenia fetida* | References |
|---|---|---|---|---|---|
| Artificial soils (ASTM) +$CaSO_4$, $PbNO_3$, $ZnSO_4$ | 0 to 48 h | Zn | 0.078-66.1 | Metal content, pH, mortality | Conder and Lanno (2000) |
| | | Cd | 0.02-41.3 | | |
| | | Pb | 0.023-43.2 | | |
| 3 artificial soils OECD + sphagnum peat + $CaCO_3$ + Metal $NO_3$ | 1, 3, 7, 10, 14, 17, 24, 28, 35, 42 days | Zn | 20.4-1420 | Accumulation of heavy metals, excretion | Spurgeon and Hopkin (1999) |
| | | Cu | 1.8-115 | | |
| | | Cd | <0.5-13.7 | | |
| | | Pb | 7.95-656 | | |
| Artificial soil (OECD) + $CdSO_4$ | 35 days | Cd | 1500, 2000, 2500, 3000, 3500, 4000 | Metal content, survival $LC_{50}$ | Reinecke *et al.* (1999) |
| Artificial soil+copper oxychloride | 28 days | Cu | 1.66-372 | Metal content, weight survival LC50, cocoon production | Maboeta *et al.* (2004) |
| 5 contaminated soils from France | 21 and 31 days | Zn | 0.4-973 | Metal and protein content, enzyme activity | Grelle and Descamps (1998) |
| | | Cd | 0.5-13 | | |
| | | Pb | 5-798 | | |
| Soils from Joplin | 0, 12, 14, 24, 44, 144, 192 days | Pb | 1150-2800 | Metal content, uptake and excretion | Maenpaa *et al.* (2002) |
| | | Zn | 3500-4200 | | |
| | | Cd | 22-29 | | |
| Soils from Aberstywyth and Ystywyth | Field study | Zn | 116-142674 | Metal content in whole earthworm | Stafford and McGrath (1986) |
| | | Cu | 22.9-207 | | |
| | | Cd | 0.9-1617 | | |
| | | Pb | 75.9-22531 | | |
| 20 soils from the Netherlands + artificial soils (OECD) | 10 weeks | Zn | 0.08-47.55 | Metal content, initial body weight BCF | Janssen *et al.* (1997) |
| | | Cd | <0.001-0.44 | | |
| | | Pb | 0.34-4.09 | | |
| | | Cr | 0.06-3.76 | | |

OECD (1984)-Guidelines for testing of chemicals by the organization for Economic, Cooperation and development, ASTM: American society for testing and materials, $ZnSO_4$: Zinc sulphate, $CdSO_4$: Cadmium sulphate, $CaCO_3$: Calcium carbonate, $NO_3$: Nitrate, Pb: Lead, Zn, Zinc, Cd: Cadmium, Cr: Chromium; Ni-Nickel, As: Arsenic, Cu: Copper, BCF: Bioconcentration factor

The possibility that earthworm activity may raise heavy metal bioavailability is of considerable relevance for the success of soil remediation, especially when the methods that are used (i.e., soil washing, phytoextraction) remove only part of the (presumably labile and bioavailable) heavy metals, or heavy metals even remain in the soil immobilized by the addition of various chemicals (solidification/stabilization). It has been reported that, after treatment with earthworms, the distribution of heavy metals in soil fractions was changed significantly, presumably increasing their bioavailability (Cheng and Wong, 2002; Ma *et al.*, 2002; Wen *et al.*, 2004). Pokarzhevskii *et al.* (1997) showed that earthworms are ecosystemivorous feeding on entire soil microbial ecosystems. For terrestrial ‘soft-bodied organisms’ (such as earthworms), the concept of equilibrium partitioning (EqP) presumes a direct relationship between the tissue concentration, taken up through the derma and the free metal ion activity. Although, EqP is able to predict metal bioavailability under laboratory conditions, it is questionable that it does so under field conditions (Peijnenburg and Jager, 2003).

The capability of earthworms to effectively compartmentalize potentially toxic metals within tissues may provide an insight into the underlying mechanisms which enable the accumulation of

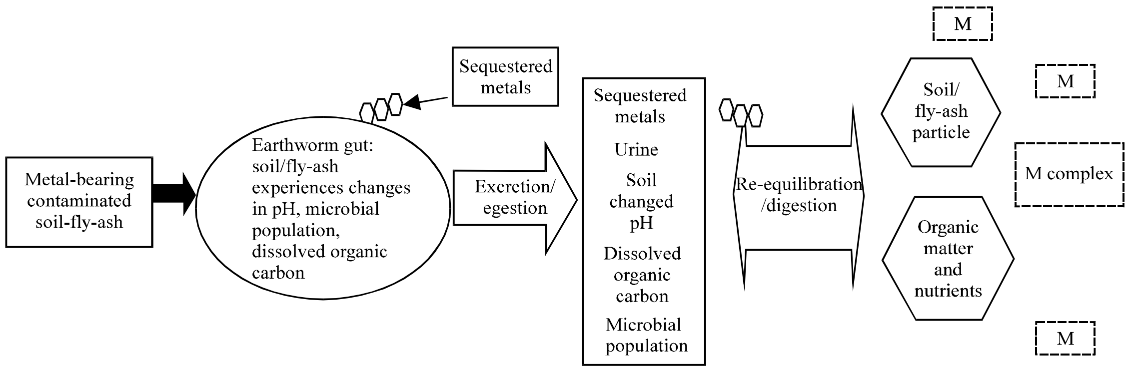


Fig. 2: Conceptual model of mechanism of metal uptake and their excretion by earthworms that are exposed to contaminated soil/fly-ash+organic matter. Contaminated soil/ fly-ash ingested moves through the gut and is finally egested. The egested soil may have a different pH, bacterial population and dissolved organic carbon content, all of which may modify soil/fly-ash/etc. Bacterial populations on modification may affect organic matter sorbed metals. pH and dissolved organic matter changes due to egestion of fly-ash and or excretion of mucus and urine may impact on sorbed metals. Some metals may be sequestered in earthworm tissues and subsequently excreted in a form different from the ingested metal. Adopted and modified by Sizmur and Hodson (2009)

high body burden surface soil dwellers) and anecic (deep burrowing). Similarly, gut-related processes in Earthworms may also increase metal availability as shown in Fig. 2. Metals taken up by earthworms in their gut are bounded by a protein called 'metallothioneins'. Ireland (1979) found that cadmium and lead are particularly concentrated in chloragogen cells in *Lumbricus terrestris* and *Dendrobaena rubidus*, where it bounds in the form of Cd-metallothioneins and Pb-metallothioneins. The chloragogen cells in earthworms appear to accumulate heavy metals absorbed by the gut and immobilize the metals in small spheroidal chloragosomes and vesicles found in these cells (Sinha *et al.*, 2008).

A suggested mechanism for an increase in the availability of metals is the stimulation of bacterial populations which enzymatically degrade organic matter, releasing the organically bound metals into solution (Rada *et al.*, 1996). An increase in the biomass of bacteria, actinomycetes and fungi have been found in the earthworm casts of soil where increase in the availability of metals to plants have been observed (Wen *et al.*, 2004). The ability of a microbial species to survive these processes depends on its ability to adapt to the conditions a particular earthworm may induce (Brown, 1995). Although, it is thought that earthworms do feed preferentially on fungal rich soil or substrate, there is also evidence to suggest that they do not gain nutrition selectively as Pokarzhevskii *et al.* (1997) showed that earthworms are ecosystemivorous feeding on entire soil microbial ecosystems. Earthworms excrete mucus and urine into the soil environment which are thought to increase microbial activity although this effect is not proportional to the size of the earthworm indicating the possibility that earthworms release other stimulating substances in addition to the mucus and urine (Binet *et al.*, 1998).

## UPTAKE PATTERNS AND ACCUMULATION AND EXCRETION

Cadmium, copper, lead and Zinc burdens are generally accumulated by the earthworms. Different time-dependent patterns of uptake are found for non-essential and essential elements.

Table 2: Studies depicting metal uptake by *Lumbricus rubellus*

| Type of exposure | Time of exposure | Metals | Soil total content (mg $kg^{-1}$) | Measure in *Lumbricus rubellus* | References |
|---|---|---|---|---|---|
| Soil samples from Broth, Cwmystwyth and Ystwyth (UK) | Field study | Zn | 100-992 | Metal content, BCF | Ireland (1979) |
| | | Ca | 998-32129 | | |
| | | Cu | 20-335 | | |
| | | Cd | 0.4-2 | | |
| | | Mn | 164-1330 | | |
| | | Pb | 42-1314 | | |
| Soil samples from different areas of braubach (Germany) | Field study | Pb | 284-1542 | Metal content, Histological examination | Janssen (1989), Kruse and Barrett (1985) |
| | | Cd | 0.08-1.29 | | |
| | | Cu | 10-17 | | |
| Agricultural soils from Cwmystwyth | At 10th day up to 90 days | Cd | 0.2-117 | Metal content, kinetic | Marino and Morgan (1999a) |
| | | Cu | 8-137.2 | | |
| | | Ca | 132-127600 | | |
| | | Pb | 3.9-6.4 | | |
| | | Zn | 17-25425 | | |
| 12 contaminated soils from UK | Field study | Cd | 0.1-350 | Metal content, weight | Morgan and Morgan (1988) |
| | | Cu | 26-2740 | | |
| | | Pb | 170-24600 | | |
| | | Zn | 160-45000 | | |
| Soil samples from 7 mines in UK | 90 days | Ca | 132-127600 | Metal content | Marino and Morgan (1999b) |
| | | Cd | 0.2-117 | | |
| | | Cu | 8-137.2 | | |
| | | Pb | 3.9-6.4 | | |
| | | Zn | 17-25425 | | |
| Contaminated soil from Waeeningen, | Field study | Zn | 10-1220 | Metal content, survival, cocoon production | Ma *et al.* (1983) |
| | | Pb | 14-430 | | |
| | | Cu | 1-130 | | |
| | | Cd | 0.1-5.7 | | |
| Agricultural soil samples from Wales | Field study | Zn | 460-1550 | Metal content | Morgan and Morgan (1992) |
| | | Cd | 2.7-14.7 | | |
| | | Cu | 23-62 | | |
| | | Pb | 570-10110 | | |
| Agricultural soils from Halkyn | Field study | Zn | 185-1870 | Metal content, pH | Morgan and Morgan (1999) |
| | | Cu | 21-60 | | |
| | | Pd | 158-10020 | | |

UK: United Kingdom, Ca: Calcium, Pb: Lead, Zn: Zinc, Cd: Cadmium, Cr: Chromium, Ni: Nickel, As: Arsenic, Cu: Copper, Mg: Magnesium, BCF: Bioconcentration factor

In case of essential elements, copper and zinc, equilibrium is reached within first seven days of exposure in all soils. Neuhauser *et al.* (1995) found similar patterns of uptake and excretion by *Eisenia fetida* exposed to these metals added singly to natural soils. Nannoni *et al.* (2011) could not observe any significant differences in terms of metal uptake and bioaccumulation between different species within a same ecological group. The metal bioavailability of earthworms can be evaluated in terms of relative toxicity (as lethality) index and through bioaccumulation determinations, yielding bioconcentration factors (BCF) and possibly tissue concentration limits (Abdul Rida and

Table 3: Studies of metal uptake by *Lumbricus terrestris*

| Type of exposure | Duration of exposure | Metals | Soil total content (mg kg$^{-1}$) | Measure in *Lumbricus terrestris* | References |
|---|---|---|---|---|---|
| Soil from South of France | Field study | Cd | 2.2-2.8 | Crop and faeces content, Metal content | Abdul Rida and Bouche (1995) |
| | | Zn | 360-489 | | |
| | | Cu | 43-84 | | |
| | | Pb | 145-1437 | | |
| | | Ni | 18-27 | | |
| | | Fe | 19965-30347 | | |
| | | Mn | 355-777 | | |
| Soil samples from Scotland and Yorkshire | | Cu, Cd, Pb | - | Metal content | Ash and Lee (1980) |
| Soil samples from Wales, England and Holland | Field study | Zn | 116-142674 | Metal content in whole earthworm | Stafford and McGrath (1986) |
| | | Cd | 0.9-1617 | | |
| | | Cu | 22.9-207 | | |
| | | Pb | 75.9-22531 | | |
| Soil samples from ashton and severnside | Field study | Cd | 1, 10 | Metal content | Wright and Stringer (1980) |
| | | Pb | 92,147 | | |
| | | Zn | 89, 617 | | |
| Soil samples from areas of Poland | Field study | Zn | 151-10154 | Metal content | Laszczyca *et al*. (2004) |
| | | Cd | 0.84-82 | | |
| | | Pb | 136-2635 | | |
| Soils samples from various areas of Russia | Field study | Mn | 20400 | Metal content | Van Straalen *et al*. (2001) |
| | | Zn | 276 | | |
| | | Ni | 43.1 | | |
| | | Pb | 54.9 | | |
| | | Cu | 58.6 | | |
| Soils from areas of Carrock fell, England | 5, 10, 15 days | Cu | 49-725 | Arsenic concentration | Langdon *et al*. (1999) |
| | | Zn | 48-1092 | | |
| | | As | <0.5-10.2 | | |

Ca: Calcium, Pb: Lead, Zn: Zinc, Cd: Cadmium, Cr: Chromium, Ni: Nickel, As: Arsenic, Cu: Copper, Mn: Manganese

Bouche, 1994). Heavy element fractionation among soil components represents one of the most significant factors influencing their mobility in soil and uptake by soil organisms. such as isopods, amphipods and earthworms (Becquer *et al*., 2005; Dai *et al*., 2004; Hobbelen *et al*., 2006; Lanno *et al*., 2004).

**Kinetics of accumulation of heavy metals:** According to authors, kinetic experiments indicate that during the uptake phase, certain metals such as Pb, Cd and Cu do not reach steady state in earthworms irrespective of exposure duration. Twenty-eight days is an appropriate duration for experiment with heavy metals that do not reach steady state. For elements in which plateau stage is already reached; 40 days is an appropriate duration. In studies involving the kinetics of uptake of heavy metals using species of earthworms-*Lumbricus rubellus*, *A. caliginosa* or *E. fetida*/*E. andrei* has been shown in Table 5. Radiotracers are generally used to follow the uptake and excretion of metals by the same earthworm (Crossley *et al*., 1995; Nahmani *et al*., 2007; Sheppard *et al*., 1997). Studies indicate that metal accumulation and excretion rates are species

Table 4: Studies showing metal uptake by *Aporrectodea* and *Allolobophora*

| Source of exposure | Duration of exposure | Metals | Soil total content (mg kg$^{-1}$) | Measure in *Aporrectodea caliginosa* | References |
|---|---|---|---|---|---|
| Soils from UK +$PbNO_3$ | 28 days | Pb | 296-6410 | Metal content, biomarker | Reid and Watson (2005) |
| Soils from areas of Galicia in Spain | Field study | Zn | 460-1550 | Metal content and Faeces content | Morgan and Morgan (1992) |
| | | Cu | 23-62 | | |
| | | Cd | 2.7-14.7 | | |
| | | Pb | 570-10110 | | |
| Soils from wales | Field study | Zn | 2250 | Metal content | Morgan and Morgan (1998) |
| | | Cd | 21 | | |
| | | Pb | 7130 | | |
| Soil from dinas powys and llanntrisant (Wales) | Field study | Zn | 193, 2034 | Metal content | Morgan and Morgan (1998) |
| | | Cu | 26, 32 | | |
| | | Pb | 166, 6930 | | |
| | | Cd | 0.9, 19 | | |
| Soil samples from Liantrisant, Halkyn, Dinas powys (Wales) | Field study | Zn | 185-1870 | Metal content, crop content, faeces content | Morgan and Morgan (1999) |
| | | Pb | 158-10020 | | |
| | | Cd | 0.8-16 | | |
| | | Cu | 21-60 | | |
| Soils from Belgium | Field study | Cu | 8.65-24.1 | Density of worms, metal content | Pizl and Josens (1995a) |
| | | Cd | 0.66-1.15 | | |
| | | Pb | 13.3-113.3 | | |
| | | Zn | 12.5-34.43 | | |
| Soils from areas of olkusz in Poland | Field study of 28 days | Zn | 151-10154 | Metal content | Pizl and Josens (1995b) |
| | | Cd | 0.84-82 | | |
| | | Pb | 296-6410 | | |

Ca: Calcium, Pb: Lead, Zn: Zinc, Cd: Cadmium, Cr: Chromium, Ni: Nickel, As: Arsenic, Cu: Copper and Mn: Manganese

dependent. Peijnenburg *et al.* (1999) studied about the rapid uptake and equilibration with Cr, Cu, Ni, Zn but little uptake of As, Cd, Pb, non-essential elements. Modelling of uptake rates is usually done involving the one compartment model of Atkins (1969), Marinussen *et al.* (1997), Peijnenburg *et al.* (1999) and Spurgeon and Hopkin (1999). Kinetics model assumes that an animal constitutes a homogeneous system with a constant excretion rate. The model has general equation:

$$Q_t = C_O + (a/k)\ (1\text{-}e^{kt})$$

where, $C_O$ is Concentration of residual metal in the animal, $Q_t$ is Concentration of metal in the animal, a is accumulation rate, k is excretion rate and t is time

Investigations are required to determine whether earthworm populations possess such genetically-determined tolerance mechanisms because their existence would have profound implications for biomonitoring and toxicity testing programmes. Indeed, the accumulation of high levels of potentially toxic metals, perhaps as a physiological expression of tolerance mechanisms, has an important and potentially far-reaching ecological implications, not least since it is evident that many toxicants are readily transferred from earthworms into biotic components higher in the food chain (Ma, 1987; Pankakoski *et al.*, 1993) thus posing a risk to the terrestrial food chain.

Table 5: Studies and the derived equations that predicts uptake of metals and excretion kinetics by earthworms

| Exposure | Species | Time of exposure | Model parameters | Equation results | References |
|---|---|---|---|---|---|
| Soil from Netherlands | *Dendrob-aena veneta* | 1, 2, 3, 7, 14, 28, 56, 112 days | $C_{cu(t)}$ = Cu concentration in the organism (mg $kg^{-1}$)<br>$C_{cu(0)}$ = Copper initial concentration in the organism (mg $kg^{-1}$)<br>$\alpha$cw = Cu uptake rate (mg $kg^{-1}$ $day^{-1}$)<br>kcu = Cu excretion rate ($day^{-1}$) | $C_{cu(t)}=C_{cu(0)} + \alpha cu/kcu\ [1-e^{-kcu(t)}]$ | Marinussen *et al.* (1997) |
| 20 samples from Dutch soils and 1 OECD | *Eisenia andrei* | 0-1-3-4-7-14-21 -28-42-63 days | $C_w$ = metal concentration in worms (mmol $kg^{-1}$ dry weight)<br>$C_{w(0)}$ = initial concentration in the organism<br>K1(x) = uptake rate constant<br>K1(x) = uptake constant rate (kg soil $kg^{-1}$ earthworm dry weight $day^{-1}$)<br>K2 = elimination rate constant ($day^{-1}$)<br>Cx = Metal concentration in soil (mmol $kg^{-1}$) | $Cw(t) = Cw(0)+(k_1Cs)/K_2$ | Peijnenburg *et al.* (1999) |
| OECD soil+$PbCl_2$ or $CdCl_2$ | *Eisenia fetida* | 7, 14, 21, 35, 42, 49, 56 days | Y = metal concentration in the worm at time x (mg $kg^{-1}$)<br>x = time(days)<br>C = constant<br>A = coefficient | For Pb = 100 and 2000 and Cd = 3 mg $kg^{-1}$<br>Y = C+Ax<br>For Cd = 80 mg $kg^{-1}$<br>$Y = C+A_1x + A_2x^2 + A_2e$ | Scaps *et al.* (1997) |
| Commercial potting soil | *Lumbricus terrestris* | 1, 3, 6, 10, 14, 20 days | A = Fraction of the radiotracer left in the gut subject to a gut clearance depuration rate constant ?g<br>1-A = remaining fraction subject to a slower depuration rate constant ?p<br>p = a rapid depuration rate | Depuration<br>$C = A(e^{?gt}) + (1-A)\ (e^{?pt})$<br>Uptake<br>$C = B[A(1-e^{?pt}) + (1-A)(e^{?pt})]$ | Sheppard *et al.* (1997) |
| ASTM soil + $Cd(NO_3)_2$ | *Eisenia fetida* | 2, 4, 7, 14 days | $C_t$ = Earthworm pellet fraction concentration (mmol metal $kg^{-1}$)<br>$M_s$= concentration of metal M in the soil (mmol $kg^{-1}$)<br>$K_u$ = the elimination rate constant ($day^{-1}$)<br>T = time (day) | $C_t = (K_u/K_e)M_s(1-e^{-ket})$ | Conder *et al.* (2002) |

OECD (2004), Guidelines for testing of chemicals by the organization for economic, Cooperation and development, $PbCl_2$-Lead chloride, $CdCl_2$: Cadmium chloride; $Cd(NO_3)_2$: Cadmium nitrate, ASTM: American society for testing and materials

## CONCLUSION

The increase in pollution levels in soil has lowered the quality of soil thus affecting crop productivity. Earthworms are crucial drivers of the remediation process as they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of contaminated soil and organic matter. The most important is the earthworms' potential of metal accumulation which has maintained the ecosystem in a balanced state. They by their metal accumulating and nutrient enriching abilities have done wonders in maintaining the soil ecosystem. Earthworms can survive in heavy-metal-contaminated soils and can even accumulate metals such as Cd, Cu, Zn and Pb and various other metals in their tissues. It has been reported in several studies that by following treatment with earthworms, the distribution of heavy metals in soil fractions was significantly changed. The increase in metal availability following gut-associated processes in earthworms has been reported by several authors. Earthworms further affect metal availability by grazing a particular functional group of soil microorganisms that play an important role in the cycling of metals. Moreover, earthworms ingest soil and various forms of biomass to produce vermicasts. Barring a few exceptions the vermicasts of most earthworm species are known to contain hormones and enzymes which stimulate plant growth and discourage pathogens.

Earthworms can safely manage all municipal and industrial organic wastes including sewage sludge and divert them from ending up in the landfills. Thus adopting this vermicomposting technology will not only prove greater availability of plant mineral nutrients but also promises more effective waste utilization for agricultural benefits by taking the advantage of increased microbial activities provided by earthworms. Moreover removal of heavy metals by biological means is more specific, eco-friendly and economical.

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