1. Introduction

The importance of biological processes in the management of industrial, municipal, domestic and agricultural organic wastes have been well recognised. Within the broad range of bioprocesses available for the reclamation of these wastes, vermitechnology has been identified as a successful technique. Biofertilisers, prepared from vermiculture have received wide acceptability all over the world because of its simple production techniques, high efficiency and environment friendliness.

Vermitechnology has been successfully used for treating effluents of several industries such as paper mills, soybean oil extraction plants, naphtha based organo-chemical plants, tanneries, sewage sludge, pesticides, hospital and surgical wastes, enzyme production units etc. In almost all the major cities, municipal wastes are treated with vermitechnology (Ghatnekar *et.al.*, 1998).

Environmental diagnostics which include the use of earthworms as bio-monitors would seem to offer a great prospect for the protection of human health and environment. In fact, earthworms have been used for pollution monitoring (Czarnowska and Zopkiewiez, 1978; Callahan, *et.al.*, 1985) and for reclaiming the open cast mining sites (Dunger, 1969; Stewart and Scollion, 1988).

It has been suggested that earthworms are excellent bio-indicators of the relative health of soil eco-system (Kuhle, 1983). As the earthworms are larger in size, more in numbers, easy to sample and identify, relatively immobile, always in contact with the substrate in which they live, they can be used as soil pollution monitoring tools (Edwards and Bohlen, 1996). The earthworms are also considered as terrestrial equivalent of aquatic filter feeders (Morgan *et. al.*, 1986). Periodical analysis of earthworm tissue may provide an excellent index of bio-availability of heavy metals in soil (Helmke *et. al.*, 1979). Earthworms host a novel suite of bio-markers that can be used as indicators of eco-toxicity, which includes bio-availability of chemicals, reproductive/developmental, neurological and immunological markers that can be interpreted as potential indicators of hazards to the organism other than earthworms (Venables *et.al.*, 1992).

Metals are integral parts of the soil matrix. In the soil, metals can exist in two forms. In the first, metals are strongly bonded to the soil matrix and are typical of the soil while in the second, metals are loosely bonded to the soil matrix either as weakly adsorbed or as organometallic complexes. This part of the metal is anthropogenic in nature and it is usually this part which when exceeds a certain limit, makes the soil polluted with respect to a particular metal. Oligochaeta being the major part of soil biota are the worst affected due to metallic pollution of soil. The first serious research on the role of "metals in the soil" on earthworm activity and vice-versa was reported in the middle of last century (Nielson, 1951). Thereafter considerable amount of work has been carried out on heavy metal accumulation, localisation, and distribution of heavy metals in the body and affinities of some of these metals to particular body parts of the earthworms. A number of interesting publications appeared during early sixties and mid seventies from J. A. van Rhee, who primarily dealt with the effect of earthworms on orchard soils (Rhee, 1963, 1967, 1975, 1977). His research on the copper toxicity in earthworms was most noteworthy wherein he inferred that long term use of copper fungicide in orchard soil resulted in complete removal of earthworm population, where copper level was more than 80 mg/kg (Rhee, 1963, 1967). In the middle of seventies, in a series of significant publications Ireland reported the accumulation pattern of some of the heavy metals in the earthworm tissues (Ireland, 1975a, 1975b, 1975c, 1976, 1979; Ireland and Wooton, 1976). For the first time he had drawn a correlation between Ca and Pb, Mn and Zn in the earthworm tissues (Ireland 1975b). Ireland further identified the earthworm tissues where heavy metals might get localised (Ireland, 1977a, 1978). He also concluded that the earthworm chloragosome functions as cation exchanger and the accumulation of Pb in chloragosome cells proceeds via Ca exchange (Ireland and Fischer, 1978). The work of Ireland and co-workers was path breaking in nature not only because they attempted the heavy metal accumulation in the earthworm tissues from a fundamental angle but because they set the trend of future research on heavy metal accumulation in the earthworms. The decade of eighties and nineties saw a distinct shift in the research of heavy metal accumulation in earthworms from conventional measurements of metals in earthworm tissues to molecular biology and genetic engineering. The most prominent group in this field was led by A. J. Morgan. The focus of the research was the calciferous glands and chloragosome tissues of the earthworms. The studies included morphological and electron microprobe study of the inorganic composition of the mineralised secretary products of the calciferous gland and chloragosome tissue of the earthworm *Lumbricus terrestris* (Morgan, 1981), the elemental composition of chloragosomes (Morgan, 1982; Morgan and Morgan 1989b), electron microprobe X-ray analysis of heavy metals in earthworm tissues (Morgan, 1984), Zn sequestration by earthworm chloragocytes (Morgan and Morgan, 1989a) and morphological and quantitative X-ray micro-analysis of cryosectioned chloragosome tissue (Morgan and Winters, 1982, 1991). Research of Morgan and co-workers also included, accumulation and compartmentation of Cd, Pb, Zn and Ca in *Dendrobaena rubida* and *L.terrestris* (Morgan and Morris, 1982), heavy metal accumulation as a function of species within and between trophic levels (Morgan *et.al.*, 1986), X-ray mapping of *in vivo* metal substitutions in metal sequestering subcellular compartments, earthworms as biological monitors of Cd, Cu, Pb and Zn in metalliferous soils (Morgan and Morgan, 1988), seasonal changes in the tissue metal concentrations in two ecophysiologically dissimilar earthworm species (Morgan and Morgan, 1993), use of earthworm indices for assessing soil metal pollution (Morgan *et.al.*, 1992), calcium-lead interactions in earthworms (Morris and Morgan, 1986), comparison of the distribution of Cd, Cu, Pb, Zn and Ca in the tissue of *L.rubellus* collected from un-contaminated and polluted sites (Morgan *and* Morgan, 1990), comparison of metal concentration in the two epigeic earthworm species, *L.rubellus* and *D.rubidus* living in same contaminated site (Morgan and Morgan, 1991)..

Late eighties saw a breakthrough in the research on accumulation of heavy metals in earthworms when cadmium binding proteins were isolated from posterior alimentary canal of *Dendrodrillus rubidus* and *L.rubellus* (Morgan *et.al.*, 1989). In a significant development Sturzenbaum and co-workers opined that by fingerprinting amplified m-RNA species by the technique of direct differential display, it was possible to pin point the genes responsible for making certain earthworms resistant to high level of heavy metals, well above the critical concentration (Sturzenbaum *et. al.*, 1998a).

It was interesting to note that from Nielson (1951) to Morgan (1999) the research on heavy metal accumulation in earthworms was mostly centred around only a few earthworm species, namely, *Aporrectodea caliginosa, D.rubida, D.veneta, L.rubellus, L.terrestris* and *Eisenia foetida*. Also, the focus of all these research had been the accumulation pattern of heavy metals in earthworm tissues and the responses generated therefrom. Except for some initial work by Ireland, very little attention was paid on the plant availability of heavy metals in the earthworm cast. Vermitechnology as a whole was also not so much considered as a potential tool for reclaming heavy metal contaminated solid wastes. It is understandable that in vermi experiments researchers justifiably prefer endemic species. Still it was surprising to note that metal accumulation research on earthworm species native to Indian subcontinent had been very little or practically non-existent though many of them were well known composting species.

Earthworm research in India till date has been confined to only ecology, energy budgeting, vermicomposting, nutrient cycling ability etc. in spite of the great promise it holds in the area of monitoring and abatement of metal pollution of soil. During a course of exploratory experiments at National Metallurgical Laboratory, Jamshedpur, it was observed that earthworms *Perionyx excavatus*, a native of India, Srilanka, New Zealand and Australia changed the plant availability of a number of heavy metals, namely, lead, manganese, zinc, cadmium, chromium and arsenic in contaminated soils. This phenomenon was investigated in greater detail and the findings of these investigations are recorded in the chapters as follows.

The aim of the present work was to understand the behavior of earthworm *P.excavatus* in soils contaminated with heavy metals like, Pb, Mn, Zn, Cd, Cr and As and explore the possibilities of vermitreatment for reclaiming heavy metal polluted solid waste sites. The objectives of the present work may be listed as follows.

- To study the growth of *P.excavatus* in soils contaminated with Pb(II), Mn(II), Zn(II), Cd(II), Cr(VI) and As(V).
- To ascertain the metal burden in contaminated earthworm tissues.
- To assess the soil parameters like, pH, organic carbon, total nitrogen, available phosphorous, potassium and heavy metals in the earthworm cast as a result of vermitreatment.
- To study the effect of pH and associated anions on *P.excavatus* in reference to metal accumulation in earthworm tissues.
- To study the synergistic effect of Pb, Mn and Zn on *P.excavatus*.
- To explore the possibility of vermitreatment in fly ash contaminated soils with *P.excavatus*

The organisation of the thesis has been done in the following manner. Besides the customary introduction, the thesis consists of six more chapters covering the entire range of objectives listed above. In addition to seven regulation chapters, the thesis contains concluding remarks and scope for further research and three appendices appendix that contain characterization of the cast of *P.excavatus*, a consolidated list of references and list of communicated, accepted and published papers.

Chapter 2 makes an extensive literature survey on the development of heavy metal accumulation research in earthworms. The Chapter briefly delves upon the history, evolution and dispersal of earthworms and the intimate relationship between the soil fertility and earthworms. Chapter 2 also make a categorical survey on Cd, Pb, Cu, Zn and a number of other heavy metals in relation to their accumulation in earthworm tissues. Materials and Methods adopted in the present study have been described in detail in Chapter 3. Role of *P.excavatus* in amending Pb, Mn and Zn contaminated soils has been studied and reported in Chapter 4A. Chapter 4A also deals with the synergistic effect of Pb, Mn and Zn on *P.excavatus*. Chapter 4B reports a similar study on *P.excavatus* in soils contaminated with Cd, Cr and As. Earthworms are in general quite sensitive towards soil pH. Response of *P.excavatus* towards varied soil pH has been studied and reported in Chapter 5. Chapter 5 also describes the effects of various anions on *P.excavatus* under identical cationic environment. Amendment of fly ash contaminated soils for agricultural purpose using *P.excavatus* has been described in Chapter 6.

Conversion of cow dung to cast as a result of vermitreatment is probably followed by some kind of mineralization, which was studied by chemical and XRD analyses of the cow dung and the cast, the results of which are given in Appendix-1. The literature base generated on heavy metal accumulation in earthworms in Chapter 2 has been arranged in a tabular form for ready reckoning in Appendix-2. Appendix-3 gives the list of communicated, accepted and published papers.