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# Bioaccumulation of zinc in earthworm *Perionyx sansibaricus* and its impacts on growth and metabolism

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**Abstract** : *Perionyx sansibaricus* one of the important earthworm species from vermicomposting viewpoint was treated with zinc chloride to study the impact of accumulation of zinc on growth and metabolism of the earthworm. Three different doses i.e. 50, 200 and 400 mg Zn $kg^{-1}$ of soil for three different duration 10, 20 and 30 days were studied to evaluate the impact along with a control experiment. No significant change was observed in the mass of *P. sansibaricus* after treatment of three different concentrations. The zinc concentration significantly ($P<0.01$) increased in the treated earthworms reaching a level of 88.4, 118.6 and 128.6 mg Zn $kg^{-1}$ dry weight at 50, 200 & 400 mg Zn $kg^{-1}$ Zn treated soil respectively. The bio-concentration factor (BCF) was estimated to be 2.43. The metabolism was found to increase at early stage of treatment and more at higher concentration. The paper deals with the details of accumulation of zinc and its effect.

**Key words:** Earthworm, bioaccumulation, growth, metabolism.

## Introduction

Earthworms are known to accumulate heavy metals from environment. In industrial and mining areas of Jharkhand where heavy metals are already present in soil in hazardous or near-hazardous concentrations (Sinha *et al.*,1990), the possibility exists for their further concentration (Lee, 1985). Zinc, an important micronutrient of soil, is vital for transformation of carbohydrates and regulate sugar in plants. The deficiency of Zn retards photosynthesis, nitrogen metabolism; flowering fruit development and growth period is prolonged resulting in delayed maturity. Zn acts as prosthetic group of metalo-enzymes in animals and plays important role in regulating vital activities. But when excess in body produces adverse effect as toxicant (Ireland and Richards, 1977; Ireland, 1983; Ma, 1982). The important source of Zn in soil is mining activity, industrial wastes, municipal refuse, sewage sludge, Zn-containing fertilizers and various insecticides & pesticides

(Petruzelli,1989). Because the earthworms constitute an important group of soil organisms sharing more than 70% of biomass and ingest large quantities of soil and decaying materials they are unusually susceptible to accumulation. The potential hazards of environmental pollutants to soil invertebrates are commonly assessed by "earthworm acute toxicity test" (Goats and Edwards, 1982). However, to arrive at a safe conclusion the acute toxicity test is not a sensitive parameter. The other parameters like bioaccumulation, growth, metabolism, reproduction and life cycle are said to be most sensitive and ecologically relevant predicting pollutional impact on field populations (Donker *et al.*, 1994). The review of literature reveals that many studies have been carried out on effect of heavy metals including Zinc on earthworm, mostly in temperate area (Ma, 1982; Malecki *et al.*, 1982; Molnar *et al.*, 1982; Ireland, 1983; Neuhauser *et al.*, 1984; Van Gestel *et al.*, 1993 and Spurgeon *et al.*, 1994), while very little information is available on tropical area. The present project has been taken to have information on accumulation of Zn, and its impact on growth and metabolism taking *Perionyx sansibaricus* a widely distributed and dominant earthworm in garbage and municipal waste rich area of Jharkhand region.

## Materials and methods

The soil used for the toxicity test, growth, accumulation and metabolism experiment had the following characteristics: Laterite type, sandy loam by texture, pH 6.9, organic matter 2.8%, N 0.22%, C/N ratio 12.7 and total Zn content 30.0 mg $kg^{-1}$.

Different age groups of earthworms were collected from a wet organically rich garbage site near university hostel. They were hand-sorted into three age groups on the basis of size and presence or absence of genital papillae and clitellum (juveniles, <2cm; immatures, $^{3}$2<4 cm; adults $^{3}$4 cm), and kept half immersed in glass Petri discs containing 30 ml of distilled water at $25 \pm 2$ °C for 24 hours to evacuate their gut contents (Dash and Patra, 1977). These gut-evacuated earthworms were used as the test specimens for various experiments. A toxicity test of each age group was first conducted at different concentrations of Zn as $ZnCl_2$-treated soil (50-1800 mg $kg^{-1}$) for 14 days. For each concentration and age group, four plastic containers filled with 1 kg treated soil were used. Ten healthy gut evacuated worms were added to each container. The experiment was maintained at $20 \pm 2\%$ (w/w) soil moisture and $22 \pm 3$ °C soil temperature. The percentage mortalities of earthworms were recorded and Finney's probit method (Finney, 1971) was followed to calculate $LC_{50}$ value for juvenile, immature and adult earthworms. Based on the results of the toxicity tests, three sub lethal concentrations of Zn were chosen (50, 200 and 400 mg $kg^{-1}$) to study its accumulation and effect on the growth and metabolism of *P. sansibaricus*. The highest concentration was chosen to mimic the level mostly found in contaminated agricultural soils (Mishra and Mani, 1991) and is very common in garbage site as well as industrial and mining areas of Jharkhand.

For the growth and accumulation experiment, 12 replicates were kept at each test concentration of Zn-treated soil (0, 50, 200 and 400 mg $kg^{-1}$) and five immature earthworms were inoculated into each replicate (containing 2 kg of soil) after being gut-evacuated and weighed. The experiment was conducted for 30 days and maintained at 20 ± 2% (w/w) soil moisture and 22 ± 3 °C soil temperature. After 10, 20 and 30 days, four replicates were removed from each test concentration. The worms were hand-sorted, then gut evacuated and weighed alive. The dry mass was determined after drying the worms at 85 °C for 48 hours (Dash and Patra, 1977). Growth was calculated as the percentage increase in initial body weight. Metabolism was determined on the basis of oxygen consumption. Zn concentrations in worms were analysed on 15th and 30th day of treatment along with control. The dried worms were digested in $HNO_3$ at a rate of 5 ml $HNO_3$ 100 $mg^{-1}$ (dry weight). After digestion, samples were diluted with distilled water up to a suitable volume, and concentrations of Zn were determined using atomic absorption spectroscopy (model no:- ECL AAS 4219 at D.K.V.K. at Ranchi).

For metabolism, experiments were also conducted in 0, 50, 200 and 400 mg $kg^{-1}$ Zn-applied soil. Four replicates were removed from each test concentration i.e. on 15th and 30th day and oxygen consumption estimated was converted in energetic value taking 4.8 KCal $l^{-1}$ of oxygen (Engelman, 1961). The bio concentration factor (BCF) was determined taking ratio of concentration of metal in earthworm and concentration in soil.

## Results

The $LC_{50}$ value, using different concentration of Zinc chloride, was determined by obtaining mortality percentage against exposures of different concentration of Zinc chloride (Fig.-1). The $LC_{50}$ values were estimated for three age groups of *Perionyx sansibaricus* i.e. juvenile, immature and mature (adult) and were found to be 805.12, 890.69 and 985.35 mg Zn $kg^{-1}$ soil respectively in their natural habitat condition. Three sub lethal concentrations i.e. 50 mg 200 mg & 400 mg $Kg^{-1}$ soil were used for experiments (Rosenthal and Alderice, 1976).

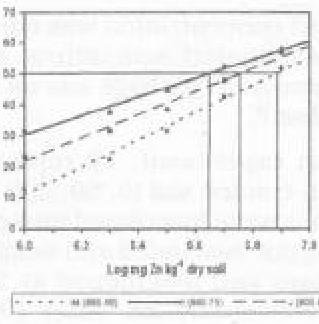


Fig. 1: Toxicity test (14-day) of *P. sansibaricus* for immature (I) Juvenile (J) and mature (M) Specimen.

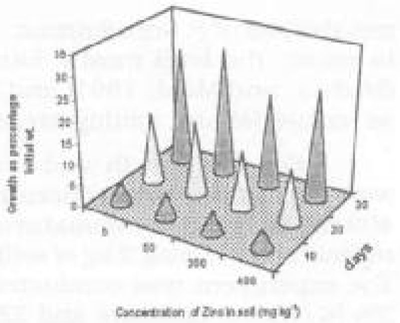


Fig. 2: Effect of different doses and duration of Zn treatment on growth of *P. sansibaricus*.

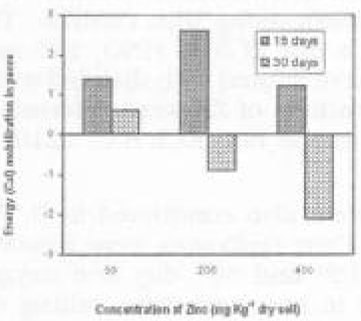


Fig. 3: Energy mobilization of *P. sansibaricus* at different doses and duration of Zn treatment.

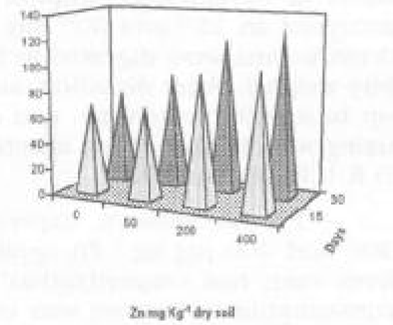


Fig.4: Accumulation of Zinc in *P. sansibaricus* at different doses and duration.

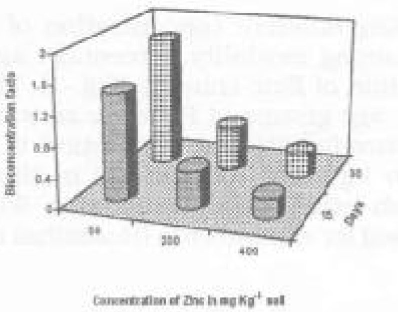


Fig. 5: Bioconcentration factors at different doses and duration of Zn treatment.

A positive as well as negative effect of Zn was observed on growth of P. sansibaricus. As shown in (Fig. 2), growth was stimulated and slightly enhanced at the lowest concentration i.e. 50 mg Zn $kg^{-1}$ soil level while lowered in comparison to control at 200 & 400 mg $kg^{-1}$ soil. The control showed 5.2, 16.5 and 27.5 per cent growth over initial state on $10^{th}$, $20^{th}$ and $30^{th}$ day of treatment while the exposed specimens at the lowest concentration (50 mg Zn $Kg^{-1}$ soil) show 5.7, 17.3 and 30.1% growth over initial state at the same intervals. At the exposure of 200 mg & 400 mg Zn $kg^{-1}$ soil level the growth was observed to be 5.2, 13.6, 21.8 and 4.9, 12.5 and 25.7 percent respectively at the 10, 20 and 30 days of interval. The growth pattern when analysed statistically revealed no significant differences ($P>0.05$) between control and various doses and duration of exposure. Oxygen consumption was taken as index of metabolism and control and treated specimens of P. sansibaricus were studied on $15^{th}$ & $30^{th}$ day for oxygen consumption. The consumed oxygen of untreated mature *P. sansibaricus* was estimated to be 337.25 $ml^{-1}g^{-1}$ dry wt $h^{-1}$which was converted for energy mobilization, which was 1.6188 Cal $g^{-1}$ dry wt $h^{-1}$. The treated specimen of *P. sansibaricus* on $15^{th}$ day of treatment showed 1.4, 3.6 and 1.2 percent high-energy mobilization over untreated at the three levels of treatment i.e. 50, 200 & 400 mg Zn $kg^{-1}$ soil. But on $30^{th}$ day an increase of 0.6% for 50 mg Zn $kg^{-1}$ soil while a decrease over untreated of the order of 0.9 and 2.1 percent was recorded for the rest two concentrations respectively (Fig. 3). Similar to growth value the increase or decrease of energy mobilization was not statistically significant ($P>0.05$). The accumulation of Zn was estimated to be 70.8, 98.8 and 103.7 mg $Kg^{-1}$ dry weight of *P. sansibaricus* on $15^{th}$ day of treatment at 50, 200 and 400 mg Zn $kg^{-1}$ concentrations in soil (Fig. 4). The bioconcentration factor at this level was found to be 1.416, 0.494 and 0.259. The bioconcentration of Zinc increased on $30^{th}$ day as 88.4, 118.6 and 128.6 mg $kg^{-1}$ dry weight of the earthworms corresponding to the three different treatments. Accordingly the BCF was found to be 1.768, 0.593 and 0.321 for 50, 200 and 400 mg Zn $kg^{-1}$ concentrations respectively (Fig. 5). The soil from where the samples were collected (garbage and municipal wastes site) had initial concentration of Zn $28.2 \pm 2.8$ while earthworms had $68.5 \pm 3.7$ mg Zn $kg^{-1}$ dry weight. On $15^{th}$ and $30^{th}$ day in the control samples the earthworm showed slight increase in accumulation as 69.6 and 70.5 mg $kg^{-1}$. The accumulation of Zn in treated earthworms over control did not show statistical significance ($P>0.05$) at different doses and duration of treatment.

## Discussion

A sublethal concentration of pollutant or toxicant is most likely to produce sublethal effects so as to alter the morphological, physiological, histological and/or ethological condition of the organism (Rosenthal and Alderice, 1976), though it may not cause immediate death of the individual, hence, more rationale than lethal or fatal or $LC_{50}$. Further more the sublethal dose mimics the hazardous condition of the habitat and allows the organism to survive for a longer period felicitating the study. Accordingly, three doses of sublethal concentrations of Zinc as 50, 200 and 400 mg $kg^{-1}$ soil have been opted for the present study while a 14-day $LC_{50}$ value for juvenile, immature and mature *Perionyx sansibaricus* was estimated to be 805.12, 890.69 and 985.35 mg $kg^{-1}$ of soil. Neuhauser *et al.* (1985) for *E. foetida* estimated 662 mg Zn $kg^{-1}$ soil for 14-day $LC_{50}$ value while Spurgeon *et al.* (1994) determined 1010 and 745 mg Zn $kg^{-1}$ soil for 14 day and 56-day duration for the same species. The present 14-day $LC_{50}$ value determined is reasonably consistent with the earlier reports of (Neuhauser *et al.*, 1985 and Spurgeon *et al.*, 1994). Panda an Sahu (1997) Also, while working on Drawida willsi determined 14-day $LC_{50}$ as 762.87, 840.69 and 907.82 mg Zn $kg^{-1}$ for juvenile, immature and mature forms respectively, which is in agreement of the present estimate.

The treated specimens did not show any statistically significant impact of treatment on growth pattern however growth of tissue was stimulated and enhanced at lowest i.e. 50 mg Zn $kg^{-1}$ concentration probably due to nutritive and increased enzymatic effect of zinc concentration on the body at that soil concentration. Though not significant statistically, the treatment at 200 mg Zn $kg^{-1}$ soil and 400 mg Zn $kg^{-1}$ soil retarded the growth differently. The inhibitory impact on growth increased with both dose and duration (Fig. 2). Underwood (1975) reported Zn to be important among the heavy metals required for normal physiological function of organisms including earthworms. Zn participates in the metabolism of earthworm as an activator of several enzymes like carboxypeptidase, carbonic anhydrase, DNA polymerase etc. (Leaverack, 1963), but produces harmful effects when present in higher amount. The present finding is in conformity with those of Leaverack (1963) and Underwood (1975).

Since oxygen consumption is directly related to energy production, it is taken as one of the important indices of metabolism in the organism. Considering respiratory activity as an important metabolic parameter many authors (Chua and Brinkhrust, 1973; Van Hook, 1974; Dash and Patra, 1977) have estimated oxygen consumption. On the basis of oxygen consumption during the study the energy mobilization of control experiment, on an average was 1.6188 Cal $g^{-1}$ dry wt $h^{-1}$. On $15^{th}$ day a rise in energy mobilization, though statistically not significant, was observed being 1.4%, 2.6% and 1.2% at the three concentrations of Zn treated samples respectively, probably due to lesser concentration of Zn accumulated in the body had positive impact on metabolic processes and enhanced it, which is reflected by increased oxygen consumption. On 30th day slight increase of 0.6% was observed for 50 mg Zn $kg^{-1}$ concentration while a lowering of energy mobilization by 0.9% and 2.1% was observed for 200 and 400 mg Zn concentration in soil. The present finding is in agreement with those of Gromadska (1962) and Panda & Sahu (1997). As the earthworms ingest high quantity of soil including organic waste material containing heavy metals they have been shown to accumulate heavy metals in their body tissue in fairly high concentration. However the rate and amount of accumulation is influenced by many edaphic factors such as pH, cation exchange capacity and organic matter content (Beyer *et al.*, 1987, Morgan and Morgan, 1988; Spurgeon and Hopkin 1996). Also, differences between metals and between species for the same metal have been reported so far accumulation is concerned (Ireland and Wooton, 1976; Morgan *et al.*, 1986 and Reinecke *et al.*, 1997). Ma (1982) reported higher accumulation of Zn in *A. caliginosa* in sandy soil than in loamy soils, though the content of Zn was higher in loamy soil. Hence the pattern and amount of heavy metal accumulation is species specific. The bioconcentration factors also vary considerably under the influence of various soil factors. Bioconcentration of Zn has been reported to vary from 2 to 8 times more in tissue than soil concentration (Ma, 1982). Ireland (1975) observed less Zn content in water extracts of casts of *D. rubidus* while 126 times more in that of body tissue. The earthworms are capable of binding Zn in their chloragogen tissue (Morgan & Morris, 1982) with involvement of metallothioneins (Suzuki, 1987). Since the binding of Zn to metallothioneins is reversible, possibly Zn-thioneins may have regulatory impact on metal concentration through facilitating rapid elimination of Zn (Van Gestel *et al.*, 1993; Marinussen *et al.*, 1997). The earthworms thus facilitate the availability of available metals to plant (Lee, 1985).

During the present study the accumulation of Zn in earthworm increased by 1.72%, 41.95% and 48.99% on 15th day of treatment at the three concentration and 25.39%, 68.22% and 82.41% on 30th day respectively, (Fig.-5), showing increase with increase in dose and duration but without statistical significance and proportion. A similar observations have been made by Morgan and Morris (1982) and Van Gestel *et al.* (1993). The amount of Zn accumulated in specimens of *P. sansibaricus* ranged between 70.8 to 128.6 mg $g^{-1}$ dry wt (Fig. 4) seems to be regulated by them as the earthworms can regulate Zn concentration in their body provided Zn is not more than 560 mg $kg^{-1}$ in soil (Van Gestel *et al.*, 1993). The present study may, however, be used for using earthworms in biomonitoring.

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