

**EFFECT OF VARIOUS CONCENTRATIONS OF ZINC AS SOIL HEALTH
INDICATOR ON EARTHWORM (*Pheretima posthuma*) AND SOME SOIL BACTERIA**

ABSTRACT

Metal toxicity is a matter of serious concern in today's time. It can affect either a whole organism or a part of it such as a cell or an organ. Zinc is a trace element that is essential for health of the organisms. It is a micronutrient and has been shown to be essential for growth, development and differentiation of all types of biota including bacteria. Zinc plays a role as an essential factor for most classes of enzymes; however, Zinc is potentially harmful at excessive levels. Toxicity studies of Zinc have mostly focused on higher trophic level species. Zinc showed a negative effect on the food web at the lower trophic level. On the other hand, bacterial isolates from natural habitats showed zinc tolerance. Soil is a good source of bacteria and earthworms and both are good indicators of the soil quality. The present study revealed that even a low concentration of zinc (*i.e.*, 0.50 mg/ml) is not suitable for the growth of soil bacteria. However, earthworms remained unaffected even at 0.75 mg/ml concentration of ZnSO₄.

Key words: Toxicity, Zinc, Bacteria, Earthworm, Soil quality

INTRODUCTION

Physiological or biological stress acts as a harmful stimulus to an organism, which responds to a stressor such as an environmental condition. Stress is a body's method of reacting to a challenge. The body's way to respond to stress is by sympathetic nervous system activation which results in the fight-or-flight response. But the body's physiological conditions soon come to normal (homeostasis), through the parasympathetic nervous system, because the body cannot keep this stressful state for long periods of time (Chetri *et al.*, 2017).

Toxicity is a function of solubility. Insoluble compounds as well as the metallic forms often exhibit negligible stress. Metal stress is the degree to which a substance or a metal can damage an organism. Some metals are toxic when they form poisonous soluble compounds. Not all heavy metals are particularly harmful, and some are essential such as iron and some, such as bismuth, have a low stress. Most often the definition of toxic metals includes at least cadmium, lead, mercury and the radioactive metals. Metalloids (arsenic, polonium) may be included in the definition. High levels of metals deposited in body tissues and subsequently in the brain, may cause significant developmental and neurological damage. Some possible sources of heavy metals poisoning include chemical products, fertilisers, industrial paint, building materials, fish that is high in mercury, silver dental fillings, and mercury preservatives in vaccines, nasal sprays, and many more.

Heavy metal(s) are widespread pollutants of great concern as they are, at high concentrations, stressful to all forms of life, including microbes, humans and animals. Heavy metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions or modifying the active conformations of biological molecules (Doelman *et al.*, 1994; Gadd and Griffiths, 1978; Wood and Wang, 1983). However, alternatively low concentrations of some heavy metal ions (e.g. Co^{2+} , Cu^{2+} , Zn^{2+} and Ni^{2+}) are essential for microorganisms since they provide vital cofactors for metallo-proteins and enzymes (Eiland, 1981; Doelman *et al.*, 1994).

Stressful metals comprise a group of minerals that have no known function in the body and, in fact, are very harmful to plant, animal and human bodies. Also, some forms of the required minerals can be highly harmful. Examples are some forms of zinc, copper, iron, manganese,

hexavalent chromium, selenium and others. The most needed minerals can also become harmful, if present in high quantity. Stressful metals replace nutrient minerals in enzyme binding sites. When this occurs, the metals inhibit, over-stimulate or otherwise alter thousands of enzymes.

Zinc (Zn) is an essential trace element required for optimal development, growth and metabolism of microorganisms (Borst-Pouwels, 1981; Ji & Silver, 1995). When organisms absorb too little Zinc they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores. Large quantities of Zinc can be found in soils. When the soils of farmland are polluted with Zinc, animals will absorb concentrations that are damaging to their health. Zinc can interrupt the activity in soils, as it negatively influences the activity of micro organisms and earthworms.

Similarly, excess load of Zinc shows stress and inhibition to microbial processes. It (Zn) is a component or activator of some enzymes of microorganisms but high concentration of zinc can cause stress on cellular activities (Babich & Stotzky, 1978; Gadd, 1993; Nweke *et al.*, 2006). Although Zinc is known to be a potent inhibitor of the respiratory electron transport system of bacteria & eukaryotic organisms, stresses of Zinc to microorganisms are metabolic changes such as, inhibition of growth, dehydrogenase activity, nitrification etc. (Babich & Stotzki, 1978; Anderson *et al.*; 1988; Kafka *et al.*, 1997; Falih, 1998; Gunditz *et al.*, 1998; Orji *et al.*, 2008). High concentration of Zinc was required to inhibit growth rates of fungi & to reduce survival of bacteria.

Earthworms have been suggested as useful indicators of soil health (de Bruyn 1997; Paoletti *et al.*, 1998). Through their feeding and burrowing activity, earthworms aid in decomposition and incorporation of organic matter, increase the number of water soluble aggregates, improve water infiltration, aeration, drainage and root penetration, and increase microbial activity (Lee, 1985). Growth and maturation in the earthworm species *Pheretima posthuma* act as a marker of sub-lethal stress of zinc. Zinc at sub-lethal concentrations affected growth and maturation in earthworms exposed to contaminated food.

Hence, the present work is designed to know the response of earthworm *Pheretima posthuma* and the bacteria present in the soil of the experimented area to different concentrations of zinc.

MATERIALS AND METHODS

ISOLATION OF BACTERIA AND EARTHWORMS

Soil samples were collected from undisturbed semi forest areas of Badarpur of the District Karimganj (presently Sribhumi), Assam, India in sterile plastic bags and brought to the laboratory. Earthworms were also collected from the same spot.

1 g of soil was added to 100 ml of sterile distilled water making a dilution of 10^{-2} . From this, 1ml was taken by pipette and added to 9 ml of sterile distilled water making a dilution of 10^{-3} . Following this technique a dilution up to 10^{-5} was prepared and 1 ml of the diluted soil sample from the concentration of 10^{-5} and 10^{-4} were plated in nutrient agar plates and incubated at $28\pm 2^{\circ}\text{C}$ for 24h. After 24h, the plates were observed for any microbial growth and the microbial colonies were isolated and sub-cultured thrice subsequently for obtaining pure cultures. These pure cultures were used for the screening. 24h old bacterial inoculation grown in liquid culture (nutrient broth) was used for the study.

The earthworms were kept in glass beakers with soil until further use.

SCREENING OF ZINC STRESS TOLERANCE LEVEL

Bacteria:

Nutrient agar (Himedia) added with ZnSO_4 solution at the concentration of 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml were prepared, autoclaved and then plated in sterilized petri dishes and allowed to solidify. On solidification, the plates were marked into several small squares on the backside and bacterial inoculums was spotted (spot test) in individual squares and numbered according to the samples spotted. After 30 minutes, the plates were sealed with parafilm and then incubated for 48hrs in inverted position at $28\pm 2^{\circ}\text{C}$ in an incubator. After the incubation period, the plates were checked for growth of bacteria and the data was tabulated (Samanta *et al.*, 2012).

Earthworm:

200g of soil sample was dried in oven at 100°C for 24 h and the moisture content was found by the formula:

$$MF = (I-F)/[A-(I-F)] \times 100 \dots \dots \dots (i)$$

where,

I = Initial wet weight of the soil taken + weight of the petri dish (g)

F = Final dry weight of the soil + weight of the petri dish (g)

A = Initial weight of the soil taken (g)

Then, 200 g of soil sample was hydrated with requisite amount of water containing ZnSO₄ at the concentration of 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml in separate glass beakers up to 45% of the soil moisture. The amount of ZnSO₄ solution to be added to the dried soil sample was found out by the following formulas:

$$H = T-MF \dots \dots \dots (ii)$$

where,

H = Hydration needed (%)

T = Target moisture content

MF = Initial moisture fraction of the soil sample.

$$W = (B \times H / 100) \times C \dots \dots \dots (iii)$$

where,

W = Amount of water to be added

H = Hydration needed (from ii)

C = Conversion factor (1ml/g)

05 earthworms were then inoculated in each beaker and allowed to stay for 14 days. After 14 days, the soil samples were emptied in trays to check the number of surviving earthworms, to study their morphological changes and to note the weight of the surviving earthworms (Norton, 1996).

RESULTS & DISCUSSION

Table 1: Showing the responses of the bacteria against different concentrations of ZnSO₄

| Isolate number | 0.25 mg/ml | 0.5 mg/ml | 0.75 mg/ml | 1.0 mg/ml | 1.25 mg/ml |
|-----------------------|-------------------|------------------|-------------------|------------------|-------------------|
| 1 | + | + | + | - | - |
| 2 | + | - | - | - | - |
| 3 | + | - | - | - | - |
| 4 | + | + | + | - | - |
| 5 | - | - | - | - | - |
| 6 | + | + | + | - | - |
| 7 | + | - | - | - | - |
| 8 | - | - | - | - | - |
| 9 | - | - | - | - | - |
| 10 | + | + | - | - | - |
| 11 | + | + | - | - | - |
| 12 | + | + | - | - | - |
| 13 | + | + | + | - | - |
| 14 | + | - | - | - | - |
| 15 | - | - | - | - | - |
| 16 | + | - | - | - | - |
| 17 | + | + | + | - | - |
| 18 | + | + | + | - | - |
| 19 | + | - | - | - | - |
| 20 | + | - | - | - | - |
| 21 | - | - | - | - | - |
| 22 | - | - | - | - | - |
| 23 | + | - | - | - | - |
| 24 | - | - | - | - | - |
| 25 | + | + | - | - | - |
| 26 | - | - | - | - | - |
| 27 | + | + | + | - | - |
| 28 | + | + | + | - | - |

Table 2: Showing the responses of the earthworm against different concentration of ZnSO₄

| Concentration (mg/ml) | Soil moisture (%) | Initial Number of worms | Initial weight of worms (g) | Final number of live worms | Final weight of worms (g) | Morphological changes |
|-----------------------|-------------------|-------------------------|-----------------------------|----------------------------|---------------------------|-----------------------------|
| 0.25 mg/ml | 45 | 5 | 25 | 5 | 25 | No significant change |
| 0.5 mg/ml | 45 | 5 | 25 | 5 | 25 | No significant change |
| 0.75 mg/ml | 45 | 5 | 24 | 5 | 24 | No significant change |
| 1.0 mg/ml | 45 | 5 | 27 | 5 | 20 | Shrinkage was seen |
| 01.25 mg/ml | 45 | 5 | 24 | 2 | 7 | Paleness, Shrinkage, Death. |

Zinc (Zn) is an essential trace element for growth and enzyme activities of heterotrophic bacteria. However, excess load of Zn shows toxicity and inhibition to microbial processes. However, Zn contamination seems to have effect on the microbial activities (Bong *et al.* 2010).

Environmentalists have made the argument that insects and other animals that reside in the soil are not able to avoid chemicals used by farmers and manufacturers. Zinc is a natural substance found in the environment and is necessary for life and our environment. However, zinc in the environment has been rising unnaturally due to human activities such as mining and steel processing. Small amounts of zinc do not affect worms, but on the other hand a large amount of zinc can impact worm growth rates and the earthworm population by decreasing it.

In table 1, five experimental concentrations of Zinc (0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml and 1.25 mg/ml) were shown. From the results it is found that zinc in low concentration (0.25 mg/ml) did not pose much threat or stress towards the bacteria (Table 1) and though there was no death of bacteria was recorded in certain media, but in some cases death of bacteria was recorded. But as the concentration was raised from 0.5 mg/ml to about 0.75 mg/ml, the bacteria failed to grow (Table 1) and there was a significant reduction in their number in the experimental media. At 1 mg/ml and 1.25 mg/ml concentration of ZnSO₄, no bacteria could survive (Table 1). Thus it can be said that high dosage of zinc is inhibitory towards the growth of bacteria. From

previous reports also it was seen that, stresses of zinc to microorganisms are metabolic changes such as, inhibition of growth, dehydrogenase activity, nitrification etc. (Babich & Stotzki 1978; Anderson *et al.* 1988; Kafka *et al.* 1997; Falih 1998, Grunditz *et al.* 1998; Orji *et al.* 2008).

In case of earthworm it was seen that at low concentration *i.e.*, up to 0.75 mg/ml, there was no inhibitory effect on the test organisms (Table 2). But with higher concentration of zinc (1 mg/ml to 1.25 mg/ml) there were changes in earthworm morphology and also on the survivability of the earthworms (Table 2). At each concentration, five earthworms were used after recording their initial body weight. After 48 hrs, each of the experimental area was checked and the earthworms were weighed again. It was observed that at 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml concentrations, there was no change in body weight and the earthworms also showed no signs of stress (Table 2).

At 1.0 mg/ml, though there was no death of any earthworms, but there was significant reduction in their body weights (Table 2). The initial weight of 05 earthworms (kept at 1.0 mg/ml of Zinc concentration) was 27 g. But after surviving 48 hrs at 1.0 mg/ml of Zinc concentration, they showed shrinkage and their body weight reduced to 20 g (table 2).

At 1.25 mg/ml out of five experimental earthworms, only two earthworms could survive (table 2) and they also showed paleness and shrinkage (Table 2). Their body weights also reduced from 24 g to 07 g, indicating that 1.25 g/ml of Zinc concentration is fatal for the earthworms. Zinc stress causes toxicity in earthworm (Garga *et al.*, 2009) and from our present experiment also these reports were proved to be applicable.

CONCLUSION

From the above experiment it can be concluded that Zinc confers stress to the micro- and macro-organisms of healthy soil system when present in large amount. So, thoughtful usage of Zinc chemicals in fields should be done. Down streaming of Zinc effluents to natural ecosystem should be avoided to maintain healthy and fertile edaphic conditions for plants, microbes and other soil dwelling organisms.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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