Investigating the Effect of Heavy Metals (Mercuric Chloride, Copper Sulphate and Zinc Sulphate) Exposure on Urea Levels in Earthworm

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Abstract: ***Urea excretion in Perionyx excavatus were analyzed with reference to toxic effects of heavy metals mercuric chloride, copper sulphate and zinc sulphate heavy metals for 96hour after exposure. The worms for this observation were exposed to different concentrations of heavy metals viz. The lower and higher concentration calculated were 25 and 30 mg/kg dry soil for mercuric chloride, 60 and 150 mg/kg dry soil for copper sulphate and 140 and 350 mg/kg dry soil for zinc sulphate respectively for 5 days of exposure. They were stressed after 96hour because of toxicity intolerance; decline in values of urea excreted under different stress situations was noted. Severe stress was caused by heavy metals mercuric chloride, copper sulphate and zinc sulphate toxicants. This is probably due to alterations in body metabolism; this may affect growth, water and salt balance and reproduction of this detrivore. So, conservation of worms against toxicants is suggested for maintaining the soil fertile. Mercuric chloride was found to be more toxic at its highest concentration followed by the highest concentration of combination of both metals Copper sulphate and zinc sulphate.***

Keywords: heavy metals, urea, conservation

1. Introduction

Earthworms are more than fish bait, they are the main contributors to enriching and improving soil for plants, animals and even humans, they create tunnels in the soil by burrowing, which aerates the soil to allow air, water and nutrients to reach deep within the soil. Earthworms eat the soil and organic matter, after it is digested, the earthworm releases waste as castings, which contain many nutrients that the plant can use. Some people even use earthworm castings as garden fertilizer. The excretory system consists of small, coiled tubes with walls that are glandular and richly supplied with blood vessels, are known as nephridia. They occur in all the segments of the body starting from the third segment downwards, and are named according to their location in the body. Nephridial excreta improve soil fertility. Integumentary nephridia are located on the inner side of the body wall in all segments except the first two. Septal nephridia are attached to both sides of the septa behind the 15th segment. Pharyngeal nephridia, these are located only in the 4th, 5th and 6th segment. (The septal nephridia may be considered as typical nephridia for de tailed description.) Most earthworms don't tolerate temperatures below freezing, nor do they tolerate high temperatures. Prolonged exposure to temperatures above 95°F kills them.

The nitrogenous excretion of earthworm may be considered as occurring in two fractions, each accounting for approximately equal portion of nitrogen removed each day. The first portion consists of a protein amounting to about 0.03mg/100ml urine/day (Bahl, 1947a) and is most probably derived from the mucus secreted the body. This mucus acts as a lubricant as the worm proceeds along its burrow. Mucus also helping to bind soil particles together and preventing the burrow wall from collapsing. Mucus probably acts as a buffer system outside the body since it is secreted in large amounts when the animal is immersed in a noxious stimulant such as acid.

This mucoid protein can account for about half the total nitrogen lost each day (Needham, Haggag El - Duweini, 1959), through this may be reflection some unnatural conditions encountered by the worms. The second fraction representing the end products metabolism is a fluid comprising ammonia and urea. In annelids the excretory processes are particularly associated with chloragogenous tissue and nephridia. The nephridia are abundantly supplied blood vessels. Their gland cells extract the excess water and nitrogenous waste from blood. The septal nephridia also eliminate the excretory material the coelomic fluid through nephrostomes. The excretory matter is discharged either directly to exterior or in to alimentary canal.

In natural conditions earthworms are exposed to variety of pathogenic substances, which may cause damage, and infection of the body cavity. When infection takes place, it is inactivated through non - specific defensive reactions, among which phagocytes, encapsulation and nodulation are of crucial importance (Glinski, 1997). The mechanism of phagocytes annelids, being a multi - phase reaction of the immune system, triggered after immunological recognition, affected by specialized cells, which are able to distinguish between the components of organism and alien substances. Subsequent stages of phagocytosis are chemotaxy and adhesion, the intensity dependent on the kind and quantity of phagocyte material, and also on the presence or absence of "plasma" factors. In annelids the factors aiding the process may include agglutinins and components of activated rophenol oxidase system (Cooper, 1996). Following adhesion phagolysosome is formed in which final enzymatic destruction of biotic factors takes place (Affar et 1998).

Ireland (1978) observed heavy metal binding properties chloragocytes of earthworm. Prento (1979) studied metal in chloragosomes *Lumbricus terrestris* and physical significance. Similar studies reported number workers to insects. Fischer *et al.* (1980) reported heavy metal effect chloragocytes earthworm *Lumbricus terrestris*. Morgan *et al.* (1981, 1982) studied excretory activity earthworm *Drawida willsi* heavy metal applied soil. Several investigators reported changes in nitrogen metabolism animals exposed pesticides herbicides. Panda (1997) studied recovery respiratory excretory activity Drawida willsi exposure malathion. Frans *et al*. (1997) studied heavy metal (copper, lead zinc) accumulation excretion by earthworm.

Affar *et al.* (1998) studied isolation, purification partial characterization chloragocytes earthworm. Morgan *et al.* (2002) observed earthworm excretory product under the stress of heavy metal. (2002) observed earthworm excretory products under the stress organic fertilizers. Morgan *et* al. (2005) studied metal in chloragosomes *Lumbricus terrestris*. In the present study it is aimed understand excretion nitrogenous waste product earthworms *Perionyx excavatus* exposed mercuric chloride, copper sulphate and zinc sulphate.

2. Material and methods

The earthworms, *Perionyx excavatus* were collected from the campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. They were maintained under normal day/night illumination at 25°±0.05°C in glass troughs experimentation. Earthworms looking healthy and having approximately equal size (10cm) and weight (3g) were selected for estimations of the excretory products. The soil characters are already given in chapter 1. Earthworms were removed from pots and kept half immersed in glass petriplates containing 30ml of tap water in 25+2°C temperature for 24 hours to evacuate their guts contents (Dash and Patra, 1977). The study was carried out in plastic culture pots under laboratory conditions following the protocol of Panda and Sahu (2002). In brief three heavy metals namely mercuric chloride (HgCl2), Copper sulphate (CuSo4.) and Zinc sulphate (ZnSo4) respectively used as the test chemicals were obtained from Ranbaxy Chemicals Ltd. S. A. S. Nagar. The pesticides were chosen on the basis of their extensive use in this area. A detailed description of these three pesticides is provided in Table 1.

Based on the results of 24 h toxicity tests (LD50), lower and higher sub lethal concentration of each heavy metal were chosen to study their impacts on excretory products of worms. The earthworm *Perionyx excavatus* were exposed for 5 days separately to lower and higher sub lethal concentrations of heavy metals. Seven sets of plastic culture pots (30cm, length X 20 cm, breadth X 20 cm, height) each with four replicates and 1 kg of soil were set up. The sub lethal concentration (lower and higher sub lethal concentration) of metal salts prepared in acetone was sprayed on to the soil surface. After evaporation of the solvent, the treated soil was thoroughly mixed to distribute the heavy metal evenly and enough water was added to bring the moisture content up to the field level. The same procedure using distilled water was applied to prepare a set of control pots. Twenty gut - evacuated earthworms were added thereafter to each pot. After exposure period of 5 days nephridia of *Perionyx excavatus* were dissected out separately in watch glasses with the help of fine scalpel, forceps, and scissors and carried out estimation of different nitrogenous excretory products like urea and uric acid using the acid was mated by using the conventional technique of Caraway (1963) as estimate described by Varley (1976). Results of three replications were averaged and expressed as mg% of a particular product.

Estimation of urea:

The urea content in the nephridia was measured by using Urease Nesslerisation method as described by Varley (1976).5% homogenate of nephridia was prepared in cold distilled water and centrifuged at 1500 rpm for 10 minutes. To 0.2 ml of supernatant 3.2 ml of distilled water and 20 mg of soyabean meal was added. The mixture was incubated for 15 minutes at 40 50°C.10% sodium tungstate and 0.3 ml 2/3 N sulphuric acid were added to incubated mixture and mixed well. After 10 minutes the mixture was centrifuged at 1500 rpm for 10 minutes again. The clear supernatant was used for the urea estimation. To 2 ml of supernatant 5 ml of ammonia free water of Nesslers reagent was added. The optical density of the colour was read immediately at 480 nm in spectrophotometer against a reagent blank. The urea content was expressed as mg of urea per 100 mg of wet tissue (mg %).

Statistical analysis

All results are expressed as mean of three replicates and the obtained data were statistically evaluated using student "t" test. Analysis the earthworm *Perionyx excavatus* clearly evidenced that the major excretory products urea uric acid. The effect of tested heavy metal sub lethal concentrations was found increase the urea uric levels nephridia exposure 5 days and given in Table. The earthworms, concentrations heavy metals analyzed urea and uric contents nephridia.

3. Results

In the nephridia of control worm 12.72% urea was found. The mercuric chloride (25 and 30 mg/kg soil) treated earthworm urea level was found 18% and 19.32% the nephridia. Urea increased significantly ($P<0.01$) by 41.50% and 51.88% in nephridia after exposed to lower and higher sublethal concentration of mercuric chloride respectively (Table). In copper sulphate (60 and 140 mg/kg soil) treated earthworm urea was found to be 14.04% and 16.68 % the nephridia. Urea increased significant at ($P<0.01$) by 10.33% and 31.13% in the nephridia after exposed lower and higher sublethal concentration of copper sulphate respectively (Table). In zinc sulphate and (140 and 350 mg/kg) treated earthworm urea level found to be 14.04% and 15.36% in the nephridia. Urea increased by 10.33% and 20.75% in the nephridia after exposed to lower and higher sublethal concentration of zinc sulphate respectively. The increased rate of urea was statistically significant at ($P<0.05$) presented in (Table).

% change in the urea in nephridia of earthworm *Perionyx excavatus* after treated to heavy metals for 5 days

Treatment		Urea
Control		12.72±0.5 *
Mercuric Chloride	Lower sublethal 25 mg/kg soil	41%
	Higher sublethal 30 mg/kg soil	51%
Copper Sulphate	Lower sublethal 60 mg/kg soil	10%
	Higher sublethal 150 mg/kg soil	31%
Zinc sulphate	Lower sublethal 140 mg/kg soil	10%
	Higher sublethal 350 mg/kg soil	20%

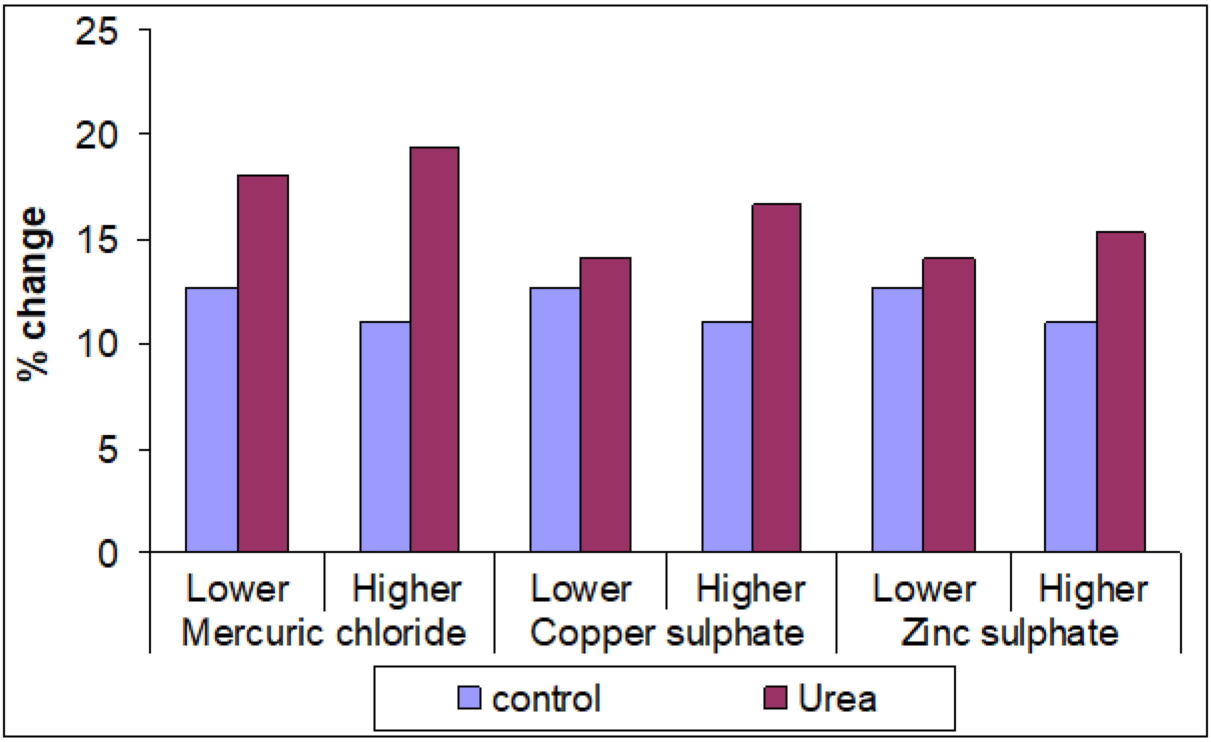

Figure: % change in the urea in nephridia of earthworm Perionyx excavatus after treated heavy metals for 5 days

4. Discussion

In the present study increases in urea levels in different tissues of *Perionyx excavatus* were observed, after exposure to mercuric chloride, copper sulphate and zinc sulphate. The present study demonstrates that worms *Perionyx excavatus* were unable to survive in heavy metal stress but they excreted urea in stressed condition. The given amount of urea excreted by the worms clearly indicates that the worms *Perionyx excavates* primarily ureotelic. The impact of stress situation on excretion in the earthworms has been studied in relation to living conditions and temperature control. Septal and supra intestinal nephridia are richly supplied with capillaries; nitrogenous wastes are re moved from the blood and coelomic fluid. Urea, ammonia and remains of dead cells, the pharyngeal and integumentary nephridia extract only waste matter from the capillaries and water is conserved. Carbon dioxide and nitrogenous wastes is excreted out from the body through moist skin by the process of diffusion. The nitrogenous wastes are excreted out of the body by special excretory organs called nephridia (singular nephridium). The nephridial tubule opens the body surface by a small opening called nephridiopore. Earthworms are primarily ammonotelic and ureotelic under stress situation, urea excretion change under different conditions of the environment like change in temperature, availability of water, food and stress conditions. The nature of excretory products also varies from species to species.

The study were investigating the effects of heavy metal exposure (mercuric chloride, copper sulphate, and zinc sulphate) on urea levels in earthworms, the significance of the findings would likely relate to understanding the toxic effects of heavy metals on the physiology of earthworms and how they affect the metabolism of the organism. One of the main significance of this research would be to understand how heavy metals affect the physiology of soil organisms, and specifically how it affects the urea metabolism in earthworm. Understanding how heavy metals affect the urea metabolism can provide insights into how heavy metals may impact overall soil health.

Additionally, the study may find that heavy metal exposure leads to changes in the urea levels of earthworms, and this information can be used as a guide for developing strategies for mitigating the effects of heavy metal pollution on soil ecosystems. Understanding how heavy metals affect urea levels can also help us to understand what effect it has on the nitrogen cycle. The study may also found that different heavy metals have different effects on the urea levels in earthworms, which can help to understand which heavy metals are more toxic and which have a lesser effect on the earthworm physiology.

Finally, urea level is a well - known biomarker to monitor the effect of pollution on soil organisms, such as earthworm, if the study found that urea level changes with heavy metal exposure, it may be possible to use urea level as biomarker for heavy metal pollution in soil.

The pesticide pollutants including heavy metals induce physiological changes in the vital systems such as respiratory, osmoregulatory, nervous etc. Many biochemical reactions are dependent on the rate of enzymatic activity. The pesticides alter the rate of biochemical reactions involved in nitrogen metabolism and augment energy demand by entering keto acids into glycolysis and triacarboxylic acid cycle (Srinivas, 1994). Needham (1957) found that during drought and starvation earthworm excrete predominantly urea, and it was established by Campbell and Bishop (1963). Prento (1989) reported distribution of arginase and other ornithine cyclic enzymes in the gut of the earthworm *Lumbricus terrestris*. Kulkami (1989) noted toxic effect of pyrethroid pesticides like cypermethrin and Fen - fen on the nitrogenous excretory product of nephridia of worms *Lampito mauritii* exposed to concentrations such as 0.007 and 0.009 ppm of cypermethrin and 0.025 and 0.03 ppm of Fen - fen. She reported a significant decrease in the ammonia content where as urea and uric acid increase. Similar reports are observed by many workers in earthworms exposed to different heavy metals (Prento, 1979). Affar *et al.* (1998) reported the increased level of urea and uric acid during exposure to zinc.

Patil (2002) reported a significant increase in urea and uric acid in earthworm Perionyx excavatus after exposing to fertilizers. Similarly Morgan *et al.* (2002) observed significant increase in excretory product of *Eisenia foetida*. Mahakur *et al.* (2004) observed that urea and uric acid excretion in increased in *Dendrobaena calebi* after exposure to fly ash amendments soil. The decreased uric acid in content in *Allolobophora caliginosa* indicates the probability of least disturbance in the formation and extraction of nitrogen products in response to toxic impact.

5. Conclusion

The conclusion of a study investigating the effect of heavy metals (mercuric chloride, copper sulphate, and zinc sulphate) exposure on urea levels in earthworms would summarize the main findings of the research, discussing how exposure to these heavy metals affected the levels of urea in the earthworms. It would also highlight any notable trends or patterns that emerged from the study, such as whether one heavy metal had a more pronounced effect on urea levels than the others, or whether there was any dose - response relationships observed. Additionally, the conclusion would relate the results to other existing studies on the topic, and discuss the ecological and environmental implications of the findings.

It could be like: "The results of this study indicate that exposure to mercuric chloride, copper sulphate, and zinc sulphate heavy metals leads to alterations in the urea levels in earthworms. The study reveals that all heavy metals tested caused an increase in the urea levels, however, copper sulphate had the most potent effect. The findings of this research have ecological and environmental implications as changes in the urea levels in earthworms can affect the overall nutrient cycling and ecosystem function. Further research should be conducted to better understand the mechanisms of heavy metal toxicity in earthworms, as well as to evaluate the potential long - term impacts of heavy metal pollution on earthworm populations and soil fertility

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