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Biochemical Response of Earthworms *Perionyx excavates* Exposed to Mercuric Chloride Contaminated Soil

Vaidya V. V*.

Dept. of Zoology, Amolak Science College, Kada, tq.Ashti, Dist. Beed. 414202
*Corresponding Author

Abstract

Toxic substance produces impairment in structural and functional organization of tissues. Toxicants may also act as a physical poison and may disrupt the integrity of membrane structure and its associated enzymes, without which the vital processes fail to, produce adequately. In the present study a megascolecid worm *Perionyx excavates* having approximately equal size (10cm long) and weight (3g) were exposed for 5 days separately to lower (60mg/kg) and higher (150mg/kg) sub lethal concentration of mercuric chloride. A correlative change in the activities of the enzyme xanthine oxidase estimated in the skin, intestine, and nephridia of *Perionyx excavates*. The activity pattern of protein measured in the present study indicates 36.31 mg% and 30.87 mg% in the ovary and 23.6 mg% in testis. The total protein level decreased significantly at ($P<0.01$) by13% and 26.05% in the ovary and 10.99 and 24.21% in the testis.

Keywords: Megascolecid, Activities, Nephridia, Xanthine oxidase, mercuric chloride.

Introduction

Earthworms are important components of terrestrial ecosystems and they represent approximately 60–80 % of the total soil biomass. They play an important role in soil processes such as improvement of soil structure, nutrient cycling and decomposition of organic matter (Yang *et al.*, 2012; Gomez-Eyles *et al.*, 2009). Some major physiological functions such as nutrition, immunity, survival, growth and reproduction have been shown to be disrupted by exposure to environmental contaminants (Bernard *et al.*, 2014). However, their successful survival under stress conditions is supported by efficient innate immune mechanisms based on cellular activities of coelomycetes and humoral immune proteins, both components of the earthworm coelomic fluid (Kauschke *et al.*, 2007). Earthworms have been widely used as an indicator organism to evaluate the soil ecological toxicity according to the American Society for Testing and Materials (ASTM), the Organization for Economic Cooperation and Development (OECD), and the International Standards Organization (ISO) (Zhang *et al.*, 2014). Heavy metals have adverse effects on vertebrate and invertebrate organisms and are known to be acutely toxic to some organisms. The level of toxicity to invertebrates, however, does not seemto be similar to that of vertebrates and needs further investigation Darvas and Polgar (1998).

As it is difficult and expensive to detect the presence and exact concentrations of these insecticides in the environment, due to their relatively short half-lives, it may be more effective to use biomarkers to assess exposure. A number of researchers have used biomarkers effectively to determine pesticide effects on earthworms (Booth *et al.*, 2000 and O'Halloran *et al.*, 1999). Biomarkers, measuring effects on sub organism levels, can provide links between a chemical and its toxic effect and are generally more sensitive than the more traditional measures of contamination, such as mortality and abundance. Biochemical reaction studies mostly focus on the evaluation of possible adverse effects of chemicals on aquatic organisms (Booth and O'Halloran, 2001: Calisi *et al.*, 2011). The severity of soil contamination caused by persistent organic pollutants is commonly assessed with standard acute and reproduction tests. These standard tests, however, are unable to provide accurate evaluation of the biochemical responses of selected organisms to a given chemical exposure. The biochemical responses, generally called early warning signals, can provide valuable information in assessing the potential risk factors of soil contamination Wu *et al.*, (2012). Proteins are the most abundant macromolecules and constitute over half of the dry weight of most organisms. Proteins are extremely complex nitrogen containing molecules, which play important role in nearly all biological processes as structural components, biocatalysts, hormones and repositories of genetic information. They also help in storage, transport, mechanical support, control of growth and differentiation (Kale, 2002). This study therefore aims at evaluating the sublethal effect of mercuric chloride on total protein of ovary and testis of earthworm *Perionyx excavatus..*

Materials and Methods

The earthworm *Perionyx excavates* having approximately equal size and weight were collected from non-irrigated field, which had the record of heavy water logging in monsoon season. The soil had the following characteristics: laterite type, sandy loam texture, pH-6.8, organic matter 2.7 g %, nitrogen 0.22 g% and a C/N ratio of 12.27. The soil was air dried and sieved before use. The earthworms were acclimated for one month with adequate provision of food (10% organic matter, cow dung +leaf litter), moisture (20g %) and temperature (25±2^{o}C). Earthworms were kept half immersed in glass petri plates containing 30ml of tap water at 25±2^{o}C temperatures for 24 hours to evacuate their guts (Dash and Patra, 1977). The study was carried out in plastic culture pots under laboratory conditions. The worms were exposed to 24 h LD_{50} sub lethal dose of mercuric chloride (25 & 30 mg/kg soil) for 5 days. The biochemical analysis of protein, done by using the method of Lowry *et al*., (1951), the changes were observed in ovary and testis of worm *Perionyx excavatus.*

Estimation of total proteins (Lowry *et al.*, 1951):

50-100 mg of tissue was homogenized in 5 ml cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected after centrifugation at 3000 rpm for 15 minutes. The pellet was repeatedly washed with distilled water to remove the traces of TCA. Precipitated protein was redissolved in 0.1N NAOH and estimated by the method of Lowry *et al.* (1951) as follows:

0.1ml of the solution was transferred into a test tube and 4 ml of alkaline mercuric chloride reagent was added, followed by 0.4 ml of diluted commercial Folins reagent. The optical density of the blue colour developed was read at 540 µm after 30 minutes of addition of the Folins reagent using UV-VIS spectrophotometer (Model Digispec 200 GL). Bovine serum albumin was used as a standard. The protein content was expressed as mg/100 mg wet weight of the tissue.

Statistical analysis:

The results were expressed as mean of three replicates and data were analyzed statistically by using student 't' test (Mungikar, 2003).

Results

Table 1. Protein levels in various tissues of *Perionyx excavates* exposed for 5 days at sub lethal concentration mercuric chloride for 5 days.

Tissue	Control	Mercuric chloride	
		Lower sub lethal	Higher sub lethal
Ovary	35.28 ± 0.4	13%	26%
Testis	28.62 ± 0.30	16%	24%

Protein

Total protein level in control earthworm *Perionyx excavatus* was found to be 66.77 mg % in skin; 41.75 mg % in ovary; 26.52 mg % in intestine, and 39.57 mg % in testis respectively.

In the mercuric chloride (25 & 30/kg soil) treated earthworm total protein level was found to be 36.31 mg % and 30.87 mg % in the ovary and 23.6 mg % in testis. The total protein level decreased significantly at ($P<0.01$) by13 % and 26.05 % in the ovary and 10.99 and 24.21 % in the testis.

Discussion

Earthworms have long been known for their tolerance to toxic chemicals present in contaminated soils and hence have been widely used as indicator organisms for ecotoxicity studies. However, earthworm survival in such stress conditions depends upon several physicochemical factors such as soil texture, pH of the medium, organic matter, nature and extent of the clay minerals (Maity *et al.,* 2008).

The Important quantities of pesticides are rejected into the environment, thus inducing a chronic contamination of an increasing number of ecosystems Sarkar *et al.,* (2006) distorting their physiological and biochemical capacities in different degrees. Tools of biosurveillance ecotoxicologique called biomarkers which reports the levels of pollution to which are subjected these bioindicators Lavado *et al.,* (2006). For ten years, the use earthworms as means of action or surveillance against the nuisances in the environment. These animals constitute today an army of cleaners to the services of the local authorities, private individuals and industrialists.

Earthworms were hardly studied in past and their use was mainly concentrated on the impact and the effect of organic pollutants such as pesticides on the earthworm **(**Bundy *et al.,* 2007; Schleifler *et al.,* 2006; Svendsen *et al.,* 2007).Whereas very few studies in past have included research on the impact and the effect of organic pollutants such as pesticides on the earthworm**.** During the exposure to pollutants, earthworms are capable to reduce the toxic effects of the chemical product by adjusting their internal biochemical responses before the growth is affected. So, the biochemical reactions are rather important to evaluate the potential adverse effects of chemicals on the environment Gao *et al.,* (2007).

In the present study the depletion in protein level was recorded in worms after 5 days of exposure to sub lethal dose of mercuric chloride in all the tissue. Decrease in protein content may be due to degradation of proteins in to amino acids to be utilized for gluconeogenesis (Begum and Dharni, 1996) to mitigate the stress. Being a part of cell membrane and as an enzyme protein level might be decreased because of its metabolism to liberate energy during pesticide stress (Chaudhari *et al.,* 1993).

Effect of various pesticides on total protein contents and different enzymatic activities in different organisms have also been extensively studied by a number of workers. Earlier Sak and coworkers (2006) reported that the level of protein in all stages and sexes of the wasp tend to be reduced after treatment with cypermethrin as compared to controls. Likewise, Mosleh and coworkers (2003) reported the effects pertaining to aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl on the soluble protein of the LC25 treated earthworm. As compared to other tested pesticides, the aldicarb appeared to be the most toxic. Vijayaraghavan and coworkers (2002) also reported that the total protein increases from larval to pupal transition whereas a decrease has been observed in larvae of *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) exposed to various insecticides. Ribeiro and coworkers (2001) also observed a reduction in protein content and associated it with physiological adaptability for compensation of insecticidal stress. In contrast, Mujeeb (2000) consider the elevated protein content in beetles of the stored grain pests *Tribolium castaneum* (Herbst.) to be due to treatment effects of malathionandprimiphosmethyl. Gill and coworkers (1990) reported the impact of an organophosphate in *Punctius conchonius* and demonstrated decline in the contents of protein. Similarly, Tripathi and Shukla (1991) observed changes in protein pattern in Clariusbatrachus after methyl parathion treatment. Saleem and coworkers (1998) also reported reduction in total protein contents of *T. castaneum* larvae after 4 days treatment of Ripcord 20EC (cypermethrin). Likewise, a decline in protein contents following pesticide treatment has been observed in different organisms (Ahmad *et al.,* 2000; Tabassum and Naqvi, 2001). In conclusion not restricted to different pesticides, even different plant extract based agro-treatments also affect proteins as well as nucleic acid contents of many secondary target organisms (Naqvi *et al.,* 1994; Azmi *et al.,* 1997; 1998; Nurulain *et al.,* 1997; 2000; Ahmad *et al.,* 2003). The slight increase of protein in control earthworms suggested that the soil nutrients were just sufficient to sustain the survival of earthworms, but insufficient to allow for additional growth. It is established fact that proteins are used as an alternative source of energy especially under stress conditions. It may be suggested that in *Perionyx excavates* also proteins must have been utilized for the production of energy to reduce the stress.

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