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Responses of the earthworm, *Eisenia foetida* coelomocytes to aluminum chloride using neutral red retention assay

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ABSTRACT

Heavy metals are one of the pollutant groups that recently have started to induce harmful effects on our ecosystems. Soil is an important sink for metals released in the environment by human activities. Therefore, soil-living organisms are exposed and potentially affected by metals accumulating in soil. This has lead to develop sub - lethal earthworm biomarkers as they are relevant indicators of environment change. In the present study the responses of coelomocytes of the earthworm, *Eisenia foetida* to exogenously applied sub lethal concentrations of Aluminum chloride have been studied, using Neutral Red Retention Assay. It was observed that 15, 30 and 45 days of exposure to Al in 200 mg and 400 mg /kg of soil significantly affected the coelomocyte viability as the neutral red retention time of lysosomal membrane significantly decreased dose dependently as well as duration - dependently in comparison to non - exposed earthworms. The present data suggest that coelomocyte responses through NRRA can be one of the sensitive biomarkers for assessing chemical contamination of soil.

KEY WORDS: ALUMINUM, COELOMOCYTES, EARTHWORMS, LYSOSOMES, NRRA.

INTRODUCTION

Earthworms represent a significant, if not a dominant part of the soil biomass, and are regarded as soil engineers regulating important soil processes and are being broadly used to assess environmental impact from heavy metal pollution (Cao X *et al.*, 2013). Various workers have shown that earthworms have been considered as useful bioindicators of soil ecosystem health, several studies have shown that worms can accumulate high

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concentrations of pollutants and therefore they have been used as bio-indicators of soil pollution by metals and pesticides (Ali *et al*., 2002, 2006, 2007, 2009, 2012; Khan *et al*., 2007).

In recent years there is a growing interest in the development of sub lethal earthworm biomarkers, one of them is Neutral Red Retention Assay which measures the membrane stability of lysosomes within the coelomocytes of earthworms in response to contaminants. Thus it can be used for evaluation of toxicity of range of toxicants under different exposure conditions.

Recently, from this laboratory, a series of publications have come up, where the effects of metals like lead, copper, aluminum and some pesticides have been studied on growth, reproduction and avoidance behavior of earthworms, *E. foetida* and *Lumbricus terrestris*, where we have reported that the hazardous pollutants significantly affect the sensitive parameters of these soil organisms (Ali, 1997; Khan *et al*., 2007; Farrukh and Ali 2011a; 2011b; Ali and Naaz 2013). In this communication, the effects of $AlCl_3$ on the coelomocyte viability of earthworm, *Eisenia foetida* using Neutral Red Retention Assay (NRRA) are reported.

MATERIAL AND METHODS

SELECTION AND CULTURE OF EARTHWORMS, *ESENIA FOETIDA*

E. foetida has been selected as test species because it has been suggested as a sensitive and standard species for ecotoxicological species by OECD (2007). These were brought from MPCST Nursery, Obedullahganj (District Raisen) M.P., prior to exposure all worms were acclimatized in the uncontaminated soil medium in the laboratory which was the mixture of cow dung manure and virgin black soil, as per the method of Rao *et al*., (2004).

For exposure to $AlCl_3$. adult earthworms with full clitellum, of weight 1.5 to 2.0 gms were used, After calculating the proper LC50 as per the method of (Rao *et al*., 2004) the earthworms were exposed to $AlCl_3$ in two concentrations (200 and 400 mg/kg dry weight of soil for periods of 15,30 and 45 days. Earthen pots of 20cm height 12 cm diameter were used for exposing the earthworms to the metal, as many as 5 to 7 earthworms were used for each concentration for varying periods of exposure ranging from 15 to 45 days, for both the control and aluminum exposed worms. Each pot was filled with 2kg of dry soil mixed with respective dose of Al, the pots were then covered with perforated filter paper sheets to minimize the loss of water by evaporation as well as movement of earthworms out of the pots. Soil temperature was maintained between 22°C to 28°C with soil P^H of 6.0 to 7.0.

A neutral red retention time assay was done according to the method described by Weeks and Sevendsen, (1996) and Booth *et al*., (2001). A neutral red working solution of 80 mg/ml was prepared in earthworm physiological Ringer solution (Speed and Smith 1975), coelomic fluid was collected from earthworms by inserting a needle containing 20 microlitres of Ringer into the coelomic cavity posterior to the clitellum and allowing it to fill by intra coelomic pressure and after a gentle drawing action on the syringe. The coelomic fluid was extracted from the control as well as from the treated worm and then placed on to a clean slide and mixed with 20 microlitres of neutral red solution, before a cover slip was placed on top. Slides were scanned for 2 minutes at 5 minutes interval and numbers of stained and unstained cells were counted until 50% of the cells became red or for 60 minutes. This time was recorded as neutral red retention time.

RESULTS AND DISCUSSION

The data presented in Figure 1 show a marked decrease in mean NRRTs of the Aluminum exposed worms in all the concentrations of the metal used. As evident from the figure, minimum concentration of 200 mg of $AlCl_3$, decreased the mean NRRT of coelomocytes of earthworms after 15 days of exposure, which decreased from a value of 57.424 ± 1.6489 to 47.28 ± 1.6489 minutes. After 30 days of exposure it was observed the NRRT decreased from a control value of 56.612 to 41.156 (min). Whereas, in case of the 45 days of Al exposure, the decrease in NRRT was from 51.96 to 30.28 (Figure 1).

Similarly as per the data of Figure 2, NRRT at the dose of 400 mg/kg of Al decreased from a control value of 56.192 to 28.458 (min) after 15 days by the double dose of Al. In an exposure period of 30 days and 45 days, of same concentration of Aluminum ie 400 mg /kg of soil, the decrease was from a control of 56.638 to 41.156 and from a control of 59.266 to 14.28 respectively (Fig 2).

In the present study sublethal doses of Al caused a significant decrease at in the mean NRRT of coelomocytes of earthworms *E. foetida* and the decrease in NRRT was found to be dose and duration dependent. From the results it has been observed that NRRT of coelomocytes of *E. foetida* is very sensitive, even to the lowest sublethal doses of aluminum and responded equally in dose dependent manner. Therefore, from the present studies it can be concluded that coelomocyte viability can be used as a sensitive parameter for assessing the toxicity of soil pollutants such as metals, both heavy and trace elements. Moore; (1985) reported that pathological alterations in lysosomes has been especially useful in the identification of adverse environmental impacts on

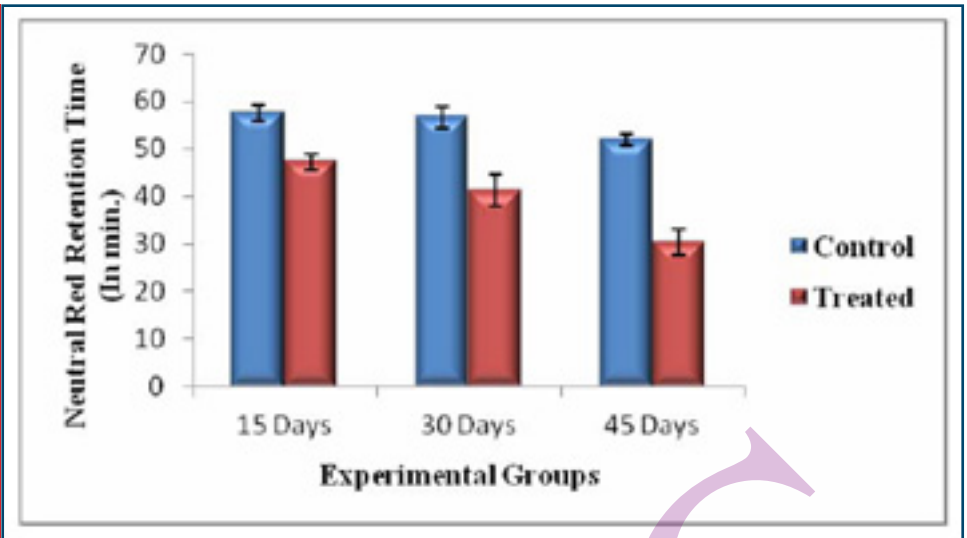

FIGURE 1: Showing the effects of Aluminium chloride (200 mg/kg of soil) on neutral red retention time in (min) of coelomocytes of earthworms, *Eisenia foetida* exposed to short and long periods, along with well matched un exposed controls (15, 30 and 45 days). Vertical bars denote ±SE.

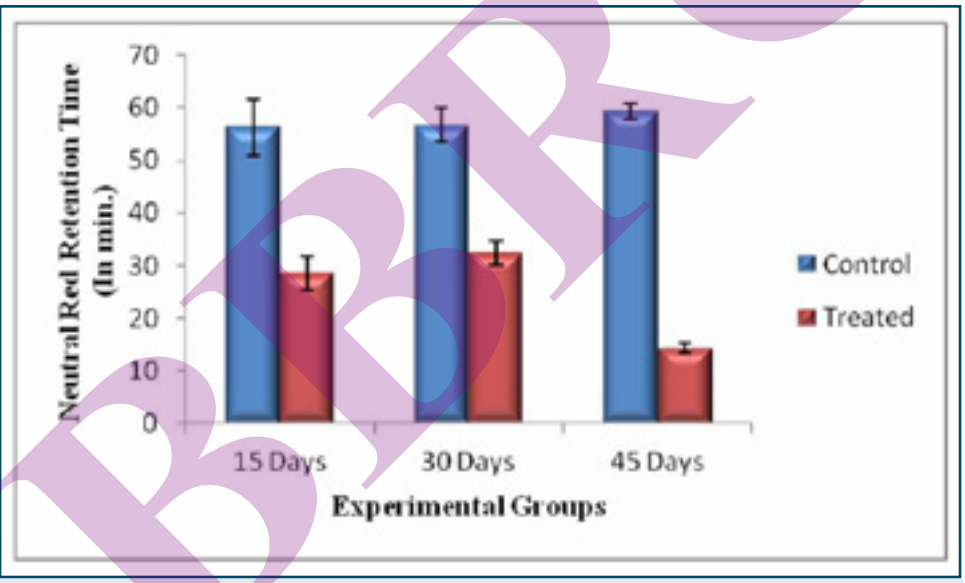

FIGURE 2: Showing the effects of Aluminium chloride (400 mg/kg of soil) on neutral red retention time in (min) of coelomocytes of earthworms, *Eisenia foetida* exposed to short and long periods, along with well matched controls (15, 30 and 45 days). Vertical bars denote ±SE.

organisms, with much evidence for aquatic organisms but with rather limited evidence for terrestrial organisms.

The present studies are in agreement with findings of Svendsen & Weeks, (1996) who reported the significant reduction in neutral red retention time of lysosomes of the coelomocytes of *L. rubellus* with increasing external copper concentrations. Later on Booth & Halloran; (2001) reported a significant reduction in NRRT of lysosomes when *A. caliginosa* was exposed to two organophosphate pesticides i.e. chlorpyrifos and diazinon. The response of neutral red retention assay to environmental contaminants occur sooner at sub cellular level than at physiological or other levels making it a useful biomarker to serve as an early warning system of stress.

CONCLUSION

In the present study, the responses of coelomocytes of the earthworm, *Eisenia foetida* to exogenously applied sub lethal concentrations of Aluminum chloride have been studied, using Neutral Red Retention Assay. It was observed that 15, 30 and 45 days of exposure to Al in 200 mg and 400 mg / kg of soil, affected the coelomocyte viability as the neutral red retention time of lysosomal membrane significantly decreased dose dependently as well as duration - dependently in comparison to non aluminum - exposed earthworms. The present data suggest that coelomocyte responses through NRRA can be one of the sensitive biomarkers for assessing chemical contamination of soil.

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