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Mercury and Cadmium Toxicity on Biomolecular parameters in *Eudrilus eugeniae*

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A B S T R A C T

Elevated level of heavy metals act as toxicants to flora and fauna of an ecosystem. Earthworms have the ability to bioaccumulate these heavy metals in yellow cells of their body thus help in bioremediation process to eliminate heavy metals from the soil. Depending upon the doses and exposure period to these heavy metals, earthworm's body find affected. That's why the investigation was conducted to check the effect of Hg and Cd on earthworm *Eudrilus eugeniae*. Six doses *viz*. Hg 0.17, 0.22 and 0.29% and Cd 0.27, 0.37 & 0.51% was sprayed for a period of 60 days. After 1st, 30th and 60th day of exposure, body tissue carbohydrate and protein were analyzed. It was concluded that Hg 0.29% affect the biomolecular concentration of body of earthworm *Eudrilus eugeniae* followed by Cd. Maximum reduction in carbohydrate i.e. 25.32% and that of protein was 32.98% at the dose of Hg 0.29%.

Keywords

Carbohydrate, Protein, Emission sources, Anthropogenic activities, Earthworm

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Introduction

Rapidly growing industries, anthropogenic activities and urbanization are rationale of various types of pollutants all over the world. Pollutants including agrochemicals, polychlorinated biphenyls, heavy metals etc. all affects the structure of soil invertebrate communities and ultimately disturbs the whole ecosystems. Earthworms are one of the first receptors to be affected by soil contamination (Urmila *et al.*, 2019). Ecologically *Eudrilus eugeniae* is an anatomically advanced earthworm having direct fertilization. This species was originating in savannah soilsof West African. *Eudrilus eugeniae* thrives on organically rich substrates and due toits faster growth rate, is the second most widely used earthworm for vermicomposting. From anthropogenic sources, the heavy metals are widely spread and caused damages to ecosystems close to emission sources (Derome and Nieminen, 1998). Although they are essential trace elements but above prescribed concentrations and exposure times, they are toxic to soil animals. These toxic heavy metals affect the distribution, abundance and diversity of almost all the animals especially the soil dwelling animals (Hopkin, 1989). As earthworms play key role in many soil processes such as soil aeration, infiltration and land reclamation, they are regarded as farmer's friend. They are relevant indicators of environmental change because they always remain in full contact with the soil; thus, they have been included in a group of five key indicators for ecotoxicological testing of industrial chemicals determined by the Organization de Cooperation et de Developpement Economiques and the European Economic Community (Capowiez *et al.,* 2003).

The present investigation was carried out to study the toxicity of two heavy metals *viz*. Hg and Cd. Mercury is widely spread heavy metal in the environment and is very toxic even at very low concentrations. These heavy metals pollutant stimulate alterations in the biomolecules and causes a wide range of adverse health effects (Sarkar, 2005). Cadmium on the other hand is also a non-essential toxic heavy metal. It has particularly harmful effects on all the organisms.

With an average concentration of about 0.1 mg/kg, it was considered that it is widely distributed in the earth's crust. Heavy metals such as Zn, Pb, Hg, Cd and Cu are bioaccumulated and results diverse eff ects in earthworms which may credits the use of earthworm as a suitable biological indicator to investigate the heavy metals pollution (Zhang *et al.,* 2009). Keeping in view the above fact, the aim of present study was to investigate alterations induced by mercury and cadmium on biomolecular parameters in *Eudrilus eugeniae.*

Materials and Methods

Procurement of test species

Healthy and Clitellated test earthworms *Eudrilus eugeniae* were collected from Vermiculture unit of Department of Zoology & Aquaculture, CCSHAU, Hisar. After collection *Eudrilus eugeniae* were cultured on cowdung.

Biochemical analysis

Mercuric chloride and Cadmium sulphate were used for assessing the heavy metals induced toxicity on earthworms. Clitellated *Eudrilus eugeniae* were exposed to different doses of Mercury and Cadmium. The detail of doses was described in table 1. Three replicate per dose were maintained along with control. After 1^{st}, 30^{th} and 60^{th} day of exposure earthworms were removed from each dose as well as from control and washed with tap water to remove dirt from their body and allowed them to feed on filter paper to void their gut content. Thereafter, earthworms were washed using distilled water and were left at 25°C for a period of 72 hours and then homogenized using pestle and mortar. The brown color paste so obtained was dried at 60±2°C for a period of 24 hours and stored at 4°C for further biochemical analysis (Jatwani *et al.,* 2016).

The total body tissue carbohydrate was analyzed by the Standard Phenol Sulphuric method (Masuko *et al.,* 2005) and protein content was estimated by Lowry method (Lowry *et al.,* 1951).

Statistical analysis

Data was analyzed by completely randomized block (CRD) with three replicates. The critical difference (CD) at 5% probability levels was worked out by utilizing Software 'OPSTAT', created at the Computer Center, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.

Results and Discussion

Dose and time dependent decrease was reported in both Hg and Cd. The investigation of 60 days revealed that amount of bio-molecules such as carbohydrates and proteins reduced gradually whereas the maximum reduction was found at the dose of mercury 0.29% i.e. 25.32% followed by Cadmium 0.51% i.e. 16.00% (Table 1). Table 2 indicate reduction in body tissue protein and utmost reduction was reported in mercury 0.29% i.e. 32.98% followed by Cadmium 0.51% i.e. 25.59%.Statistically significant reduction in body tissue carbohydrate and protein was reported as compared to control. The body tissue carbohydrates decreased to 7.671 and 8.837µg mg^{-1} as compared to control (10.765 µg mg^{-1}) after 60 days of exposure to higher dose of mercury and cadmium.

Accordingly, after 60^{th} day of exposure to higher dose of both the heavy metals the body tissue protein decreased upto 17.841 and 19.839µg mg^{-1} as control (27.048µg mg^{-1}). Biochemical changes in the body of living organisms which was induced due to any pollutant act as fateful indicators for the impendence of contaminants in environment. Notwithstanding, this kind of studies helps in establishment of threshold levels for pollution and pollutants. This study also helps for the better understanding of detrimental impacts of pollution. The body tissue carbohydrate content was decreased from 10.272 µg $mg^{-1}$to 7.671 µg mg^{-1} and 10.521 µg $mg^{-1}$to 8.837 µg mg^{-1} at the dose of Hg 0.29% and Cd 0.51% respectively. This gradual decrease in carbohydrate content was supported by the investigation of Hem Lata *et al.,* (2017) and Dezwaan and Zandee (1972). Pollutant may lead to the occurrence of hypoxia that in turn increases the carbohydrate utilization (Dezwaan and Zandee, 1972). Carbohydrates act as principal immediate source of energy and the stress induced due to pollutant has resulted in increased carbohydrate catabolism (Umminger, 1970). The body tissue protein was reduced from 26.619µg $mg^{-1}$to 17.841 µg mg^{-1} and 26.661 µg $mg^{-1}$to 19.839 µg mg^{-1}at the dose of Hg 0.29% and Cd 0.51% respectively. This present study investigated dose and time dependent reduction in protein content which was supported by investigation of Jatwani *et al.,* (2016).

The present study also supported by the findings of Mosleh *et al.,* (2003). The decreased level of proteins may be due to the increased turnover of protein by the interference of toxicant. Beforehand it has also been reported that protein may act as alternate source of energy during prolonged stress condition that also vindicates the present findings (Sturzenbaum *et al.,* 2001).

Table.1 Description of doses given to test species *Eudrilus eugeniae* along with control

Sr. No.	Doses	Concentration (in %)
1.	Control	Control
2.	Mercury	0.17, 0.22 & 0.29
3.	Cadmium	0.27, 0.37 & 0.51

Table.2 Impact of mercury and cadmium on carbohydrate content of *Eudrilus eugeniae*

Sr. No.	Doses (in %)	Carbohydrate (μg mg^{-1} body tissue)		
		1 day	**30 day**	**60 day**
1.	Control	10.865 ± 0.017	10.665 ± 0.019	10.765 ± 0.027
2.	Mercury (0.17)	10.254 ± 0.008	10.305 ± 0.338	8.847 ± 0.023
3.	Mercury (0.22)	10.351 ± 0.015	9.636 ± 0.005	8.253 ± 0.010
4.	**Mercury (0.29)**	10.272 ± 0.006	9.406 ± 0.003	7.671 ± 0.013
5.	Cadmium (0.27)	10.453 ± 0.013	10.021 ± 0.011	9.639 ± 0.009
6.	Cadmium (0.37)	10.412 ± 0.006	9.764 ± 0.015	9.117 ± 0.004
7.	Cadmium (0.51)	10.521 ± 0.006	9.558 ± 0.008	8.837 ± 0.013
	CD (P=0.05)	0.033	0.393	0.049

Table.3 Impact of mercury and cadmium on protein content of *Eudrilus eugeniae*

Sr. No.	Doses (in %)	Protein (μg mg^{-1} body tissue)		
		1 day	**30 day**	**60 day**
1.	Control	26.861 ± 0.009	26.839 ± 0.012	27.048 ± 0.017
2.	Mercury (0.17)	26.532 ± 0.005	24.157 ± 0.012	21.239 ± 0.014
3.	Mercury (0.22)	26.583 ± 0.004	23.359 ± 0.020	20.135 ± 0.019
4.	**Mercury (0.29)**	26.619 ± 0.006	21.047 ± 0.036	17.841 ± 0.000
5.	Cadmium (0.27)	26.871 ± 0.005	25.145 ± 0.018	23.653 ± 0.008
6.	Cadmium (0.37)	26.746 ± 0.012	23.241 ± 0.009	20.868 ± 0.019
7.	Cadmium (0.51)	26.661 ± 0.015	22.509 ± 0.065	19.839 ± 0.023
	CD (P=0.05)	0.028	0.094	0.049

The decadence proteins level may also be due to catalysis of proteins to amino acids to reduce the stress conditions because the proteins act as disjunctive source of energy specifically during stress conditions (Table 3). The detrimental effects of heavy metals on the biomolecules of earthworms may be stamped by the present findings. Not only the biomolecules but growth and fecundity also affected by these heavy metals (Pb and Ni) as reported by Urmila *et al.,* (2019). Hereby, the heavy metals pose threat to soil quality and fertility by diminishing the survivability of soil dweller creatures, the earthworms. It can be concluded that both mercury and cadmium induce stress which leads to alterations in the biomolecular composition of *Eudrilus eugeniae*. Although both the heavy metals are detrimental to biomolecules of earthworms but mercury was found to be more toxic. Hereby, emphatic management strategies have to be developed in order to implement the proper disposal of pollutants especially heavy metals to enhance earthworms' survivability, thereby improving the sustainability of agroecosystems.

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