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Effect of lead and Nickel on growth and fecundity of earthworm, *Eisenia fetida*

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Abstract
Expeditiously growing industries, anthropogenic activities and urbanization are rationale of various types of pollutants all over the world. Pollutants whether 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. Due to this reason the current disquisition was carried out to scrutinize the effect of Pb and Ni on growth and fecundity of earthworm, *Eisenia fetida*. The results demonstrate toxicity of two heavy metals *viz.* Lead and Nickel individually as well as in combination of different concentrations on earthworms in terms of survivability, growth (length and weight) and cocoon production. During the investigation, dose and time dependent reduction was observed in growth and fecundity of *Eisenia fetida*. In term of growth, maximum rebate in length (4.783 cm) and weight (0.487 g) of earthworm was remarked in soil treated with Pb-0.06ppm as compared to control where approximate length and weight was 8.660cm and 1.020g respectively. Similar trend was remarked in the population and fecundity of earthworm. Maximum rebate in population in terms of survivability was reported in Pb-0.06ppm i.e. 51.67% chased by Pb+Ni -0.03+0.03ppm i.e. 61.67% and Ni -0.06ppm i.e. 65%. After 90 days of treatment maximum decline (33.89%) in number of cocoon production was seen in Pb-0.06ppm trailed by Pb+Ni -0.03+0.03ppm i.e. 22.54%.

Keywords: *Eisenia fetida*, heavy metals, growth and fecundity

Introduction

Earthworms due to presence of metameric segmentation grouped under phylum Annelida. They have long, narrow, cylindrical, bilaterally symmetrical body covered with delicate cuticle. Although they are hermaphrodite but self-fertilization usually does not take place. The sexually mature worms exhibit ring shaped structure termed as clitellum. The clitellar region possess gland cells that secrete material to form a viscid, girdle like structure known as cocoon. The size cocoons varying with species. Growth rate is very fast as the mature worm can grow up to 1400 - 1500 mg of body weight during initial 70 days of life span. The prolificacy of mature worm is one cocoon every third day and one to three hatchlings come out from each cocoon after three to four weeks of incubation (Das *et al.*, 2002) [16]. As we all know that earthworms are a soil dwelling organism that’s why they are directly affected by heavy metal pollutions discharged by industries on land. Multifold industries such as electroplating, metal finishing, metallurgy, tannery, chemical battery manufacturing and mining usually produce huge bulks of heavy metal inclusive wastewater. The atmospheric input of heavy metals to agricultural systems also extensively assembled to metal loading in soil (Vidovic *et al.*, 2005) [17]. Heavy metals are generic adulterators of the soil ecosystem as a consequence of several industrial and agrarian activities (Vandana and Keshav, 2015) [18]. These heavy metal takes their way in earthworm’s body by ingesting polluted soil. In nature if one component of ecosystem disturbs then whole the ecosystem ultimately disturbs. Earthworms are one of the first addressees to be affected by soil contamination. Earthworms have bio-accumulative caliber that entitle them to be used for the bio-monitoring of soil pollution (Hirano and Tamae, 2011) [4]. Exalted concentration of heavy metals such as cadmium (Cd), mercury (Hg), copper (Cu), lead (Pb) and zinc (Zn) affects the density, viability, cocoon production, growth and sexual development of earthworms. Metals pollution cause mortality of earthworms within few days (Davies *et al.*, 2003) [3] and affects their population size and their species diversity (Spurgeon *et al.*, 2005) [13]. Reproduction is the factor which have remarkable importance in assessment of eco-toxicology because it influences population dynamics. Higher level of heavy metals lowers the cocoon production while medium level of heavy metals delayed cocoon production (Maleri *et al*., 2007) [8]. Earthworms are excellent bio-indicator of soil eco-toxicity according to the Organization for Economic Cooperation and Development (OECD) (Zhang *et al*., 2014) [19] and their relative health signalize relative health of ecosystem considerably soil ecosystem. Earthworms enlist a number of qualities that helps us to predispose them for use in environmental monitoring and remediation of soil (Reinecke and Reinecke, 2004; Ricketts *et al*., 2004) [10, 12]. A wide range of biomarkers such as behavioral, reproductive, enzymological, lysosomal, genetic, immunological, neurological and histopathological have been exalted in earthworms against pollution which helps us in assessment of soil health and soil ecosystem (Ali *et al*., 2012) [1]. *Eisenia fetida*, popularly known as red wriggler considered as convenient earthworm species for carrying out eco-toxicological studies as per recommendations of OECD. The worm fecundity test is fateful bioindicator for assessing the sublethal effects of soil pollution (Haeba *et al*., 2013) [20]. Hereupon, this disquisition focused on assessment of effects of Lead and Nickel contamination on the growth and fecundity of *Eisenia fetida*.

2. Materials and Methods

2.1. Collection of test species

The toxicity test species *Eisenia fetida* was procured from Vermicomposting unit of Department of Zoology and Aquaculture, CCSHAU, Hisar, Haryana, India.

2.2. Experimental Framework

The current disquisition was carried out in Vermi-fish technology laboratory in Department of Zoology and Aquaculture, CCSHAU, Hisar, Haryana, India. The experiment of growth and reproduction was carried out for the duration of 90 days in tubs of 40L capacity. The predecomposed cow-dung was used as substrate. Twenty healthy and clitellated *Eisenia fetida* were added in each tub. The experiment was conducted in triplicate. The heavy metals Pb and Ni were selected for this study. Cow dung alone was kept as control.

Table 1: Illustration of treatments of Pb and Ni to test species *Eisenia fetida* along with control

Sr. No.	Treatments	Concentration (in ppm)
1.	Lead	0.02, 0.04 and 0.06
2.	Nickel	0.02, 0.04 and 0.06
3.	Lead + Nickel	0.01+0.01,0.02+0.02 and 0.03+0.03

2.3. Growth parameter study

Growth parameters were measured at different time interval (30, 60 and 90 days) by the method of Lapinski and Rosciszewska, 2008 [15]. Growth was measured in term of alterations in body weight and body length of survived worms. Body weight (in gm) was measured by weighing the surviving worms on weighing balance and body length was measured in cm with the help of scale.

2.4. Reproductive parameter study

Reproductive parameters (survivability and cocoon production) were also measured at different time interval. At regular interval of 30, 60 and 90 days manual sorting was applied to determine the number of individuals and age structure, depicted by two age classes: adults and cocoons (Lapinski and Rosciszewska, 2008) [15].

2.5. Statistical Analysis

The standard statistical tools were used for analysis of data recorded during experiment. Critical difference (CD at 0.05%) was calculated between the treatments by CRD, accordingly, using software 'OPSTAT', developed at the Computer Center, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.

3. Results

Dose and time dependent changes was reported in both the heavy metals *viz.* Pb and Ni individually as well as in combination. The growth was measured in term of weight and length of earthworm. Significant difference in body weight and length (0.487gm and 4.783 cm) were measured in Pb-0.06ppm chased by Pb+Ni -0.03+0.03ppm (0.517gm and 5.957cm) and Ni -0.06ppm (0.547gm and 6.267cm) as compared to control after 90th day of experiment as demonstrated in Table 3 & 4. The present study lighted 51.67%, 61.67% and 65% survivability in Pb (0.06ppm), Pb+Ni (0.03+0.03ppm) and Ni (0.06ppm) after 90th day as demonstrated in Table 1. Significant decline in the total number of cocoon production was reported in all the treatments but maximum diminution (33.89%) was reported in the case of Pb-0.06ppm followed by Pb+Ni -0.03+0.03ppm (22.54%) and Ni -0.06ppm (17.72%) whereas the utmost cocoon production was observed in control having nil heavy metals as decoded in Table 2.

Table 1: Effect of different concentrations of heavy metals on the survivability of the earthworm, *Eisenia fetida*

Sr. No.	Treatments	Adults		
		Day 30	Day 60	Day 90
1.	Control	22.000±1.155	24.000±1.155	26.000±1.155
2.	Pb (0.02) ppm	19.667±0.882	18.667±1.453	17.333±1.764
3.	Pb (0.04) ppm	16.000±1.000	15.333±1.202	13.667±0.882
4.	Pb (0.06) ppm	12.667±0.882	12.333±0.882	10.333±1.202
5.	Ni (0.02) ppm	19.333±1.202	18.333±1.453	17.333±1.453
6.	Ni (0.04) ppm	17.667±0.882	15.000±1.000	15.667±1.453
7.	Ni (0.06) ppm	16.000±1.155	14.000±1.155	13.000±1.155
8.	Pb+Ni (0.01+0.01) ppm	18.333±1.453	17.333±1.202	15.000±1.000
9.	Pb+Ni (0.02+0.02) ppm	16.667±1.453	15.667±0.882	13.667±0.882
10.	Pb+Ni (0.03+0.03) ppm	15.000±1.000	12.667±1.453	12.333±0.882
	SE(m) ± CD (P=0.05)	1.128 3.330	1.198 3.535	1.210 3.572

Table 2: Effect of different concentrations of heavy metals on the cocoon production of the earthworm, *Eisenia fetida*

Sr. No.	Treatments	Cocoons per twenty worms		
		30 Day	Day 60	90 Day
1.	Control	46.000±1.155	55.000±1.155	60.000±1.155
2.	Pb (0.02) ppm	35.000±1.732	30.333±1.202	28.333±1.453
3.	Pb (0.04) ppm	25.667±1.453	25.667±1.764	23.667±1.453
4.	Pb (0.06) ppm	19.667±2.028	15.667±0.333	13.000±1.000
5.	Ni (0.02) ppm	32.667±1.453	30.667±0.882	27.333±0.882
6.	Ni (0.04) ppm	29.333±1.453	27.000±0.577	24.000±1.155
7.	Ni (0.06) ppm	26.333±1.202	22.333±1.764	21.667±0.882
8.	Pb+Ni (0.01+0.01) ppm	30.000±1.000	25.667±0.882	24.000±1.732
9.	Pb+Ni (0.02+0.02) ppm	28.667±1.764	23.333±0.667	22.333±1.202
10.	Pb+Ni (0.03+0.03) ppm	23.667±1.453	20.667±0.882	18.333±1.202
	SE(m) ± CD (P=0.05)	1.470 4.340	1.110 3.277	1.231 3.633

Table 3: Effect of different concentrations of heavy metals on the body weight of earthworm, *Eisenia fetida*

Sr. No.	Treatments	Body weight of earthworms (in g)		
		30 Day	60 Day	90 Day
1.	Control	0.900±0.012	0.950 ±0.012	1.020±0.012
2.	Pb (0.02) ppm	0.867±0.018	0.617 ±0.018	0.587±0.012
3.	Pb (0.04) ppm	0.813±0.009	0.603 ±0.012	0.573±0.015
4.	Pb (0.06) ppm	0.673±0.015	0.553 ±0.012	0.487±0.015
5.	Ni (0.02) ppm	0.853±0.020	0.723 ±0.015	0.677±0.018
6.	Ni (0.04) ppm	0.830±0.020	0.673 ±0.015	0.627±0.015
7.	Ni (0.06) ppm	0.767±0.023	0.647 ±0.013	0.547±0.015
8.	Pb+Ni (0.01+0.01) ppm	0.853±0.012	0.763 ±0.018	0.697±0.013
9.	Pb+Ni (0.02+0.02) ppm	0.797±0.012	0.727 ±0.012	0.657±0.007
10.	Pb+Ni (0.03+0.03) ppm	0.713±0.012	0.620 ±0.006	0.517±0.018
	SE(m) ± CD (P=0.05)	0.016 0.047	0.013 0.039	0.014 0.041

Table 4: Effect of different concentrations of heavy metals on the body length of earthworm, *Eisenia fetida*

Sr. No.	Treatments	Body length of earthworms (in cm)		
		30 Day	60 Day	90 Day
1.	Control	8.000±0.012	8.430 ±0.011	8.660±0.012
2.	Pb (0.02) ppm	7.827±0.020	7.870 ±0.011	7.783±0.015
3.	Pb (0.04) ppm	6.343±0.023	6.357 ±0.007	6.300±0.005
4.	Pb (0.06) ppm	4.843±0.026	4.900 ±0.012	4.783±0.027
5.	Ni (0.02) ppm	7.520±0.006	7.540 ±0.011	7.460±0.015
6.	Ni (0.04) ppm	6.663±0.022	6.670 ±0.012	6.620±0.006
7.	Ni (0.06) ppm	6.300±0.010	6.300 ±0.011	6.267±0.015
8.	Pb+Ni (0.01+0.01) ppm	6.673±0.018	6.680 ±0.012	6.620±0.021
9.	Pb+Ni (0.02+0.02) ppm	6.033±0.024	6.030 ±0.012	5.953±0.009
10.	Pb+Ni (0.03+0.03) ppm	5.963±0.020	6.020 ±0.012	5.957±0.012
	SE(m) ± CD (P=0.05)	0.019 0.057	0.011 0.033	0.016 0.049

4. Discussion

Growth was measured in term of alterations in body weight and body length of survived worms in each treatment whereas the fecundity was apprized by counting cocoons and the number of adults in each treatment.

4.1. Growth parameter

When the earthworms were continuously subjected to heavy metal treatment, they signalize the patterns of retarded growth in all the treatments. The result of current disquisition was supported by the study of Kaur and Hundal (2016) [5] which indicate significant decrease ($p>0.05$) in body weight when earthworms reared in contaminated samples of soil. Detention in growth of worms when exposed to heavy metals has also previously been reported by Lapinski and Rosciszewska (2008) [15]; Miguel *et al*. (2012) [26] and Bilialis *et al*. (2013) [27] which may be alleged to the avoidance behavior and low feeding activity on metal contaminated substrate. Gomez-Eyles *et al*. (2009) [28] reported loss in body weight due to reduced feeding of worms. Discontinuous pattern of body weight was observed in worms reared in Pb, Cd and Ni contaminated soil (Kaur and Sangha, 2014) [6]. In the filter paper contact test, the order of toxicity from most toxic to least toxic was Cu > Zn > Ni > Cd > Pb. In artificial soil test, the order of toxicity from most toxic to least toxic was Cu ~ Zn ~ Ni> Cd > Pb (Neuhauser *et al*., 1985) [9]. Malecki *et al*. (1982) [7] identified the concentrations of the five metals tested in these experiments that inhibit earthworm growth and reproduction. Reduction in growth rate in the present investigation was possibly due to depression of food intake and utilization of major proportion of the absorbed food energy into metabolism. If the food intake or assimilation is reduced than there will be less energy and protein available for growth so the rate will decline. However, heavy metal exposure induced certain physiological changes may also be attributed to biomass reduction, as worm feeding in treated and control soils was reported to be similar by Kreutzweiser *et al*. (2008) [29].

4.2. Reproductive parameter
Significant decline in the total number of cocoon production was reported in all the treatments but maximum diminution was reported in the case of Pb-0.06ppm. Lower reproduction rates in heavy metal exposed worms may be due to detention in copulation (Novais *et al*., 2011) [21], demritorious gamete development (Rongquan and Canyang, 2009) [22], egregious embryo development (Kumar *et al*., 2008) [23], lesser hatchling success and anon growth due to stress (Sivakumar *et al*., 2009) [24]. Kaur and Hundal (2016) [5] reported least numbers of cocoons in soil samples which had very high concentrations of the Pb, Cr and Ni. Kaur and Sangha (2014) [6] reported least number of cocoons in sample 3, probably due to the high concentration of heavy metals Pb and Cd in this sample of soil. Cocoon production was observed to be the most susceptible and impressed parameter of heavy metal toxicity which may also be due to deferred maturation of worms and contaminated substrate as stated by Garg *et al*. (2009) [25]. Kaur and Sangha, 2014 [6] reported mortality of earthworm *Eisenia fetida* when exposed to Pb, Cd and Ni contaminated soil. This current disquisition is also supported by previous findings of Reinecke *et al*. (2001) [11], indicating that heavy metals affect cocoon viability. Van Straalen *et al*. (1989) [14] calibrated that the contamination of heavy metals over a long time alter the ultimate survival of earthworms. Many workers reported that heavy metals effect on the survival and cocoon production increases the rate of mortality (Malecki *et al*., 1982; Bengtsson *et al*., 1986) [7, 2].

5. Conclusion
The current disquisition adduced fecundity was more susceptible to Pb and Ni contamination than growth. Although, growth was found affected but acute effect was perceived on reproduction i.e. cocoon production. Both the heavy metals (Pb and Ni) individually as well as in combination displayed negative effect on growth and reproduction. Disquisition of the current discipline can be used to framework and execution of remediation strategies for the metal polluted soils in areas where risk of metal contaminated soil is associated with population dynamics of soil invertebrates.

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