Journal of Applied and Natural Science 8 (1): 208 - 212 (2016)

JANS

Effect of heavy metals on the survival, growth and development of earthworm *Eisenia fetida*

Gaganmeet Kaur* and S.S. Hundal[1]

[1]Department of Zoology, Punjab Agricultural University, Ludhiana – 141004 (Punjab), INDIA
*Corresponding author. Email: gaganmeet02@gmail.com

Received: May 28, 2015; Revised received: September 24, 2015; Accepted: February 15, 2016

Abstract: The present laboratory study was carried out to determine the effect of the metal contaminated soil on the survival, growth and development of *Eisenia fetida.* Metal contaminated soil samples were collected from five different sites of Ludhiana city which is an industrial hub of Punjab. Maximum heavy metal concentrations were recorded in soil sample taken from hambran around the Buddha Nullah and minimum concentration of heavy metal from haibowal. The level of heavy metals chromium and nickel (347 ppm and 189 ppm) were higher than permissible limits according to CCME (2001), whereas lead (42.6 ppm) was within permissible limits. Adult *E. fetida* were exposed to different concentrations of heavy metals in artificial soil for 28 days. Significant effects were observed on growth and reproduction of the tested organisms. There was 100% survival of *E. fetida* in soil amended with lead, chromium and nickel along with morphological abnormalities such as extrusion of coelomic fluid. It was observed that the worms in the low and high doses of heavy metal treated substrate produced cocoons with a one week and two week delay, respectively, *vis-a-vis* the control group. Furthermore, the emergence of hatchlings started one week earlier in control than in soil treated with medium and high concentration of metals. It is safely concluded that presence of heavy metal in soils adversely affects the growth, reproduction and development of *E. fetida.* Results of the study indicated that reproduction was more sensitive to higher concentrations of heavy metal contaminated soil than survival or weight change.

Keywords: *Eisenia fetida*, Heavy metals, Reproduction, Soil

INTRODUCTION

Soil is a dynamic and complex system functioning as habitat for microorganisms, plants, micro and macro fauna which has a direct relation with humans. Now a days, contaminated soils have become of concern, since they lead to groundwater contamination and biomagnification of chemical compounds through food webs and effect human health (Loureiro *et al.,* 2005). Metal pollution may disturb soil ecosystems by affecting the structure of soil invertebrate populations. Generally, the potential hazards of various environmental toxicants to soil invertebrates are assessed by bioassays with the keystone species – earthworms. Earthworms are one of the first receptors affected by soil contamination. They are more susceptible to metal pollution than many other groups of soil invertebrates. Elevated concentration of heavy metals in soils can affect the density, viability, cocoon production, growth and sexual development of earthworms. Heavy metals cause mortality (Davies *et al.,* 2003) and affect the population size, avoidance and species diversity of earthworms (Spurgeon *et al.,* 2005). Reproduction is likely to be of particular importance in eco-toxicological assessment because of its influence on population dynamics. The main aim of the present study was to determine the effect of heavy metal exposure on survival, growth and development in the earthworm with *Eisenia fetida* as a model species.

MATERIALS AND METHODS

The present study was conducted by collection of metal contaminated soil samples from five different sites viz., Tajpur Road, Jalandhar bypass, Hambran, Haibowal, Field Ganj in the vicinity of Buddha Nullah, Ludhiana (Punjab, India) (30° 4' N, 75° 5' E). All the soil samples were collected at least 2 meters away from the main stream and about 5 kg of soil was collected from top 2 cm layer at each site, after removal of surface vegetation and litter, in transparent polythene bags. Coloured bags were not used to avoid any extra contamination of soil samples. All the bags were marked with respect to the sites from which the samples were collected. The collected soil samples were brought to the laboratory and the soil aggregates were broken up while still damp and placed in an oven at 60ºC to dry and later sieved. Metal content in soil was analysed by taking approximately 2g of test sample of soil with 20ml aqua regia and placed overnight. The digested matter was then diluted to 50ml with distilled water. The solution was analysed by Inductively Coupled Plasma-Atomic Emission Spectrometry

Table 1. Concentrations of different heavy metals in soil samples.

Site Location (Soil Sample)	Concentration of heavy metals (ppm)*		
	Lead (Pb)	Chromium (Cr)	Nickel (Ni)
Tajpur road (Samrala chowk) (S1)	27.14	99.86	78.93
Jalandhar bypass (S2)	23.59	95.41	108.3
Hambran (S3)	42.6	347	189
Haibowal (S4)	13.2	46.0	42.6
Field ganj (S5)	31.0	146	117

(ppm)*is the concentration parts per million

(ICP-AES) in the Department of Soil Science, Punjab Agricultural University, Ludhiana.

Preparation of artificial soil: The artificial soil was prepared following OECD (Organisation for Economic Cooperation and Development) guideline No.207. The medium consisted of 70% sand, 20% clay (kaolin clay) and 10% organic matter (as *Sphagnum* peat) on dry weight basis. Sand was obtained from a local market, the kaolin clay from a pottery supplier and the *Sphagnum* peat from a local garden centre and the pH of the soil was adjusted to 6.1 with calcium carbonate. To allow accumulation rates in the artificial soil to be compared with the contaminated soils, metals were added to the soil in similar ratios to those found at collection sites. Salts of lead, chromium and nickel nitrates were mixed with soil to give dry weight concentrations of 42.6 mg/kg (0.0426g in 1000g soil), 70.0 mg/kg (0.07g in 1000g soil), 112 mg/kg (0.112g in 1000g soil) lead, 64.0 mg/kg (0.064 g in 1000 g soil), 173.5 mg/kg (0.1735g in 1000g soil), 347 mg/kg (0.347g in 1000g soil) chromium, 50.0 mg/kg (0.050g in 1000g soil), 94.5 mg/kg (0.0945 g in 1000g soil), 189 mg/kg (0.189g in 1000g soil) nickel. The dry soil was moistened with distilled water to obtain approximately half of the final required water content. Three replicates were used for each test and a control test was maintained for comparison.

Study of reproductive parameters: Juvenile forms of *Eisenia fetida* were obtained from the stock culture maintained at the laboratory. The selected worms (10 in number) were acclimatized for seven days, washed and weighed. These worms were placed into each of three replicate trays and after 3 hours they were checked to ensure that all the worms had burrowed into the soil. This was designated as day one of the experiment. The substrate was supplemented with dried and sterilized FYM every week. Plastic trays were covered with a muslin cloth to avoid the moisture loss. Optimum growth conditions of temperature, pH and moisture were maintained and worms were hand sorted from all the soil samples and monitored regularly for survival, weight change, cocoons laid and hatchlings present on days 7, 14, 21 and 28 of the experiment. Mortality was determined by counting the number of dead earthworms. Worms were considered dead if they lacked any movement and did not respond to a definite tactile stimulus to the anterior end. Cocoons were separated from each container by hand sorting. These cocoons were further used for studying different life stages of *E. fetida.* Cocoons were observed after every second day to observe hatching. Number of juveniles hatched per cocoon per week were also recorded in all samples of soil.

Statistical analysis: The data was statistically analyzed by analysis of variance (ANOVA) using computer software CPCS1.

RESULTS AND DISCUSSION

Survey of soil: The soil samples were analyzed and the values of predominant heavy metals - lead, chromium and nickel - are depicted in Table 1. Maximum concentration was of lead, chromium and nickel i.e. 42.6 ppm, 347 ppm and 189 ppm in soil samples taken from Hambran area followed by Field Ganj (centre of Ludhiana city), Tajpur Road and Jalandhar Bypass

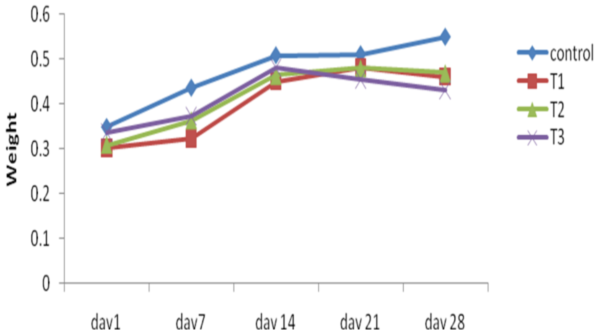

Fig. 1. *Weekly changes in body weights (g) of adults of E. fetida reared in different treated samples of soil.*

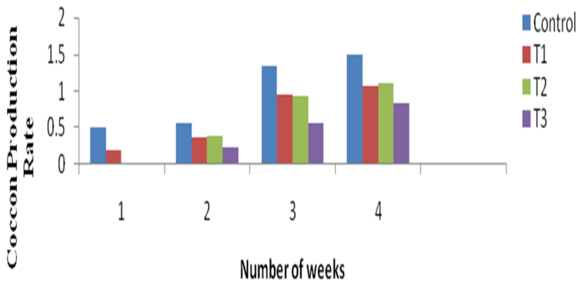

Fig. 2. *Cocoon production rate of E.fetida in metal contaminated and control soil samples.*

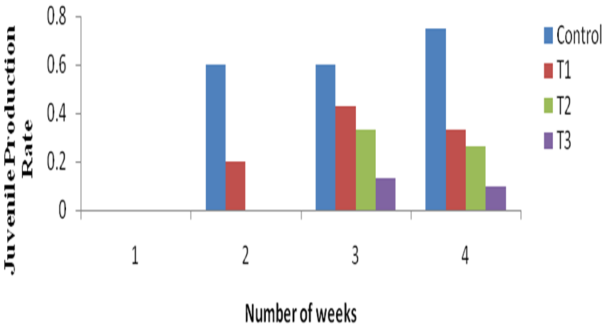

Fig.3. *Juvenile production rate of E. fetida in metal contaminated and control soil samples.*

areas of Ludhiana city. Minimum concentration of lead, nickel and chromium was found in soil sample taken from Haibowal. The levels of concentration of heavy metals in various areas were probably due to the establishment of dyeing and electroplating industries in those areas as has also been recorded by Kaur and Sangha (2014a) who also recorded cadmium to be a pollutant in addition to lead and nickel whose concentrations were 4.18 ppm for lead, 2.29 ppm for nickel and 0.29 ppm for cadmium. Our study, however, revealed chromium to be at higher concentration than cadmium, lead and nickel. The results of present study showed the values of chromium and nickel concentration to be higher than their permissible limits according to CCME (2001) whereas, lead was within permissible limits. Earlier, Hirano and Tamae (2011) have suggested that metal pollution affects the structure of soil invertebrate communities and the bioaccumulation ability of earthworms enables them to be used as organisms for biomonitoring of soil pollution, thus supporting our choice of earthworm to monitor heavy metal concentration.

Survival: The near natural mixture of heavy metals in the artificial soil prepared, mimics the natural composition available in the soil of the localities sampled, which had heavy metal concentration due to various anthrogenic activities. *E. fetida* survived in all samples treated with lead, chromium and nickel with mixtures of doses - low (42.6 mg/kg lead, 64.0 mg/kg chromium and 50.0 mg/kg nickel), high (70.3 mg/kg lead, 173.5 mg/kg chromium and 94.5 mg/kg nickel) and very high (112 mg/kg lead, 347 mg/kg chromium and 189 mg/kg nickel). There was 100% survival of *E. fetida* in all soil samples. The results of the present study are in agreement with Honsi *et al.* (2003) who reported that there is no effect on survival of earthworm *E. fetida* exposed to heavy-metal (Cu, Zn, Cd and Pb) contaminated soils in Norway despite the fact that the concentrations of metals were high (max Pb – 8750 µg g–1, Cd – 110 µg g–1 soil). These concentrations were 78 times more than of present study indicating the high levels of tolerance exhibited by the earthworm. Extrusion of coelomic fluid as a response was observed in contaminated soil samples of present study which has been reported earlier too (Rao *et al.,* 2003). Though the reaction of coelomic fluid extrusion seemed to be a common factor, survival of the worms was of prime importance. High level of aluminium in acidic red soil was found to be harmful to *E. fetida* (Zhang *et al.,* 2013) and led to certain morphological abnormalities like tail shedding, body fragmentation have also been recorded by Kaur and Sangha (2014a). No such morphological abnormalities were observed during the present study.

Changes in body weight: In control sample of soil, non-significant decrease ($p<0.05$) in body weight was observed, while a significant decrease ($p>0.05$) in body weight was observed in worms reared in all the contaminated samples of soil as shown in Fig. 1. There have been reports which support the findings of body weight reduction due to metal exposure in the present study (Berthelot *et al.,* 2008; van Gestel *et al.,* 2009). The worms living in metal polluted soils reached the lower weight or needed more time to reach the maximum weight than in non-polluted sites (Spurgeon and Hopkin, 1996). Heavy metal concentration in the substrate significantly reduced the growth of *E. fetida* (Matuseviciute *et al.,* 2005). The results of the present study are in agreement with the work of Zaltauskaite (2010) who reported that *E. fetida* exposed to different concentrations of lead in soil tended to lose more weight than those in control.

A study by Shahmansowri and coworkers (2005) revealed that though heavy metals like of Cr, Cd, Pb, Cu and Zn were bioaccumulated by *E. fetida,* there was a decrease in body weight of worms when exposed to higher concentration of heavy metals. Similar decrease in body weight of earthworm *Pheretima guillelmi* at high concentration of lead in soil has been recorded by Zheng and Canyang (2009). Among *E. albidus, E. crypticus* and *E. fetida, E. fetida* has been found to be the most sensitive species with decrease in weight when the industrial waste material avoidance tests were conducted by Kobeticova *et al.* (2010). The weight of worms was significantly affected by high levels of heavy metals in the study of Miguel *et al.* (2012).

Cocoon production rates: During our experiments, the cocoons were recorded one week after earthworms (ten in each tray) were introduced in the soil samples. Maximum cocoon production was observed in control soil sample, due to the least or near nil concentration of heavy metals which favoured their growth and reproduction and is depicted in Fig.2. Minimum numbers of cocoons were collected from those soil samples which had very high concentrations of the heavy metals lead, chromium and nickel. Delayed cocoon production in all the contaminated soil samples showed that cocoon production rates were particularly sensitive during early period of reproduction as also reported by Spurgeon and Hopkin (1995) and later Fordsmand *et al.* (1998) had observed that reproduction was the most sensitive parameter which reduced at high nickel concentration in soil. Reduction in cocoon production due to lead and cadmium exposure has been reported in several studies (Spurgeon *et al.,* 1994; Savard *et al.,* 2007). Low cocoon production rate in heavy metal polluted soils by *E. fetida* has also been reported by Maleri *et al.* (2007).

Emergence of hatchlings: In control sample, hatchlings started emerging one week earlier than treatment samples, and the total number of hatchlings was also more in case of uncontaminated soil sample (Fig. 3). Lesser number of hatchlings in each contaminated soil sample was due to the elevated level of heavy metals (lead, chromium and nickel), which was directly proportional to the concentration of heavy metals.

Several studies have noted that lead nitrate significantly reduces the hatching success of cocoon produced by *E. fetida* (Reinecke and Reinecke 1996; Reinecke *et al.,* 1997). The effects of chromium on growth and reproduction of *E. fetida* was examined by Molnar *et al*. (1989) which showed that reproduction after 8 weeks was the measure most sensitive to chromium with 55% decrease in number of cocoons and hatchlings at 625ppm chromium. Cocoon viability was affected negatively by lead, making it a sensitivity endpoint (Maboeta *et al.,* 1999), while Nahmani *et al.* (2007) suggested that life cycle parameters, such as cocoon production and hatching rate, of *E. fetida* were more sensitive to metal pollution than survival or weight change. While studying the effect of metal contaminated soil on *E. fetida* (Kaur and Sangha, 2014b) observed that the elevated metal concentration of nickel and lead in soils added to the sensitivity of the worms which resulted in the increase in duration to reach sexual maturity in juveniles. These observations are in close association to the record of the present study.

Conclusion

The study indicates that the presence of heavy metals in soils is detrimental to the growth of the *E. fetida* and acute effects were seen on cocoon production and hatchlings produced. The results of the study provide evidence that reproduction of *E. fetida* is more sensitive than survival and weight change. The population and presence of earthworms is, considered to be, an indicator of soil health and these findings could be related to remediation strategies of metal polluted soils.

REFERENCES

Berthelot, Y., Valton, E., Auroy, A., Trottier, B. and Robidoux, P.Y. (2008). Integration of toxicological and chemical tools to assess the bioavailability of metals and energetic compoundsin contaminated soils. *Chemosphere,* 74 : 66–177.

CCME. (2001). Canadian water quality guidelines for the protection of aquatic life: Canadian Water Quality Index 1.0 Technical Report. In Canadian environmental quality guidelines, 1999. Winnipeg, Manitoba.

Davies, N. A., Hodson, M. E. and Black, S. (2003). The influence of time on lead toxicity and bioaccumulation determined by the OECD earthworm toxicity test. *Environ Pollut.,* 21:55-61.

Fordsmand, S. J. J., Weeks, M. J. and Hopkin, S. P. (1998). Toxicity of Nickel to the Earthworm and the Applicability of the Neutral Red Retention Assay. *Ecotoxicol.,* 7 (5): 291-95.

Hirano, T. and Tamae, K. (2011). Earthworms and soil pollutants. *Sensors (Basel*). 11 (12): 11157-67.

Honsi, T. G., Stubberud, H. E., Andersen, S. and Stenersen, J. (2003). Lysosomal fragility in earthworms (*Eisenia veneta*) exposed to heavy metal contaminated soils from two abandoned pyrite ore mines in Southern Norway. *Water, Air Soil Pollut.,* 142 : 27–37.

Kaur, K. and Sangha, G. K. (2014a). Effects of metal contaminated soils on *Eisenia fetida* (Savigny) at Ludhiana (Punjab), India. *J. Appl. Nat. Sci.,* 6 (2): 519-523.

Kaur, K. and Sangha, G.K. (2014b) Effects of metal contaminated soil on the survival, growth and duration of life span of juveniles of earthworm *Eisenia fetida* (Savigny). *Ind J Ecol.,* 41(2): 316-319.

Kobeticova, K., Hofman, J. and Holoubek, I. (2010). Ecotoxicity of wastes in avoidance tests with *Enchytraeus albidus, Enchytraeus crypticus* and *Eisenia fetida* (Oligochaeta). *Waste Management.* 30 (4) : 558-64.

Loureiro, S., Soares, A. M. V. M and Nogueira, A. J. A. (2005). Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. *Environ Pollut.,* 138: 121-31.

Maboeta, M. S., Reinecke, A. J. and Reinecke, S.A. (1999). Effects of low levels of lead on growth and reproduction of Asian earthworm *Perionyx excavatus* (Oligochaeta). *Ecotoxicol Environ Saf.,* 44(3): 236-40.

Maleri, R., Reinecke, S. A., Przybylowicz, J. M. and Reinecke, A. J. (2007). Growth and reproduction of earthworms in ultramafic soils. *Arch Environ Contam Toxicol*. 52(3): 363-70.

Matuseviciute, A. and Eitminavicivte, I. (2005). Effects of different cadmium concentrations on survival, reproduction and adaptation of *Eisenia fetida* Californica. *Acta Zool lit.,* 15: 361-69.

Miguel, A., Domínguez, C. Z., Hernández, E. S., Aidé, M., Huerta, T., De la Luz, X. M., Rodríguez, N., Barajas, C. E. and Vela, A. F. (2012). Effect of the heavy metals Cu, Ni, Cd and Zn on the growth and reproduction of epigeic earthworms (*E. fetida*) during the vermistabilization of municipal sewage sludge. *Water Air Soil Pollut.,* 223(2):915-31.

Molnar, L., Fischer, E. and Kallay, M. (1989). Laboratory studies on the effect, uptake and distribution of chromium in *Eisenia foetida* (Annelida, Oligochaeta). 223: 57-66.

Nahmani, J., Hodson, M. E. and Black, S. (2007). Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. *Environ Pollut.,* 49: 44-58.

Rao, J. V., Kavitha, P. and Rao, A. P. (2003). Comparative toxicity of tetraethyl lead and lead oxide to earthworms, *Eisenia fetida. Environ Res.,* 92 : 271-76.

Reinecke, A. J. and Reinecke, S. A. (1996). The influence of heavy metals on the growth and reproduction of the compost worm *Eisenia fetida* (Oligochaeta). *Pedobiologia*., 40: 439-48.

Reinecke, A. J., Maboeta, M. S. and Reinecke, S. A. (1997). Stimulating effect of low lead concentrations on growth and cocoon production of *Eisenia fetida* (Oligochaeta). *South Afr J Zool.,* 32: 72–75.

Savard, K., Berthelot,Y., Auroy, A., Spear, P. A., Trottier, B. and Robidoux, P. Y. (2007). Effects of HMX-lead mixtures on reproduction of the earthworm *Eisenia andrei*. *Arch Environ Contam Toxicol.,* 53: 351–58.

Shahmansouri, M. R., Pourmoghadas, H., Parvaresh, A. R. and Alidadi, H. (2005). Heavy metals bioaccumulation by Iranian and Australian earthworms (*Eisenia fetida*) in the sewage sludge vermicomposting. *Iranian J Environ Health Sci Engg.,* 2: 28-32.

Spurgeon, D. J. and Hopkin, S. P. (1995). Extrapolation of the laboratory based OECD earthworm toxicity test to metal-contaminated field sites. *Ecotoxicol.,* 4: 190-205.

Spurgeon, D. J. and Hopkin, S. P. (1996). Effects of metal-contaminated soils on the growth, sexual development, and early cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. *Ecotoxicol Environ Saf.,* 35: 86-95.

Spurgeon, D. J., Hopkin, S. P. and Jones, D. T. (1994). Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ Pollut.,* 84:123–30.

Spurgeon, D. J., Ricketts, H., Svendsen, C., Morgan, A. J. and Kille, P. (2005). Hierarchial responses of soil invertebrates (earthworms) to toxic metal stress. *Environ Sci Tech.*, 39:5327-34.

van Gestel, C. A. M., Koolhaas, J. E., Hamers, T., van Hopper, M., van Roovert, M., Korsman, C. and Reinecke, S. A. (2009). Effects of metal pollution on earthworm communities in a contaminated floodplain area: linking biomarker, community and functional responses. *Environ Pollut.,* 157: 895–903.

Zaltauskaite, J. and Sodiene, I. (2010). Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm *Eisenia fetida. Ekol.,* 56: 10-16.

Zhang, J., Yu, J., Ouyang, Y. H. and Xu, H. (2013). Responses of earthworms to aluminum toxicity in latosol. *Environ Sci Pollut Res.,* 20 (2): 1135-1141.

Zheng, R. and Canyang, L. (2009). Effect of lead on survival, locomotion and sperm morphology of Asian earthworm, *Pheretima guillelmi. J Environ Sci.,* 21 (5):691-95.