ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/357527802

Relative toxicity of Lead and Nickel on acid and alkaline phosphatase in epigeic earthworm Eisenia fetida (Savigny, 1826).

Article · January 2021

CITATIONS
0

READS
22

3 authors, including:

Partha Pratim Chakravorty
Raja Narendralal Khan Women's College(Autonomous)
70 PUBLICATIONS **221** CITATIONS
SEE PROFILE

Atanu Dey
Vidyasagar University
3 PUBLICATIONS **1** CITATION
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

1. Ecophysiological---Soil microarthropod . View project

Biomonitoring of selected heavy metals in fly ash contaminated soil using non-target selected species of soil Collembola Submitted by: Priyanka Sarang View project

All content following this page was uploaded by Partha Pratim Chakravorty on 03 January 2022.

The user has requested enhancement of the downloaded file.

Relative toxicity of Lead and Nickel on acid and alkaline phosphatase in epigeic earthworm *Eisenia fetida* (*Savigny*, 1826)

Atanu Dey[a], Partha Pratim Chakravorty[a,*], Subrata Kumar De[b]

[a]*PG Department of Zoology, Raja Narendra Lal Khan Women's College (Autonomous), Paschim Midnapore, West Bengal 721102, India.*

[b]*Department of Zoology, Vidyasagar University, Midnapore (West), West Bengal 721102, India,*
E-mail:parthapratimchakravorty@yahoo.in

ARTICLE INFO

Received: 05.10.2020;
Revised: 02.01.2021;
Accepted: 10.01.2021

Keywords: *Lead, Nickel, Eisenia fetida,acid phosphatase (ACP), alkaline phosphatase (ALP).*

ABSTRACT

Among the soil fauna, earthworms are not only acting as bioindicator to determine soil pollution but also they provided with some specific enzymatic biomarker to decode the soil contamination. In the present heavy metal toxicity study, the LC50 of lead (Pb) and nickel (Ni) were determined in both artificial and natural ground soil by acute toxicity test (14 days) in *Eisenia fetida.* Low observed effective concentration (LOEC) of mixture of both metals (Pb and Ni) were also determined through repetitive experimental acute toxicity test. In the chronic toxicity test (28 days), the experimental set up had been arranged as control (C), T1(1506.25 mg Pb), T2 (3012.5mg Pb), T3 (193.75mg Ni), T4 (387.5mg Ni), T5 (753.125mg Pb and 96.875mg Ni) and T6(1506.25mg Pb and 193.75mg Ni) per Kg of dry soil. After end of chronic periods, specific activity of acid and alkaline phosphatase were determined in the earthworm tissue. The mean difference of recorded specific activity values of both enzymes were significant ($P<0.05$) and also showed a significant negative correlation ($P<0.05$) between the specific activity both enzymes.

1. Introduction

Several kinds of anthropogenic activity and use of agrochemicals greatly affects the normal ecological health of soil that is correlated with physiological health of terrestrial biome. Heavy metal pollution is an ecophysiological threat to paedo-fauna that increases in now days.This type of pollution of soil is responsible for beginning of bioaccumulations followed by biomagnifications in terrestrial ecosystem. In the last decades, soil pollution has been enhanced enormously due to industrial activities, urban sewage, intensive use of biocides and chemical fertilizers. Diffusible heavy metals contamination occurs in soil from variable sources, such as agricultural field,

industrial wastes, mining and sludge residues (Wuana et al., 2011). Mandal and Sengupta (2002) reported that the coal fly ash contains heavy and trace metals like arsenic, beryllium, cadmium, chromium, copper, cobalt, nickel (Ni), lead (Pb) etc. Heavy metals released from coal-fired thermal power station and deposited in soil, significantly responsible for environmental degradation of surrounding areas (Khillare et al., 2012). Heavy metals do not decompose or disappear from soil although their release to the ecosystem can be controlled by specific way of treatment (Brusseau, 1997). The sources of metal pollution include natural sources (Hobbelen et al., 2006), agricultural activities, municipal, industrial waste, sewage sludge etc (Salehi and Tabari, 2008), and also comes from particulates from aerial deposition of vehicular emission (Ward and Savage, 1994). The heavy metals are diffused in the soil rapidly (Lanno et al., 2004). Previous studies exhibited that earthworms accumulate metals, such as Cd, Cr, Cu, Co, Ni, Pb, Zn from soil under both field and laboratory conditions (Li et al., 2010). Kammenga et al. (2000) reported that earthworms acts as good sentinel organisms of soil pollution because they contact with soil pore water directly. In the environment, earthworms are frequently exposed to heavy metals and so their efficient detoxification systems maintain certain molecules that acts as molecular biomarkers of soil contamination. The earthworm acts as good bioindicator in terrestrial ecosystem due to interest of pollution (Suthar et al. 2008). Different kinds of soil bioindicator and biochemical indicator has been worked out in soil fauna by many researcher. Enzymes are called as biological catalysts that catalyzed several reactions on its substrate molecule to produce product, in association with specific specificity and kinetics under suitable environmental (external and internal) parameters. Biochemically, the enzymes are simple or complex protein molecules produced in the living cells where they were performing various physiological reactions. The assessment of any the environmental effect of a pollutant on mortality test of animal was a colloquial measure. However, sublethal effects of heavy metals acts as gradual and indicative physiological modification can be found as detrimental as mortality to the animal's survivability (Sonawane, 2017).Quantitative assessment of biocatalyst is a plausible indicator of environmental pollution such as heavy metal contamination that affect physiological and biochemical condition of organisms. Physiological activity of many enzymes including lysosomal hydrolytic enzymes are inhibited by heavy metals(Cheng, 1983).Lysosome contains different kinds of acid hydrolases are responsible for intracellular digestion (Berhet, 1965; de Durve and

Wattiaux 1966).The distribution of both acid phosphatase (ACP) (EC 3.1.3.2) and alkaline phosphatase (ALP) (EC 3.1.3.1) are differing in the cellular and sub cellular localization. Lysosomal enzyme, ACP catalyzes the phosphomonoester substrates in acidic condition pH, 4.8. ALP ismultifunctional enzyme, catalyzes phosphomonoester substrates in alkaline medium pH, 9.8 (Bhargavan, 2010; Sadasivam and Manickam, 2015). The specific activity of acid phosphatase and alkaline phosphatase is a biochemical indicator during hyperactivity of lysosomal digestion process that also involving with the metallic stress (Bhargavan, 2010). ACP is an important lysosomal marker enzyme and ALP is frequently occurs in the fraction containing plasmalemma. These enzymes are associated with the cell differentiation and growth of organisms. (Ram and Sathyanesan, 1985). Bhattacharya et al. (1975) and Anees (1976) reported that the activity of ACP and ALP enzymes being changed with the treatment of endrin and heavy metals like Cu, Hg and Zn on fish *Clarius batrachus* and in the fish *Channa punctatus* respectively. The enzymatic efficiency become changed by metallic exposure in some way associated with the response of physical and physiological parameters, co-factors, and also its Michaelis-Menten constant (K_m) (Jackim, 1974). The inhibition of acid phosphatase activity and the acceleration of the alkaline phosphatase activity were found after fungicide exposure (Chakravorty et. al., 2017). Ukpabi et al. (2013) reported that the activity of ALP and ACP could be used as biomarkers for detection of cellular damage after the treatment of metal contaminated fertilizer in *E. fetida* or for soil contamination surveys.

This experiment a attempt to study the effect of selected heavy metals on the activity of acid phosphatase and alkaline phosphatase. The present work has been done for evaluating the LC50 of Lead and Nickel in acute toxicity followed by chronic toxicity that determine the specific activity of acid phosphatase and alkaline phosphatase in *Eisenia fetid* after exposure with sublethal doses of lead, nickel and their combination of lowest observed effective concentration (LOEC) value.

2. Materials and methods

2.1. Sample collection and culture

The sample specimens *(Eisenia fetida,* red wiggler worm*)* was collected from WBCADC (*West Bengal Comprehensive Area Development Corporation*) Tamluk Project that is regulated under Panchayet & Rural Development Department, Govt. of West Bengal, India. The specimens has been cultured in vermicomposting pit in Raja Narendra Lal Khan Women's College. Gope Palace, P.O- Vidyasagar University, Dist: Paschim Midnapore, Pin: 721102. The vermiculture

unit located in natural environment with shadow shade area. The vermicomposting cum cultural pit had been covered by fine meshed iron net to avoid unwanted contamination. The substrate medium consists of pesticide free fine ground soil with dried cow dung manure. Finely grinded soil particles was mixed with cow farmyard manure as 1:1 ratio and was used as the culture medium for the specimens (Ismail, 1997). The hand sorted cocoons became separated and cultured in a separate pots, those were used as experimental specimens later. The refined water has been used for maintaining moisture. The dust cow dung manure has been utilized as food for the growing test specimen and gave at a specific interval of time. The compost use as biofertilizer in the flower garden of this college. Chakravorty et al., (2017) pointed out that *Eisenia fetida* are easily breed and culture at the Environmental Test Chamber in laboratory with different kinds of organic medium. Therefore, the species is very scientifically specific for ecophysiological and toxicological studies.

2.2. Acute toxicity test

Adult age synchronized *Eisenia fetida* worms (with visible clitellum) were blotted on filter paper and weighed individually. The worms were acclimated for 24 hrs after removal from the mother culture prior to experimental use, washed with redistilled water and then hold on wet filter paper in the dark environmental chamber at desirable temperature (28°C ±0.5°C) and humidity (60-65%). This process was allowed to defecation of gut contents (Rathi et al., 2011). Acclimated *Eisenia fetida* was used for the acute toxicity test in both artificial soil and natural ground soil medium. Age synchronized individual worm weighing about 270-290 mg was selected for exposure in this test. Experimental culture of specimens were done in inert polythene boxes (16 X 12 X 1 cm, total area, 192 cm^2). The artificial soil was comprised (by dry weight) of 70% quartz sand, 20% kaolinite, and 10% finely sieved paddy husk, with the pH adjusted to 6.0 ± 0.5 by the addition of calcium carbonate ($CaCO^3$) (De Silva *et al.*, 2009; Sanyal et al., 2015). The LC50 of lead (Pb) and nickel (Ni) was also determined in the ground soil. The LC50 of lead (Pb) and nickel (Ni) were determined in both artificial and natural ground soil as per acute toxicity test, OECD guideline 207. From pesticide free grassland, the collected soil samples were sun-dried, grinded and sieved as particle size of 0.25 mm that acts as the test medium or substrate. Individually Lead acetate trihydrate [$(CH_3COO)_2Pb._3H_2O$] and *Nickel acetate tetrahydrate* [$(CH_3.COO)2Ni.4H_2O$)] are used for contamination of the experimental soil. Three replicates was kept up for each set of experiments together with control set simultaneously. The experimental inert boxes

were remained as undisturbed for 48 hours before inoculation of worms for softening of medium and thermo-stabilization. Defecated and acclimated earthworms (10 pieces per box) were inoculated in each experimental boxes.

The experimental set up was performed in an Environmental Test Chamber at a constant temperature of 28 ± 0.5°C and 60-65% relative humidity. The entire experiment was performed for three times (Dasgupta et al., 2010).

The physiochemical parameters of both artificial soil and natural soil such as Organic carbon Content, moisture content, and pH were determined in constant room temperature and moisture content. Infrared Torsion balance moisture meter utilized for determination of moisture content of the soil (Chakravorty, 1990). The pH and organic carbon content of both soil were determined by the method of Piper (1942) in stable temperature and moisture. Those specimens showed no observable evidence of life after every 7days of interval, even when poked with a blunt needle, were considered as dead and were removed from the box due to avoid unwanted contamination. The soil was checked at specific regular interval (weekly) for detection of moisture loss by weighing the test containers and replenished with redistilled water if required. After end of the study (14 days), the mortality were assessed by EPA probit analysis program 1.5 (US EPA, 2006).

2.3. Chronic toxicity test

The sublethal doses of LC50 of two selected heavy metals (Pb and Ni) were used individually and jointly for chronic toxicological study of bioaccumulation and metallothionein response in the above described specimen. The chronic toxicity test was performed in similar way as described above in acute toxicity test in ground soil for a period of 28 days of study. Very finely grinded (0.25 mm) and dried cow dung manure (5 g dry weight)) was added to the test soil medium weekly to provide food for the growing worms (Chakravorty et al., 2017). Two individual sub-lethal doses of lead(Pb), nickel(Ni), and combination of lead with nickel (Pb-Ni) were applied in ground soil, along with control (C) for determining the bioconcentration factor and metallothionein response. 25% (T1 -1506.25 mg) and 50% (T2 - 3012.5 mg) of the LC50 values of the lead(Pb) and 25% (T3 – 193.75 mg) and 50% (T4 – 387.5 mg) of the LC50 values of the nickel(Ni) were applied on garden soil (Kg) for metallic exposure. The test concentrations of heavy metals combination were determined after repetitive conduction of preparatory trial experiment. The lowest observed effective concentration (LOEC) for each metals was chosen for final experimentation (Todorova et al. 2015). The mixture of both heavy metals,

lead and nickel was applied on soil (Kg) as 12.5% (T5 - 753.125 mg Pb and 96.875 mg Ni) and 25%(T6 – 1506.25 mg Pb and 193.75 mg Ni) of the LC50 values of the respective lead and nickel metals. The specimens were introduced in the experimental boxes, after that the boxes are placed in an Environmental Test Chamber and maintaining constant temperature of 28°C (±0.5°C) and 60-65% relative humidity. The earthworms were removed from the container after the end of chronic experimental period. The worms were cleanup with redistilled water followed by blotting with paper towels and then the worms were subjected for experimental findings.

Instruments Used:

Instruments	Company and Model N
ectronic Balance	Mettler Toledo (New Classic MS)
Environmental Test Chamber	IIC-INSTIND
Homogenizer	Remi Electrotechnik L (Type RQP-127/A).
Centrifuge	Remi Cooling Centrifu (C-24BL).
ectrophotometer	Systronics (UV-VIS Spectrophotometer 11

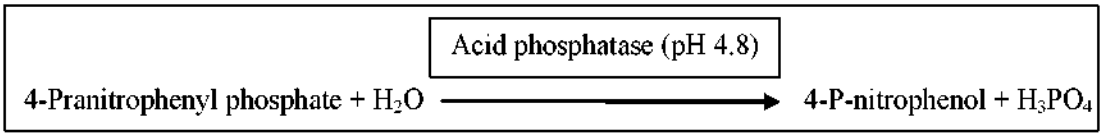
2.4. Determination of acid phosphatase activity (ACP)

The activity of acid phosphatase was measured as described by Walter and Schutt, (1974). 250 mg fresh earthworm tissue was taken in homogenizing tube and subjected to homogenization in 5 ml normal saline. The homogenized sample was then centrifuged at 10000 rpm for 10 minutes at normal room temperature. 0.2 ml supernatant was taken in a test tube in which 1 ml acid buffer was added and mixed thoroughly. A blank was prepared by giving 0.2 ml of 0.7% saline to 1 ml acid buffer. The test tubes were keep in the incubator for 30 minutes at 37° C. After the incubation period, 2 ml of 0.1 N NaOH solution was added to the test tubes and were mixed absolutely. The amount of liberated p-nitrophenol in tissue sample mixture gives an intense yellow colour that was measured spectrophotometrically at 405 nm wave length after adjusting the absorbance of the blank. A standard curve was drawn with the known amount of paranitrophenyl phosphate in the same procedure and the values of liberated p-nitrophenol were determined from the standard curve.

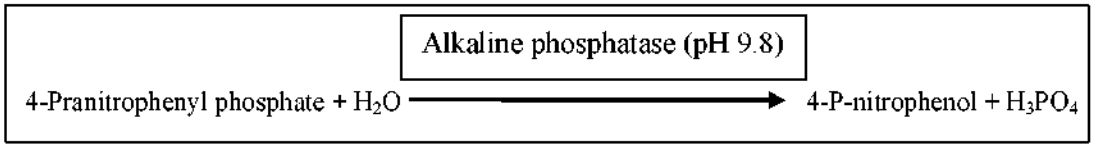
Protein concentration of each sample was quantified by the Lowry's method. A standard curve was drawn using BSA and the amount of protein in our earthworm tissue was calculated from the liner regression equation based on the standard curve (Lowry et al., 1951). The acid phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

2.5. Determination of alkaline phosphataseactivity (ALP)

The activity of alkaline phosphatase was measured as described by Walter and Schutt,



(1974). 250 mg fresh earthworm tissue was taken in homogenizing tube and subjected to homogenization in 5 ml normal saline. The homogenized sample was then centrifuged at 10000 rpm for 10 minutes at normal room temperature. 0.05 ml supernatant was taken in a test tube in which 2 ml alkaline buffer was added and mixed thoroughly. A blank was prepared by giving 0.05 ml of 0.7% saline to 2 ml alkaline buffer. The test tubes were keep in the incubator for 30 minutes at 37° C. After



the incubation period, 10 ml of 0.05 N NaOH solution was added to the test tubes and were mixed absolutely. The amount of liberated p-nitrophenol in tissue sample mixture gives an intense yellow colour that was measured spectrophotometrically at 405 nm wave length after adjusting the absorbance of the blank. A standard curve was drawn with the known amount of p-nitrophenol phosphate in the same procedure and the values of liberated p-nitrophenol were determined from the standard curve.

Protein concentration of each sample was quantified by the Lowry's method. A standard curve was drawn using BSA and the amount of protein in our earthworm tissue was calculated from the liner regression equation based on the standard curve (Lowry et al., 1951). The alkaline phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

3. Results

In our experiment, some important physicochemical parameters of both artificial and artificial soil had been determined which are given in table 1. The pH and organic carbon content of natural ground soil was slightly higher than artificial soil. But moisture content of ground soil was lower than artificial soil.

The acute toxicity test was performed for 14 days to determine the LC50 of lead and nickel in both natural garden soil and artificial soil and the LC50 value were given in table 2. In this experiment nickel shows more toxic than lead in both soil experiment. On the other hand, both metals revealed more toxic in ground soil than artificial soil.

The acid phosphatase, alkaline phosphatase and acetylcholinesterase activity of *E. fetida*

Table 1: Physicochemical parameters of the artificial soil and natural soil acts as test medium used in our experiment.

Soil parameters	Artificial soil	Natural soil
P^H	6.40	6.80
Organic Carbon Content	0.76%	0.88%
Moisture	62.2%	61.4%

were determined after exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb with Ni (T5 & T6). The activity of acid and alkaline phosphatases were significantly different among all sublethal doses of metallic treatments ($P<0.05$). Activity of acid and alkaline phosphatases per mg of protein in all different treatment of soil was graphically represented in figure 1 and figure 2 respectively. The highest level of acid phosphatase activity was recorded in T4 and lowest was recorded in control set (C). Induction of acid phosphatase activity in worm was greater in nickel treated soil than the lead. The alkaline phosphatase activity was decreased in all metallic exposure than control set (C). The highest and lowest level of activity of alkaline phosphatase had been recorded in control set (C) and in T6 respectively. Inhibition of alkaline phosphatase activity in worm was greater in nickel treated soil than the lead. The activity of both phosphatases were more affected by combined treatment of

Table 2: LC50 values of the two heavy metals (mg/kg) used in the Acute Toxicity test.

Heavy metals	Commercial compound	LC50(14 days) (mg/kg)					
		Artificial soil			Ground soil		
		LC50	95% Confidence Limit		LC50	95% Confidence Limit	
			Lower	Upper		Lower	Upper
Lead(Pb)	Lead acetate trihydrate $[(CH_3COO)_2 Pb.{}_3H_2O]$	6250	1.065	1.484	6025	0.755	1.994
Nickel(Ni)	Nickel acetate Tetrahydrate $[(CH_3.COO)_2 Ni.4H_2O)]$	790	0.137	0.183	775	0.135	0.179

lead and nickel comparatively than the treatment of metal intoxication individually, lead or nickel.

4. Data analysis:

The LC50 of individual metal was determined through probit analysis by EPA probit analysis program, version 1.5 (US EPA; 2006) in 95% confidence limit of each metal. Statistical analyses for other measurements were performed by Statistical Package for Social Sciences (SPSS) version 20.0.After metallic exposure, the mean activity of acid and alkaline phosphatases were different significantly by the analysis of variance (ANOVA) followed by Post Hoc test ($P<0.05$). The values of both phosphatases of earthworm tissue after the metallic exposure were analyzed by correlation and showed negative type of correlation ($P<0.01$).

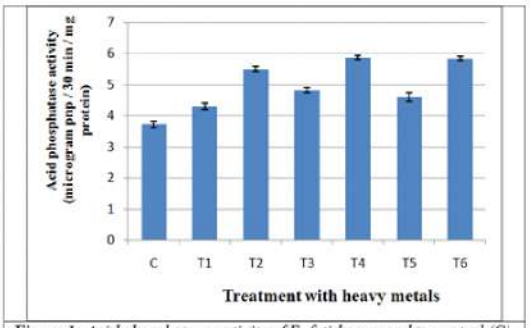


Figure 1: Acid phosphatase activity of E. fetida exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb+Ni (T5 & T6). Error bar represent the standard deviation.

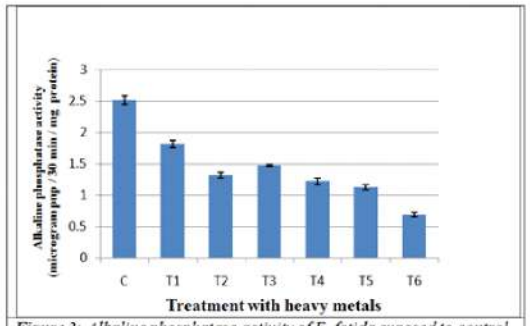


Figure 2: Alkaline phosphatase activity of E. fetida exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb+Ni (T5 & T6). Error bar represent the standard deviation.

5. Discussion:

Digestion and metabolism are essential part in the nutrition of animals. Both are sequential processes and maintained by several types of extra and intra cellular enzymes. Investigation of the physiological deviation from normal state made by the pollutants such as heavy metal can alter the enzymatic activity. Recent studies showed that environmental monitoring had been assessed quickly by use of enzymatic biomarkers and the increase or decrease of the activity of specific enzymes could detect a possible environment stress. The enzymatic activity acts as the excellent hallmark for potential biomonitoring out of the many biological devices to assess the metabolic alteration caused pollutants (Filimon et al., 2013). Several research had been explain alterations in the enzymatic activity were occurred (superoxide dismutase, catalase, gluthathione reductase and gluthathione peroxidise etc) in several organisms under metallic stress conditions and use of

enzymeacts as efficient molecular biomarkers in evaluation of environmental impacts associated with heavy metals. (Bocchetti et al., 2008; Cogo et al., 2009).Heikens et al. (2001) pointed out a review to explain the concentration of heavy metals in most groups of soil invertebrates were happened in the order as Pb>Cd>Cu. Alnuaimi et al. (2012) crried out the activity of acid and alkaline phosphatase in the clam, *Scrobicularia plana* were altered with the treatment of heavy metals. Elevation of acid phosphatase activity indicates hyper activity of Lysosome that generally occurs in prenecrotic changes (Novikoff 1961; De Duve 1968). Khan et al. (2014) showed that lead acetate induce the histopathological changes in brain and hepatocytes in crucian carp along with the inhibition of neural enzymes. Alam (1984) reported that the acute and chronic exposures with metals, the activity of acid phosphatase became enhanced and decrease in alkaline phosphatase activityin *Viviparous bengalensis*. Dalela et al. (1980) opined that oxidative phosphorylation became uncoupled with the inhibition of acid and alkaline phosphatases. Acid and alkaline phoasphatase became decreased and increased respectively after the exposure of insecticide and herbicide (Chakravorty et al. 2015 and 2017). But Sonawane reported that (2017) after the heavy metal stress (copper, mercury and cadmium), the activity of acid and alkaline phosphatase showed increased and decreased level respectively than control set of experiment on fresh water bivalve, *Lamellidens marginalis* and this observation support the present investigation. The both enzymes, acid and alkaline phosphatases were showed a reverse image on their specific activity after the exposure of insecticide or herbicide and heavy metals (lead and nickel). In this experimental findings, it was clearly indicated that LC50 value of lead was higher than nickel, that means nickel was more toxic than lead. After the metallic exposure, it has been also established a negative correlation between the specific activity of both acid and alkaline phosphatases of earthworm's tissue. The activity of both acid and alkaline phosphatases of earthworm's tissue were increased and decreased respectively after treatment the soil with heavy metals and acts as biochemical and physiological biomarker to identify soil pollution. The effect of nickel was more effective than lead on both phosphatases. The combination of heavy metals had synergistic effect to the activity of both phosphatases than individual metal also.

Conflict of interest: The authors declare that there are no conflicts of interest in the above specified investigation.

Acknowledgement:

Atanu Dey and Partha Pratim Chakravorty thankfully acknowledge the Principal of Raja

N. L. Khan Women's College, Midnapore, West Bengal, India for providing us with the necessary laboratory facilities and other support those are associated with this regards. Atanu Dey acknowledge the *University* Scientific Instrumentation Centre (*USIC*) in Vidyasagar University, Midnapore, West Bengal, India for laboratory instrumental support.

References:

Alam, S.M. Some aspects of physiology of *Viviparus bengalensis*. *Ph.D. Thesis*. Marathwada University.Aurangabad (M.S., India), 1984.

Ali, S. and Kashem, M.A. Life Cycle of Vermicomposting Earthworms *Eisenia fetida* and *Eudrilus eugeniae* Under Laboratory Controlled Condition. *Biomed J Sci & Tech Res,* 2018; 10(5): 8110-8113.

Alnuaimi, M.M., Saeed, I.A. and Ashraf, S.S. Effect of various heavy metals on the enzymatic activity of E. coli alkaline phosphatase. *Int J Biotechnol Biochem, 2012;* 8:47–59.

Anees, M.A. Intestinal pathology in fresh water teleost *Channa punctatus* (Bloch) exposed sublethal and chronic levels of three organophosphorus insecticides. *Acta. Physiol. Latino – Amer.* 1976; 26(1): 63-67.

Berhet, J. La digestion intracellulaire et les lysosomes. *Archs. Biol.* (Liege), 1965; 76: 387-388.

Bhargavan, P.and Salih, K. Haematological responses of green mussel Perna viridis (Linnaeus) to heavy metals copper and mercury. *Ph.D thesis submitted to Cochin University of Science and Technology*, Kochi, India. 2010; pp 22-23.

Bhattacharya, S., Mukherjee, S. and Bhattacharya S. Toxic effects of endrin on hepatopancreas of the fish Clarius bactrachus (Lin.) *Ind. J. Exp. Biol.* 1975; 13: 185-186.

Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D. and Regoli, F. Contamination accumulation and biomarker responses in caged mussels, Mytilus galloprovincialis, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas. *Aquatic Toxicology.* 2008; vol.89; 257-266.

Brusseau, M.L. Transport and fate of toxicants in soils.*Soil Ecotoxicology*, J. Tarradellas, G. B., and D. Rossel, Eds., Lewis Publishers, New York, NY, USA, 1997; pp.33–53.

Chakravorty, P.P. Studies on the ecological hazards and residual toxicity of some insecticide on non-target soil micro

arthropod fauna. *PhD Thesis*. Visva-Bharati University, Santiniketan. 1990; 268-270.

Chakravorty, P.P. Chronic toxicity of cypermethrin, a pyrethroid insecticide to epigeic earthworm eisenia fetida. *International Journal of Current Research*. 2015; 07. 17693-17696.

Chakravorty, P. P., Mandal. S. and Kundu. J. K. Relative Toxicity of two Selected Fungicides on Acid Phosphatase and Alkaline Phosphatase activity of Epigeic Earthworm Eisenia Fetida (Oligochaeta) *World Wide Journal of Multidisciplinary Research and Development* . 2017; 4(2): 14-17.

Cheng, T. C. Effects of *in vivo* exposure of *Crassostrea virginica* to heavy metals on hemocyte viability and activity levels of lysosomal enzymes. *Pathol. Mar. Sci.* 1990; 513–524.

Cogo, A.J.D., Siqueira, A.F., Ramos, A.C., Cruz, Z.M.A. and Silva, A.G. Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais. *Natureza on line*. 2009; 7(1); 37-42.

Dalela, R.C., Rani, S. and Verma, S.R. Physiological stress induced by Sublethal concentrations of Phenol and Pentachlorophenol in Notopterus notopterus; Hepatic acid and alkaline phosphate axes and a succinic dehydrogenase. *Environ. Pollut.* 1980; 213-218.

Dasgupta, R., Chakravorty, P.P. and Kaviraj, A. Studies on relative toxicities of six insecticides on epigeic earthworm, Perionyx excavates. *Bull. Environ. Contam. Toxicol.* 2010; 85, 83-86.

De Duve, C. and Wattiaux, R. Functions of Lysosomes. *A Rev. Physiol.* 1966; 28: 435-492.

De Duve, C. In lysosomes a new group of cytoplasmic particles. Subcellular particles edited by T. Hayashi, *Renald Press*, New Yo4k, 1988; 128.

De Silva, P.M.C.S. and van Gestel, C.A.M. Development of an alternative artificial soil for earthworm toxicity testing in tropical countries. *Applied Soil Ecology*. 2009; 43: 170–174.

Filimon, M.N., Nica, D.V., Ostafe, V., Bordean. D.M., Borozan, A.B., Vlad, D.C. and Popescu. R. Use of enzymatic tools for biomonitoring inorganic pollution in aquatic sediments: a case study (Bor, Serbia). *Chem Cent J.* 2013; 7(1): 59.

Heikens, A., Peijnenburg, W.J.G.M. and Hendriks, A.J. Bioaccumulation of heavy metals in terrestrial invertebrates.*Environmental Pollution*. 2001; 113 (3): 385- 393.

Hobbelen, P.H.F., Koolhaas, J.E., Van Gestel,

C.A.M., Bioaccumulation of heavy metals in the earthworms Lumbricus rubellus and Aporrectodea caliginosa in relation to total and available metal concentrations in field soils. *Environ. Pollut.* 2006; 144 (2): 639–646.

Ismail, S.A. *Vermicology-the Biology of Earthworms*. Orient Longman, 1997.

Jackim. F. Enzyme responses to metals in fish. In : Pollution and physiology of marine organisms. *Eds. Vernberg F.J. and Vernberg, W.B., Academic Press, New York and London*, 1974.

Kammenga, J.E., Dallinger, R., Donker, M.H., Köhler, H.R., Simonsen, V., Triebskorn, R. and Weeks, J.M. Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. *Rev. Environ. Contam. Toxicol.* 2000; 164: 93–147.

Khan, S. A., Liu. X. and Shah, B. R. Impact of acute toxicity of lead acetate on the level of essential trace metals and histopathological changes in crucian carp. (*Carassius auratus gibelio*) *The Journal of Animal & Plant Sciences*. 2014; 24(5): 1018-7081

Khillare, P.S., Jyethi, D.S. ad Sarkar, S. Health risk assessment of polycyclic aromatic hydrocarbons and heavy metals via dietary intake of vegetables grown in the vicinity of thermal power plants. *Food Chem. Toxicol.* 2012; 50: 1642–1652.

Lanno, R., Wells, J., Conder, J., Bradham, K. and Basta, N. The bioavailability of chemicals in soil for earthworms. *Ecotoxicol. Environ. Saf.* 2004; 57(1): 39–47.

Li, L., Xu, Z., Wu, J. and Tian, G. Bioaccumulation of heavy metals in the earthworm *Eisenia fetida* in relation to bioavailable metal concentrations in pig manure.*Bioresource Technology*, 2010; 101(10): 3430–3436.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 1951; 193: 265-275.

Mandal, A., and D, Sengupta., Characterization of coal and fly ash from coalfired thermal power plant at Kolaghat possible environmental hazards. *Indian J EnvironProt*, 2002; 22 (8): 885-891.

Mazorra, M.T., Rubio, J.A., and Blasco, J. Acid and alkaline phosphatase activities in the clam Scrobicularia plana: kinetic characteristics and effects of heavy metals.*Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* 2002; 131: 241–249.

Novikoff, A.B. The cells (Eds. J. Brachent and A.P. Mirsky) *New York. Academic Press*, 1961; 423.

OECD, Guideline for testing of chemicals No. 207, Earthworm Acute Toxicity Test.

Organization for Economic Co-operation and Development. Paris, France, 1984.

OECD, Guideline for testing of chemicals No. 222, Earthworm Reproduction Test (*Eisenia fetida/andrei*). Organization for Economic Co-operation and Development. Paris, France, 2004.

Piper, C.S. Soil and Plant Analysis. Hans Publ. Bombay. 1942; p.368.

Ram, R. N. and Sathyanesan, A. G. Mercuric chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa puntae- tus. Envir.Ecol.* 1985; 3:263-268.

Rathi, V., Sambyal, S.S., Kulshreshtha, H. and Satvat, P.S. Heavy metal bioaccumulation by *Eisenia fetida*, cynodon dactylon and Vigna radiata in single, bi and tri-metal soil systems.*International Journal of Technology And Engineering System(IJTES) 2011;* 23.

Sadasivam, S., Manickam, M. Biochemical methods. *New age international publishers*. Third edition. 2015; 111.

Salehi, A. and Tabari, M., Accumulation of Zn, Cu, Ni and Pb in soil and leaf of Pinus elderica Medw. Following irrigation with municipal effluent. *Res. J. Environ. Sci.* 2008; 2(4): 291–297.

Sanyal, S., Dasgupta, R., Kaviraj, A., Chakravorty, P.P., Comparative toxicity studies of four pesticides on the epigeic earthworm *Perionyx excavatus* in two different soil media. *EM International.* 2015; 34(1): 47-51.

Sonawane, S.M. Effect of Heavy Metals on Lysosomal Enzyme Acid Phosphatase Activity of Bivalve L. marginalis. *International Journal of Engineering Science Invention.* 2017; 6 (8): 65-70.

Sonawane S M Effect of Heavy Metals on Lysosomal Enzyme Alkaline Phosphatase Activity of Bivalve *L. marginalis. IOSR Journal Of Pharmacy.* 2017; 7: 53-58.

Suthar, S., Singh, S. and Dhawan, S. Earthworm as bioindicators of metals (Zn, Fe, Mn, Cu: pb and Cd) in soils: is metal bioaccumulation affected by their ecological categories. *Ecol. Eng.* 2008; 32: 99–107.

Todorova, K., Velcheva, I., Yancheva, V., Stoyanova, S., Petrova, S., Georgieva, E., Effects of nickel and its combination with other heavy metals (cd, pb, zn) on common carp (*Cyprinus carpio* Linnaeus, 1785). *Trakia Journal of Sciences*, 2015; 13(2): 324-328.

Ukpabi, C. F., Akubugwo, E. I., Ibiam, U. A., Agbafor, K. N., Ezeigbo, R. O. and Wogu, C. Fertilizer Application Effect on Heavy Metal Composition and Biochemical Responses of *Earthworm Eisenia Fetida* (Savigny) in a Sandy Farm

Soil. *American Journal of Biochemistry*. 2013; 3 (3): 74-79

US EPA, Statistical analysis for biological methods. Available at http:// www.epa.gov/nerleerd /stat2.htm . Ecological exposure research division, US EPA. Available via DIALOG, 2006.

Walter, K. and Schutt, C. Acid and alkaline phosphatase in serum. In: Bergmeyer, H. U. (ed.), Verlag Chenie Weinheim, Methods of Enzymatic analysis. Academic Press Inc, New York, 1974; 856-864.

Ward, N.I. and Savage, J.M. Elemental status of grazing animals located adjacent to the London Orbital (M25) motorway. *Sci. Total Environ.* 1994; 147: 185–189.

Wuana, R.A. and Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Notices: Ecology - Hindawi*, Article ID 402647, 2011; 20.

View publication stats