**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 9[11] October 2020 : 89-98
©2020 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95


**ORIGINAL ARTICLE** **OPEN ACCESS**

# Relative toxicity of Lead and Nickel on some enzymatic biomarkers in epigeic earthworm *Eisenia fetida* (*Savigny*, 1826)

**Atanu Dey[1], Partha Pratim Chakravorty[1,*], Subrata Kumar De[2]**
[1]PG Department of Zoology, Raja Narendra Lal Khan Women's College (Autonomous), Paschim Midnapore, West Bengal 721102, India.
[2]Department of Zoology, Vidyasagar University, Midnapore (West), West Bengal 721102, India.
[*]**Corresponding author email:** parthapratimchakravorty@yahoo.in

**ABSTRACT**
*Among all soil fauna, the 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 of acid phosphatase, alkaline phosphatase and acetylcholinesterase were significant (P<0.05) and also showed a significant negative correlation (P<0.01) between the specific activity of acid phosphatase and alkaline phosphatase.*
***Keywords:*** *Lead, Nickel, Eisenia fetida, Enzymatic biomarkers.*

Received 23.08.2020 Revised 12.09.2020 Accepted 09.10.2020

## 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 [1]. Heavy metals do not decompose or disappear from soil although their release to the ecosystem can be controlled by specific way of treatment [2]. The heavy metals are diffused in the soil rapidly [3]. Previous studies exhibited that earthworms accumulate metals, such as Cadmium, Chromium, Copper, Cobalt, *Ni*, Pb, Zinc from soil under both field and laboratory conditions [4]. The earthworms acts as good sentinel organisms of soil pollution because they contact with soil pore water directly[5]. 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 [6]. 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. Quantitative assessment of biocatalyst is a plausible indicator of environmental pollution such as heavy metal contamination that affect physiological and biochemical condition of organisms. Very low amount of heavy metals are exist within the biological systems in the form of metallic ions that facilitates biochemical reactions [7-9]. High amount of heavy metals accumulated in major organs that

alter the physiological homeostatic function including excess amount of reactive oxygen species had been created [10-12]. Few metal ions react with the -Oh and -SH groups of active site of enzyme leading to conformational changes and inhibit the formation of enzyme substrate complex[13]. Physiological activity of many enzymes including lysosomal hydrolytic enzymes are inhibited by heavy metals [14]. 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 medium pH, 4.8. ALP is multifunctional enzyme, catalyzes phosphomonoester substrates in alkaline medium pH, 9.8. The specific activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) is a biochemical indicator during hyperactivity of lysosomal digestion process that also involving with the metallic stress [15-16]. ACP is 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. The ALP and ACP could be used as biomarkers to determine the pollution level and for detection of cellular damage after the treatment of metal contaminated fertilizer in *E. fetida* or for soil contamination surveys respectively [17-19]. Acetylcholinesterase (AChE, EC 3.1.1.7) is regarded as the vital cholinesterase in earthworms [20-21]. This is a major enzyme that helps neural transmission in nervous system and after neurotransmission occurs in cholinergic synapses then acetylcholinesterase hydrolyze the acetylcholine (neurotransmitter) into choline and acetate [22]. The pesticides mainly inhibit AChE such as Organophosphorus and carbamate. AChE in earthworms considered as vital enzymatic biomarker of pesticide pollution in soil [23-24].

This experiment was conducted to study the effect of selected heavy metals on the activity of acid phosphatase (ACP), alkaline phosphatase (ALP) and acetylcholinesterase (AChE). 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 (ACP), alkaline phosphatase (ALP) and acetylcholinesterase (AChE) in *Eisenia fetida* after exposure with sublethal doses of lead, nickel and their combination of lowest observed effective concentration (LOEC) value.

## MATERIALS AND METHODS

### 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 [25]. 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. The *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 [26].

### 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 [27]. 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($CaCO^3$) [28-29]. 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 [30]. 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.4$H_2O$)] 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 [31]. 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 [32]. The pH and organic carbon content of both soil were determined by the method of Piper [33] 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 [34].

**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 [26]. 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 [35]. 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 No. |
|---|---|
| Electronic Balance | Mettler Toledo (New Classic MS) |
| Environmental Test Chamber | IIC-INSTIND |
| Homogenizer | Remi Electrotechnik Ltd (Type RQP-127/A). |
| Centrifuge | Remi Cooling Centrifuge (C-24BL). |
| Spectrophotometer | Systronics (UV-VIS Spectrophotometer 117) |

**Determination of acid phosphatase activity (ACP)**

The activity of acid phosphatase was measured as described by Walter and Schutt [36]. 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 $37^0$ 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 [37]. The acid phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

**Determination of alkaline phosphatase activity (ALP)**

The activity of alkaline phosphatase was measured as described by Walter and Schutt [36]. 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 $37^0$ 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 [37]. The alkaline phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

**Determination of acetylcholinesterase activity (AChE)**

The activity of acetylcholinesterase was measured as described by Ellman [38]. First seven segments from the anterior part of the surviving earthworms were separated and the tissue piece was homogenized in 10% (w/v) 0.1 M, phosphate buffer (pH 7.5) using a suitable homogenizer. The homogenized solution was centrifuged at 10,000g for 10 min and the supernatant was again centrifuged at 10,000g for 10 minutes at $4^oC$. The resultant supernatants were stored in ice and used for enzymatic assay. A Kinetic measurements were performed with acetylthiocholine iodide (substrate) and the reactions were performed in 300 µl of 0.1 M Phophate buffer, pH-8 containing 20 µl of 0.01 M DTNB [(5,5'-dithio-(2-nitrobenzoic acid)], 20 µl 0f 0.075 M substrate and 10 µl tissue extracts. The whole reactants were thoroughly mixed and substrates were continued to be added untill a stable reading was recorded in spectrophotometer at 412 nm. After addition of the substrate again and again, the change in absorbance was recorded for total period of 10 mins at an interval of 2 mins. Change in absorbance reading per minute was thus determined. The acetylcholinestarase activity was finally expressed in nmoles thiocholine /min/mg of protein after estimation of protein content of the samples.

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 [37]. The alkaline phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

**Data analysis:**

The LC50 of individual metal was determined through probit analysis by EPA probit analysis program, version 1.5 [34] 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, alkaline phosphatases and acetylcholinesterase were different significantly by the analysis of variance (ANOVA) followed by least significant difference (LSD) ($P<0.05$). The values of both phosphatases of earthworm tissue after the metallic exposure were analyzed by correlation and showed negative type of correlation ($P<0.01$).

## RESULTS AND DISCUSSION

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* 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 lead and nickel comparatively than the treatment of metal intoxication individually, lead or nickel. Activity of Acetylcholinesterase (nmole thiocholine/min/mg protein) in all different metallic treatment of soil was graphically represented in figure 3. The activity of acetylcholinesterase was significantly different between the control set with the other sublethal doses of metallic treatments ($P<0.05$). The acetylcholinesterase activity was clearly inhibited after lead and nickel exposure individually or their combination than control. Highest inhibitory effect was showed after intoxication of higher sublethal dose of lead (T2) and lowest inhibitory effect was showed after exposure of combined lower sublethal dose of lead and nickel (T5). During the combined treatment of lower sublethal doses of lead and nickel (T5 and T6), the inhibition of AChE activity was almost alike as after the treatment of individual sublethal dose of nickel (T3 and T4).

Generally biomarkers indicate any kind of pollutant exposure on a specific environment. Earthworm's biomarkers acts as new tools of environment to analyze the impact of soil pollutants on paedofauna. Several earthworm species acts as suitable bioindicator that detect metallic pollution in pedological system. The earthworm, *Eisenia fetida* is frequently applicable species in ecotoxicological experiment [24]. Biomarkers that deals with metal pollution such as Metallothionein induction have been thoroughly investigated. Generally other biomarkers (e.g., ACP, ALP, AChE) has been used in biomonitoring programs with vertebrates but little attention have been done in earthworm studies. The metal pollution has been interfere with the several physiological system such as digestive system, nervous system etc. 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 [39]. 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 enzyme acts as efficient molecular biomarkers in evaluation of environmental impacts associated with heavy metals. The concentration of heavy metals in most groups of soil invertebrates were happened in the order as Pb>Cd>Cu. 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 [40-45]. The lead acetate induce the histopathological changes in brain and hepatocytes in crucian carp along with the inhibition of neural enzymes. The acute and chronic exposures with metals, the activity of acid phosphatase became enhanced and decrease in alkaline phosphatase activity in *Viviparous bengalensis* [46-47]. Acid and alkaline phoasphatase became decreased and increased respectively after the exposure of insecticide and herbicide [26, 48]. But 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 [49-50]. 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). AChE is main enzyme in neuronal system, that propagating impulses by the hydrolysis of acetylcholine [51]. The acetylcholinesterase activity had been altered in fish and birds after the exposure of cholinergic poisons [52]. Few metals inhibit the activity of AChE during nerve nerve inpule propagation [53]. In fish, both AChE and Catalase activities are inhibited by metallic exposure[54]. The inhibition of AChE in earthworms is indicating early warning of contamination of pesticides in soil that affect wildlife in terrestrial ecosystems. Several scientists established that AChE was affected in earthworms by the exposure of pesticides in both laboratory experiment and natural field experiment [25, 55- 58]. The AChE activity was strongly reduced by nickel intoxication in snails and lead acetate inhibit the activity of acetylcholinesterase [59-60]. In *Eisenia fetida*, the AChE activity became affected by nickel and greatly affected by nickel with diazinon [61]. In our experimental study, it is clear that acetylcholinesterase activity was inhibited after lead and nickel exposure individually or their

combination and highest inhibition occurred after the application of lead exposure. During the combined treatment of lower dose of lead and nickel, the inhibition of AChE activity was nearly same as after the treatment of nickel intoxication that's why combined metallic exposure (lead with nickel) seems to be showed that synergistic inhibitory effect on the AChE activity.

**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 |
|---|---|---|
| PH | 6.40 | 6.80 |
| Organic Carbon Content | 0.76% | 0.88% |
| Moisture | 62.2% | 61.4% |

**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 $[(CH_3COO)_2Pb.3H_2O]$ | 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 |

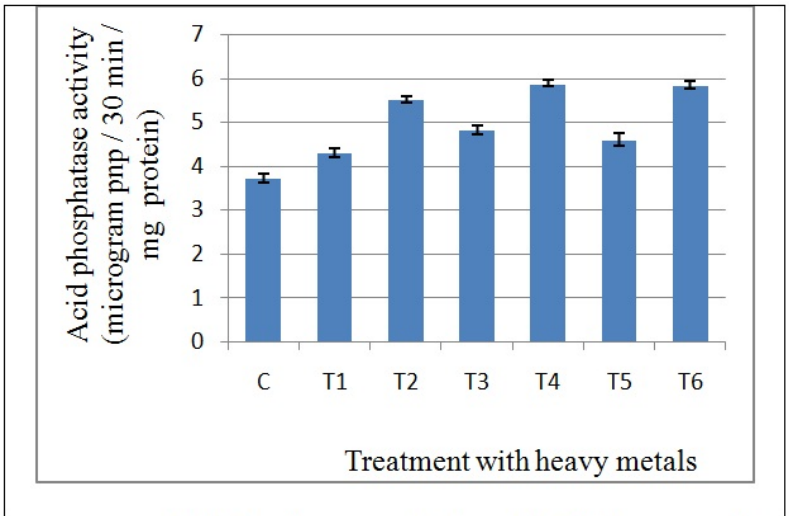


**Figure 1**: Acid phosphatase activity of *E. fetida exposed* to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb with Ni (T5 & T6). Error bar represents the standard deviation.

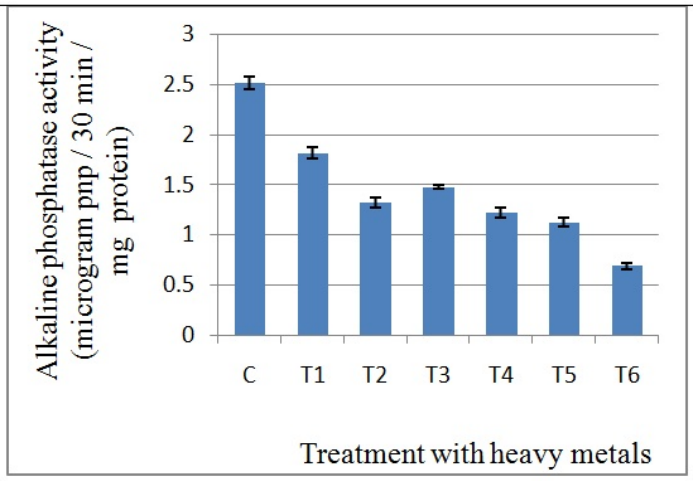


**Figure 2**: Alkaline phosphatase activity of *E. fetida exposed* to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb with Ni (T5 & T6). Error bar represents the standard deviation.

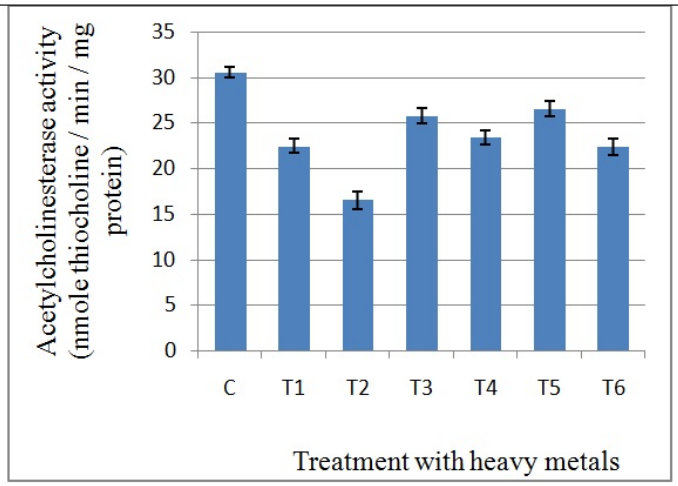


**Figure 3**: Acetylcholinesterase activity of *E. fetida* exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb+Ni (T5 & T6). Error bar represents the standard deviation.

## CONCLUSION

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. In case of acetylcholinesterase, lead showed highest inhibition than nickel but inhibitory effect on acetylcholinesterase by the combination of lower doses of lead and nickel were almost alike as nickel alone. So the enzymatic biomarker, acid phosphatase, alkaline phosphatase and acetylcholinesterase of *Eisenia fetida* could act as sensitive biological tools for the detection of metal contamination in terrestrial ecosystem.

## 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

1. Wuana, R.A. & Okieimen, F.E. (2011). 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, 20.
2. Brusseau, M.L. (1997). Transport and fate of toxicants in soils. *Soil Ecotoxicology*, J. Tarradellas, G. B., and D. Rossel, Eds., Lewis Publishers, New York, NY, USA., pp.33-53.
3. Lanno, R., Wells, J., Conder, J., Bradham, K. & Basta, N. (2004). The bioavailability of chemicals in soil for earthworms. Ecotoxicol. Environ. Saf., 57(1): 39–47.
4. Li, L., Xu, Z., Wu, J. & Tian, G. (2010). Bioaccumulation of heavy metals in the earthworm *Eisenia fetida* in relation to bioavailable metal concentrations in pig manure. Bioresource Technology., 101(10): 3430–3436.
5. Kammenga, J.E., Dallinger, R., Donker, M.H., Köhler, H.R., Simonsen, V., Triebskorn, R. & Weeks, J.M. (2000). Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. Rev. Environ. Contam. Toxicol., 164: 93–147.
6. Suthar, S., Singh, S. & Dhawan, S. 2008. Earthworm as bioindicators of metals (Zn, Fe, Mn, Cu: pb and Cd) in soils: is metal bioaccumulation affected by their ecological categories. Ecol. Eng., 32: 99–107.
7. Cohen, A., Nelson, H., Nelson, N. (2000). Metal-ion transporters— from yeast to human diseases. Madame Curie Bioscience Database [Internet], Landes Bioscience, Austin, Tex, USA.
8. Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. Planta, 212(4): 475–486.
9. Ma, Z., Jacobsen, F. E., & Giedroc, D. P. (2009). Metal transporters and metal sensors: how coordination chemistry controls bacterial metal homeostasis. Chemical Reviews, vol. 109(10): 4644–4681.
10. Flora, S.J.S., Mittal, M. & Mehta, A. (2008). Heavy metal induced oxidative stress & it's possible reversal by chelation therapy. Indian Journal of Medical Research, 128(4): 501–523.
11. Rico, D., Mart´ın-Gonz´alez, A., D´ıaz, S., de Lucas, P., & Guti´errez, J. C. (2009). Heavy metals generate reactive oxygen species in terrestrial and aquatic ciliated protozoa. Comparative Biochemistry and Physiology :Toxicology and Pharmacology, 149(1):90–96.
12. Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavymetals and living systems: an overview. Indian Journal of Pharmacology, 43(3): 246–253.
13. Glusker, J.P., Katz, A.K., Bock, C.W. (1999). Metal ions in biological systems. The Rigaku Journal, 16(2): 381–412.
14. Cheng, T. C. (1990). Effects of *in vivo* exposure of *Crassostrea virginica* to heavy metals on hemocyte viability and activity levels of lysosomal enzymes. Pathol. Mar. Sci. 513–524.
15. Bhargavan, P. and Salih, K. (2010). 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. pp 22-23.
16. Sadasivam, S. & Manickam, M. (2015). Biochemical methods. New age international publishers. Third edition. pp 111.
17. Ram, R. N. & Sathyanesan, A. G. (1985). 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. 3: 263-268.
18. Basaglia, F. 2000. Isozyme distribution of ten enzymes and their loci in South American lungfish, Lepidosiren paradoxa (Osteichtyes, Dipnoi). Comp. Biochem. Physiol. Part B 126: 503–510.
19. Ukpabi, C. F., Akubugwo, E. I., Ibiam, U. A., Agbafor, K. N., Ezeigbo, R. O. & Wogu, C. (2013). Fertilizer Application Effect on Heavy Metal Composition and Biochemical Responses of *Earthworm Eisenia Fetida* (Savigny) in a Sandy Farm Soil. American Journal of Biochemistry. 3(3): 74-79
20. Rault, M., Mazzia, C. & Capowiez, Y. (2007). Tissue Distribution and Characterization of Cholinesterase Activity in Six Earthworm Species. Comparative Biochemistry and Physiology Part B : Biochemistry & Molecular Biology, 147: 340-346.
21. Lionetto, M.G., Calisi, A. & Schettino, T. (2012). Chapter 16. Earthworm Biomarkers as Tools for Soil Pollution Assessment. In: Hernandez-Soriano, M.C., Ed., Soil Health and Land Use Management , InTech, Italy.
22. Soreq, H. & Zakut, H. (1993). Human Cholinesterase and Anticholinesterase. Academic Press, New York.
23. Calisi, A., Lionetto, M.G. & Schettino, T. (2011). Biomarker Response in the Earthworm Lumbricus terrestris Exposed to Chemical Pollutants. Science of the Total Environment. 409: 4456-4464.
24. Scott-Fordsmand, J.J., Weeks, J.M. (2000). Biomarkers in earthworms. Reviews of *Environmental Contamination and Toxicology*. 165:117-159.

25. Ismail, S.A. (1997). Vermicology-the Biology of Earthworms. Orient Longman.
26. Chakravorty, P. P., Mandal. S. and Kundu. J. K. (2017). 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. 4(2): 14-17.
27. Rathi, V., Sambyal, S.S., Kulshreshtha, H. & Satvat, P.S. (2011). 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) 23.
28. De Silva, P.M.C.S. and van Gestel, C.A.M. (2009). Development of an alternative artificial soil for earthworm toxicity testing in tropical countries. Applied Soil Ecology. 43: 170–174.
29. Sanyal, S., Dasgupta, R., Kaviraj, A., & Chakravorty, P.P. (2015). Comparative toxicity studies of four pesticides on the epigeic earthworm *Perionyx excavatus* in two different soil media. EM International. 34(1): 47-51.
30. OECD, (1984). Guideline for testing of chemicals No. 207, Earthworm Acute Toxicity Test. Organization for Economic Co-operation and Development. Paris, France.
31. Dasgupta, R., Chakravorty, P.P. & Kaviraj, A. (2010). Studies on relative toxicities of six insecticides on epigeic earthworm, Perionyx excavates. Bull. Environ. Contam. Toxicol. 85: 83-86.
32. Chakravorty, P.P. (1990). Studies on the ecological hazards and residual toxicity of some insecticide on non-target soil micro arthropod fauna. PhD Thesis. Visva-Bharati University, Santiniketan. pp 268-270.
33. Piper, C.S. (1942). Soil and Plant Analysis. Hans Publ. Bombay. pp368.
34. US EPA, (2006). Statistical analysis for biological methods. Available at http://www.epa.gov/nerleerd /stat2.htm . Ecological exposure research division, US EPA.
35. Todorova, K., Velcheva, I., Yancheva, V., Stoyanova, S., Petrova, S. & Georgieva, E. (2015). Effects of nickel and its combination with other heavy metals (cd, pb, zn) on common carp (*Cyprinus carpio* Linnaeus, 1785). Trakia Journal of Sciences, 13(2): 324-328.
36. Walter, K. & Schutt, C. (1974). Acid and alkaline phosphatase in serum. In: Bergmeyer, H. U. (ed.), Verlag Chenie Weinheim, Methods of Enzymatic analysis. Academic Press Inc, New York, pp 856-864.
37. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R. J. (1951). Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265-275.
38. Ellman, G.L., Courtney, K.D., Andres, V., & Jr, Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7: 88–95.
39. Filimon, M.N., Nica, D.V., Ostafe, V., Bordean. D.M., Borozan, A.B., Vlad, D.C. & Popescu. R. (2013). Use of enzymatic tools for biomonitoring inorganic pollution in aquatic sediments: a case study (Bor, Serbia). Chem Cent J. 7(1): 59
40. Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D. & Regoli, F. (2008). 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. 89: 257-266.
41. Cogo, A.J.D., Siqueira, A.F., Ramos, A.C., Cruz, Z.M.A. & Silva, A.G. (2009). Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais. Natureza on line. 7(1): 37-42.
42. Heikens, A., Peijnenburg, W.J.G.M. & Hendriks, A.J. (2001). Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution. 113(3): 385- 393.
43. Alnuaimi, M.M., Saeed, I.A. & Ashraf, S.S. (2012). Effect of various heavy metals on the enzymatic activity of E. coli alkaline phosphatase. Int J Biotechnol Biochem 8: 47–59.
44. Novikoff, A.B. (1961). The cells (Eds. J. Brachent and A.P. Mirsky) New York, Academic Press, 423.
45. De Duve, C. (1968). In lysosomes a new group of cytoplasmic particles. Subcellular particles edited by T. Hayashi, Renald Press, New Yo4k, 128.
46. Khan, S. A., Liu. X. & Shah, B. R. (2014). 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. 24(5): 1018-7081.
47. Alam, S.M. (1984). Some aspects of physiology of *Viviparus bengalensis*. Ph.D. Thesis. Marathwada University. Aurangabad (M.S., India).
48. Chakravorty, P.P. (2015). Chronic toxicity of cypermethrin, a pyrethroid insecticide to epigeic earthworm eisenia fetida. International Journal of Current Research. 07: 17693-17696.
49. Sonawane, S.M. (2017). Effect of Heavy Metals on Lysosomal Enzyme Alkaline Phosphatase Activity of Bivalve *L. marginalis.* IOSR Journal Of Pharmacy. 7: 53-58.
50. Sonawane, S.M. (2017). Effect of Heavy Metals on Lysosomal Enzyme Acid Phosphatase Activity of Bivalve L. marginalis. International Journal of Engineering Science Invention. 6(8): 65-70.
51. Thompson, C.M. & Richardson, R.J. (2004). Anticholinesterase insecticides, In: Pesticide Toxicology and International Regulation, Marrs, T. C. and Ballantyne, B. (Eds.), John Wiley & Sons, West Sussex, England, 89-127.
52. Greig-Smith, P.W. (1991). Use of cholinesterase measurements in surveillance of wildlife poisoning in farmland. In: Cholinesterase inhibiting insecticides: Their impact on wildlife and the environment. Mineau P (Ed.), Elsevier, Amsterdam, The Netherlands. pp. 127-150.
53. Heywood, R., James, R.W., Street, A.E., Rudd, C.J., & Barry, P.S.I. (1978). In vitro and in vivo study of the ability of the tetraethyl lead to inhibit cholinesterase activity. Toxicology Letters. 2: 11-16.

54. Olson, D., & Christensen, G.M., (1980). Effects of water pollutants and other chemicals on fish acetylcholinesterase (in vitro). Environmental Research 21: 327-335.
55. Booth, L.H., & O'Halloran, K. (2001). A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos. Environmental Toxicology and Chemistry. 20(11): 2494-2502.
56. Rao, J.V., Kavitha, P. (2004). Toxicity of azodrin on the morphology and acetylcholinesterase activity of the earthworm *Eisenia foetida*. Environmental Research. 96(3): 323327.
57. Gambi, N., Pasteris, A., & Fabbri, E. (2007). Acetylcholinesterase activity in the earthworm *Eisenia andrei* at different conditions of carbaryl exposure. Comparative Biochemistry and Physiology Part C. 145(4): 678-685.
58. Calisi, A., Lionetto, M.G. & Schettino, T. (2009). Pollutant-induced alterations of granulocyte morphology in the earthworm *Eisenia foetida*. Ecotoxicology and Environmental Safety. 72(5): 1369-1377.
59. Zawisza-Raszka, A., Dolezych, B., Dolezych, S., Migula, P. & Ligaszewski, M. (2010). Effects of nickel exposure and acute pesticide intoxication on acetylcholinesterase, catalase and glutathione S-transferase activity and glucose absorption in the digestive tract of *Helix aspersa* (Pulmonata, Helicidae). International Journal of Environment and Pollution. 40: 380-390.
60. Ribera, D., Narbonne, J., Arnaud, C., & Saint-Denis, M. (2001). Biochemical responses of the earthworm Eisenia fetida andrei exposed to contaminated artificial soil, Effects of carbaryl. Soil Biology and Biochemistry. 33: 1123-1130.
61. Zawisza-Raszka, A., & Dolezych, B. (2013). Nickel in Soil Modifies Sensitivity to Diazinon Measured by the Activity of Acetylcholinesterase, Catalase, and Glutathione S-Transferase in Earthworm Eisenia fetida. Applied and Environmental Soil Science. pp 5-6.



**CITATION OF THIS ARTICLE**

A Dey, P P Chakravorty, S Kumar De. Relative toxicity of Lead and Nickel on some enzymatic biomarkers in epigeic earthworm *Eisenia fetida* (*Savigny*, 1826). Bull. Env. Pharmacol. Life Sci., Vol 9[11] October 2020 : 89-98