

Levels of Some Metals and Earthworm Phosphatase Activities in the Soil around Odo-Efo River, Ilorin, Kwara State

* R.O. Arise, O.A. Idowu², S.T. Farohunbi¹, F. A. Abubakar,¹
J.A. Aboyewa²

¹Department of Biochemistry, Faculty of Science, University of Ilorin,
Ilorin, Nigeria

²Department of Biochemistry, South-Western University, Okun-Owa, Ogun
State, Nigeria

*Corresponding Author Email: arisedshine@yahoo.com; Tel: 08033869546

Abstract

Soil pollution has increased in recent years thereby causing the accumulation of heavy metals. In this study, the levels of some metals in the soil collected from four different sites S1 (400-500m), S2 (300-250m), S3 (150-75m) and S4 (10-25m) around Odo-Efo River that receives effluent from Global Soap and Detergents Industry located at Asa-Dam, Ilorin, Kwara State in north central Nigeria and their effects on biomarker enzymes (alkaline phosphatase and acid phosphatase) in earthworms collected from each sites were investigated. They were compared to a control (S_c) soil sample from a forested area at the University of Ilorin Botanical Garden. The soil samples were assayed for Cd, Cu, Hg and Pb by flame atomic absorption spectrophotometer after aqua regia digestion. Results of analysis revealed that metal concentration ranged as: Cd (0.003-0.004mg/kg), Cu (0.04-0.21 mg/kg), Hg (0.001-0.003 mg/kg) and Pb (0.007-0.008 mg/kg) with Cd, Cu and Hg found to be significantly higher ($p < 0.05$) in all the sampling sites when compared to the control but with a significant decrease ($p < 0.05$) in the concentration of Pb when compared to the control. The increase in some metal concentrations was accompanied by a corresponding increase in the concentration of the earthworm biomarker enzymes and

malondialdehyde concentration. The study concluded that the effluents may contain some heavy metals with long term effects on soil characteristics and food chain.

Keywords: Soil pollution, heavy metals, effluents, earthworms, biomarkers enzymes, food chain.

Introduction

Soil pollution is the build-up of persistent toxic compounds, chemicals, salts, radioactive materials, or disease causing agents which have adverse effects on plant growth, animal and human health. Soil becomes polluted through several ways among which is seepage from a landfill, discharge of industrial waste (effluent) into the soil, rupture of underground storage tanks, excess application of pesticides, herbicides or fertilizers [1]. Heavy metals are also considered one of the major sources of soil pollution. Heavy metal pollution of soil is caused by various metals among which are Cu, Ni, Cd, Cr and Pb which have no bio-importance in human biochemistry and physiology while consumption even at very low concentrations can be toxic. However, some others like Fe, Zn, Ca and Mg have been reported to be of bio-importance to man and their daily medicinal and dietary allowances have been recommended. Heavy metals could enter the aquatic environment from both natural and anthropogenic sources. The most anthropogenic sources of metals are industrial, petroleum and sewage disposal [2].

Heavy metals are highly hazardous to the environment and organisms. Once the soil suffers from heavy metal contamination, remediation may be difficult [3]. Metal uptake by plant from soil is usually at high concentrations and subsequent accumulation along the food chain. These result in toxic effects towards biota by affecting key microbial processes, decreasing the number and activity of micro-organisms and also acting as a potential threat to animal and human health. Harmfulness of metals to decomposer organisms and other micro-organisms are assessed with methods ranging from chemical analyses to different kinds of bio tests. Some of these have been standardized and some are currently undergoing standardization processes in the International Organization for

Standardization (IOS) and other regulatory bodies. One approach to assessing changes in environmental quality is the use of bio-markers. Biomarkers are measurements at low levels of biological organization (molecular, cellular and tissue levels) that indicate exposure to particular compounds or elements and the effects of such exposure. Because of their relative response, these bio-markers have also been used as tools for biological effect measurement in anticipation of changes at higher levels of biological organization [4-5].

Karaca *et al.* [6] reported that enzymatic activities are influenced in different ways by different metals due to different chemical activities of enzymes in the soil system. Several studies conducted have been aimed at identifying the bio-indicators of soil and water quality in order to determine the effects of pollution by heavy metals. There have been reports that associated the effect of pollution on the enzymatic activities which contribute to the degradation of plant residues and soil organic materials. Phosphatase activities (including alkaline phosphatase, ALP and acid phosphatase, ACP) involved in the energy metabolism of the soil flora have thus been studied [7-9]. The toxicity of heavy metals can result in a dysfunction of the enzymatic systems of the organisms [10].

Phosphomonoesterases, also called "phosphatases", catalyse the hydrolysis of orthophosphoric monoesters and of H_3PO_4 anhydrides. They are acid and alkaline phosphatases whose activities are optimal in the acid (pH 6.5) and alkaline (pH 11) environments respectively [11]. Phosphatases are involved in the organic phase of the phosphorus cycle which leads to the release of inorganic phosphorus that can be assimilated by living organisms. Acid and alkaline phosphatases are also involved in the energy metabolism [12]. Furthermore, Navarro *et al.* [13] found that high levels of alkaline phosphatase indicated cellular damage, as long as phosphatase removes phosphates from membrane phospholipids. Therefore, phosphatase activities are often studied in order to determine the impact of heavy metals on the biological quality of soil and water toxicity.

Heavy metals can reduce enzyme activity by interacting with the enzyme-substrate complex, denaturing the enzyme protein, interacting with its active sites, or by affecting the synthesis of the enzymes within the cells of soil and water organisms. Metal-induced changes in the community structure can also modify enzyme activity [14]. When Cadmium (Cd), for example, binds to active sites, enzymes such as ALP are inactivated and metabolism is disrupted [15]. Cd, besides being an enzyme inhibitor, can have deleterious effects on membrane structure and function by binding to the ligands such as phosphate and the cysteinyl and histidyl groups of proteins [16-17]. Also, it was reported by Khan *et al.* [18] that the consumption of heavy metal contaminated food can seriously deplete some essential nutrients in the body that may further be responsible for decreased immunological defences, intrauterine growth retardation, disabilities associated with malnutrition and higher prevalence of upper gastrointestinal cancer rates.

Earthworms are key species within terrestrial food webs and perform a number of essential functions like decomposition of organic litter, tillage, aeration of soil and mixing of their organic and inorganic fractions [19-20]. Previous studies have shown how some invertebrates e.g. nematodes, arthropods, and earthworms are able to tolerate high concentrations of metals in the soil [21] and that the posterior alimentary canal is their main site of metal accumulation, where the chloragogenous tissue separating the absorptive epithelium from the coelom is a major metal depository [22-23]. It is well known that earthworms play an important role in soil macrofauna biomass. The bioaccumulation ability of earthworm is essential for bio-monitoring. The bio-accumulative ability of the earthworm is well known and thus, it could be a useful living organism for bio-monitoring of soil pollution. Stubberud *et al.* [24] reported the isolation of ACP from an earthworm species, *Eiseniaveneta*, which has two isoenzymes: one is 113 kDa composed of identical peptide chains of 36 kDa.

Karaca *et al.* [6], Zhang *et al.* [25] and Lukkari [26] reported the changes in ACP and ALP activities of earthworms in metal contaminated soils. The aim of this study therefore was to investigate levels of metals and earthworm

phosphatase activities from the soil around the industrially polluted Odo-Efo River in Ilorin, Kwara State. This is because the river area is known for commercial production of vegetables consumed in Ilorin. Soil from Botanical Garden of the University of Ilorin was chosen as the control because it is a forested area that is free from any industrial wastes or effluents.

Materials and Methods

Study Area

The study was conducted on the soil around Odo-Efo River in Osere area of Ilorin, Kwara State. The river is located in Ilorin South LGA which is one of the 16 Local Government Areas and the capital city of Kwara State (Figure 1). It receives effluent from Global Soaps and Detergents Industry in Asa dam, Ilorin. Soil sample from the river bank flowing in the forested area at the University of Ilorin Botanical was used as the reference control group. This forested area is free from industrial wastes or effluents.

GPS READING: $+8^{\circ} 27' 47.69''$, $+4^{\circ} 32' 12.03''$

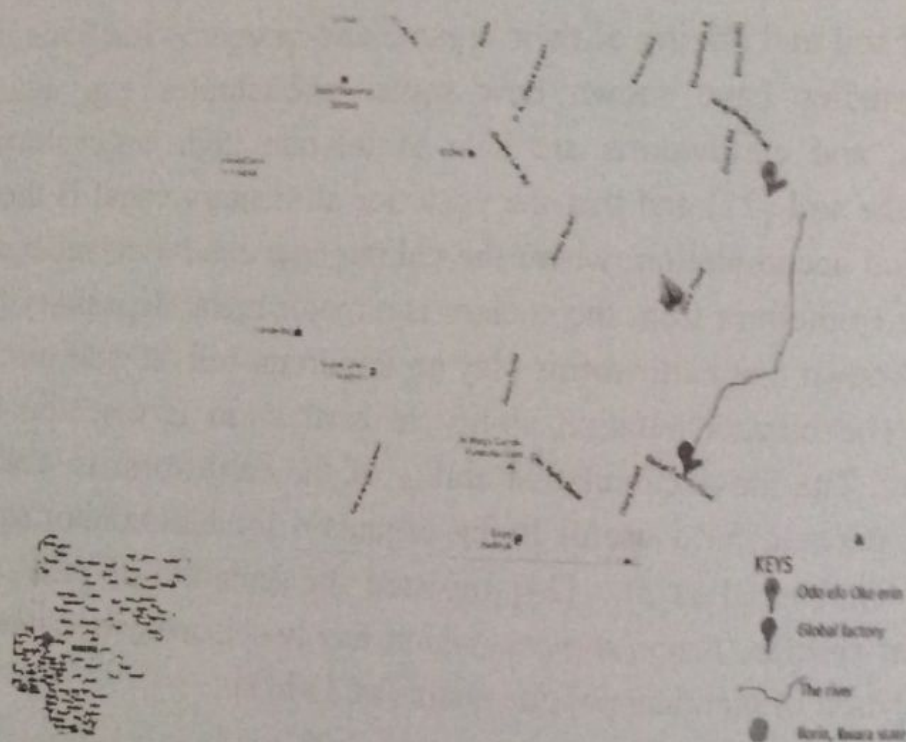


Figure 1: Schematic Map of Sampling Site

Source: Kwara State Ministry of Lands, Ilorin, Kwara State

Sampling and Analysis

Soil and earthworm samples were collected from 4 different sites (S1, S2, S3, S4) at a distance of 400-500m, 300-250m, 150-75m and 10-25m respectively from the river and a control (Sc) from the soil of Botanical Garden, University of Ilorin respectively for analyses. A 10 g portion of each soil sample was weighed into 100ml capacity beaker and digested with aqua regia (HNO_3 and HCl ; 1:3) for 2 hrs. The mixture was filtered with Whatman No. 42 filter paper and 5 ml of the digested (filtrate) was made up to 100 ml with de-ionized water in a volumetric flask to remove silicate and other insoluble materials. All samples were analyzed for Cu, Cd, Hg, Pb and Zn concentrations using a flame atomic absorption spectrophotometer [27]. Earthworms were collected from each sample site by hand sorting and kept in soil from the sampling location after which they were homogenized in sucrose solution and stored at 4°C prior to enzyme assay.

Enzyme Assay

Activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were assayed in the earthworm samples. The method described by Armstrong [28] was employed to assay for ACP while the method described by Bassey *et al.* [29] as modified by Wright *et al.* [30] was employed for ALP enzyme assay.

Statistical Analysis

Analysis of variance (ANOVA, significant level $p < 0.05$) followed by the Duncan Multiple Range Test was used to analyse the derived data from the sampling sites. Values are presented as mean $\pm$ SD. Differences with values of $p < 0.05$ were considered statistically significant.

Results and Discussions

The results of the concentration of the heavy metals of soils from all the sampling sites (S1, S2, S3, S4) around Odo-Efo River are shown in Figures II, III, IV and V while Figures VI, VII and VIII show the activities of alkaline phosphatase, acid phosphatase and malondialdehyde concentration respectively. It was observed that the metal with the highest concentration in all the four sampling sites is Cu, with the lowest concentration of 0.04mg/kg in S4 and the highest concentration of 0.21mg/kg in S1. The metal with the lowest concentration was found to be Hg with the minimum concentration of 0.001mg/kg in S1 and maximum concentration of 0.003mg/kg in S4.

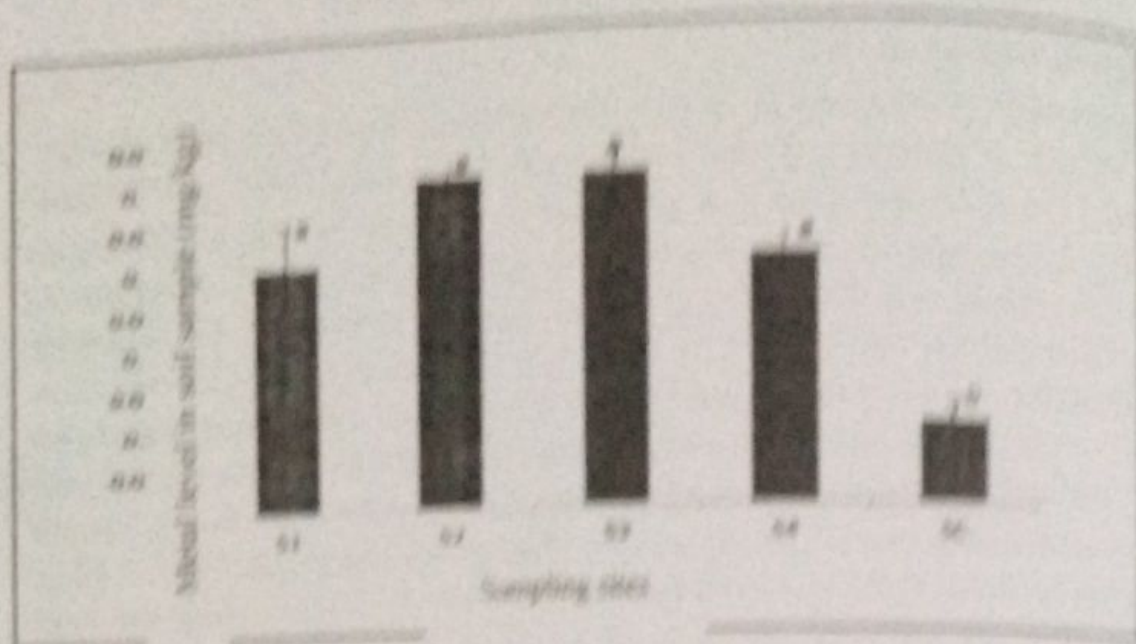


Figure II: Cadmium content of soil samples from sites around Odo-Eto River Ilorin, Kwara State

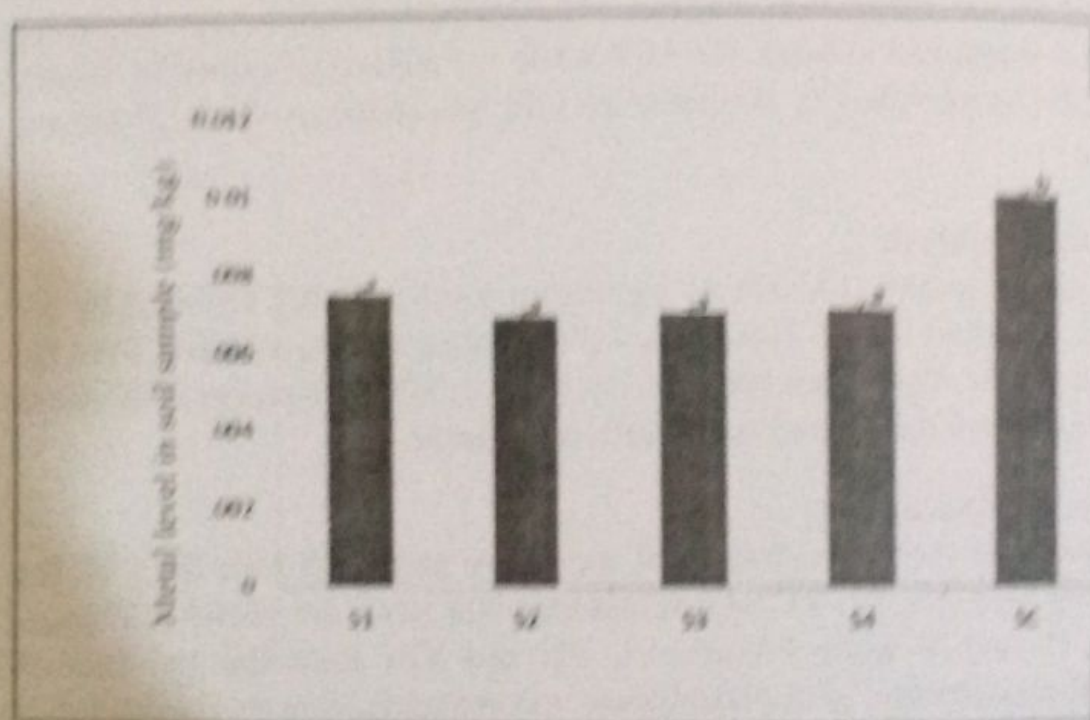


Figure III: Lead content of soil samples from sites around Odo-Eto River, Ilorin, Kwara State

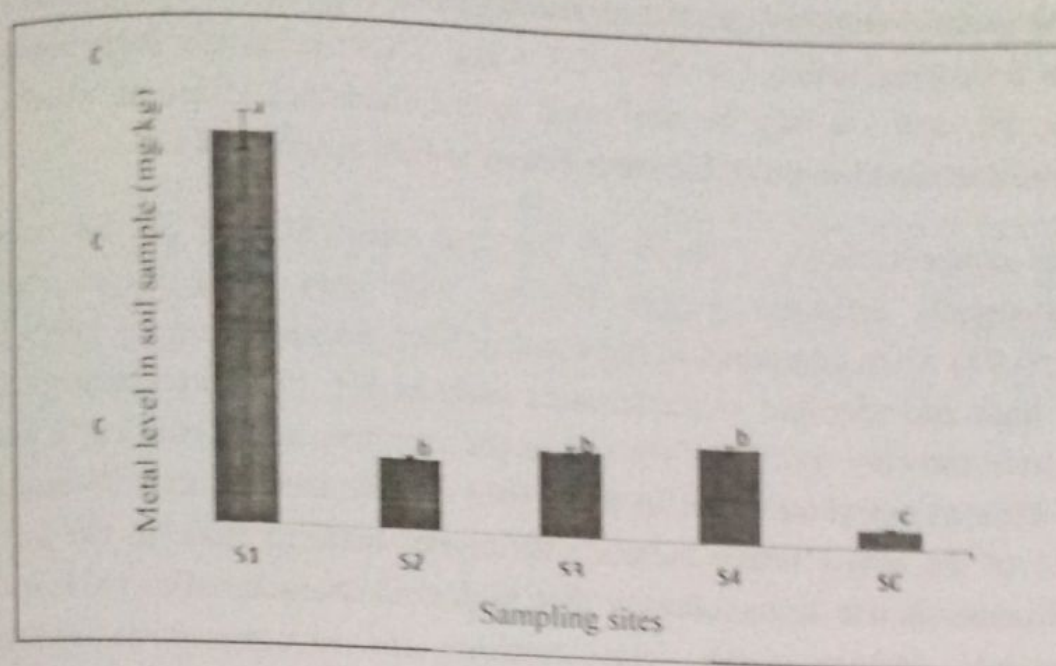


Figure IV: Copper content of soil samples from sites around Odo-Efo River, Ilorin, Kwara State

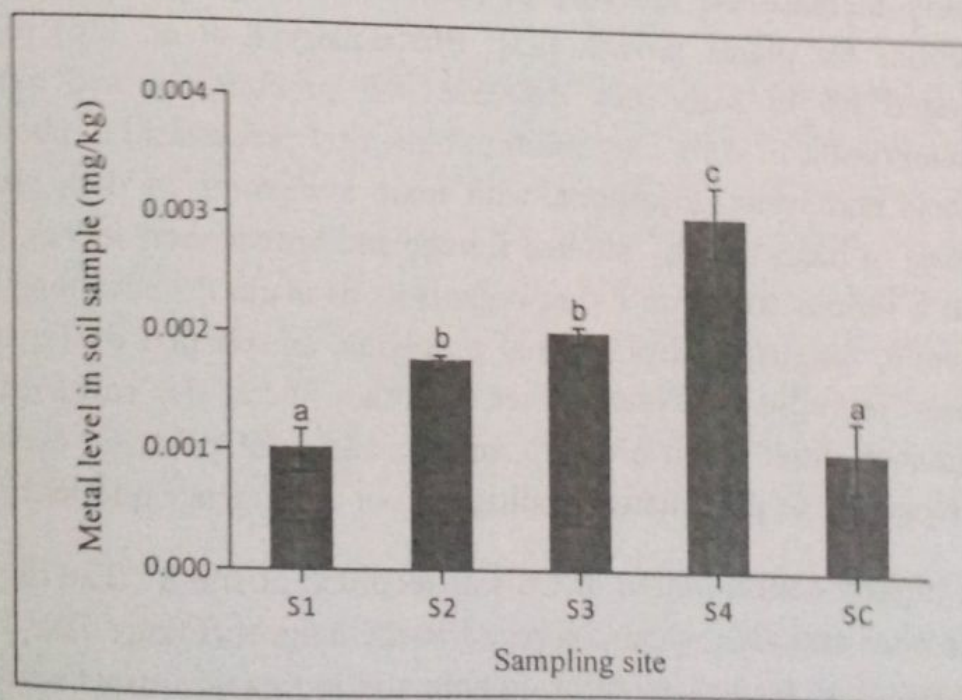


Figure V: Mercury content of soil samples from sites around Odo-Efo River, Ilorin, Kwara State

The general trend of metal concentrations in (mg/kg) of soil sample was in the following order: $Cu > Pb > Cd > Hg$. These relatively high contents of Cu, Pb, and Cd may be attributed to the industrial effluents which might have contained more of the three heavy metals and less of Hg.

The concentrations of Pb in all the four sites (S1, S2, S3, S4) were not statistically different (Figure III) but with only a significant reduction ($p < 0.05$) when compared to the control. Such decrease may probably be due to high soil physical characteristics such as pH, moisture content, organic matter and clay content of the soil in the sampling sites resulting to decrease in Pb concentration found in these sites despite the fact that Pb constituted one of the major heavy metals with higher concentration in the industrial effluents. It has been reported that high soil characteristics (pH, moisture content, organic matter, clay content) are recognized to reduce the bioavailability of some metals in soil [31-33]. Pb is known to have strong affinity for organic ligands and the formation of such complexes may greatly increase the mobility of Pb in the soil [34]. However, Pb is not essential for plants growth [35]. Bhattacharyya *et al.* [36] reported that elevated Pb in soils may decrease soil productivity and very low Pb concentration in soils may inhibit some vital processes i.e. photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short leaves. It may also pose a serious threat on living organisms as acute Pb poisoning may cause anaemia, fatigue, gastrointestinal problems, anoxia and dysfunction of the kidney, reproductive system, liver and brain. It can also cause difficulties in pregnancy, high blood pressure, muscle and joint pain and even affect the development of gray matter leading to poor intelligence quotient (IQ) [37].

The highest concentration of Cu was recorded in site S1 than the remaining three sites and also when compared to the control (Figure IV). This maybe as a result of accumulation of organic and inorganic wastes since S1 is the farthest from the point of the effluent discharges though metal bioavailability in soils and factors determining it are not fully understood. High decomposition rate of soil organic matter which influences the release

of heavy metals in soil solution for possible uptake by plants may also likely be responsible for this.

It was observed that there was high Hg concentration in S4 than in S1, S2 and S3 even when compared to the control (Figure V), this maybe because S4 is the closest to Odo-Efa river where the effluents are discarded thus resulting to the accumulation of Hg in S4 and this is in support of Spurgeon and Hopkin [38] who reported that a sampling site closest to a factory contains higher metal concentration.

A significant increase ($p < 0.05$) was recorded in the concentration of cadmium in sampled sites when compared to the control (Figure II). The significant increase may be as a result of accumulation of cadmium ions from the effluents and industrial discharge deposited in the soils. Cadmium concentrations have been shown to be limited by CdCO_3 in neutral and alkaline soils [39] and the average natural abundance of Cd in earth crust has been reported to range from 0.1 mg/kg to 0.5 mg/kg [40].

The uptake of heavy metals by plants from soil and successive accumulation along the food chain result in a great health risk considering food-chain implications [41]. Asharf and Ali [42] also reported that heavy metals exert toxic effects on soil micro-organisms in the change of the diversity, population size and overall activity of the soil microbial communities. Microbial enzymes might be affected by heavy metals due to the potential inhibition of both enzymatic reactions and metabolic processes [43]. Chronic level ingestion of toxic elements has undesirable impacts on the human body and can seriously deplete some essential nutrients in the body [18].

Although some of these metals like Cu are essential elements for normal growth and also act as components of metalloenzymes, these elements can easily lead to poisoning when their concentration is greater than the optimal values [44-45]. Excessive human intake of Cu may lead to severe mucosal irritation followed by depression, severe gastrointestinal irritation while

narcotic changes in the liver and kidney can also occur. Cadmium is a well-known heavy metal toxicant with a specific gravity of 8.65 times greater than water and it is more toxic to enzymes than Pb because of its greater ability and lower affinity for soil colloids. The target organs of Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones [46]. Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Cadmium has also been associated with many clinical conditions but it has been difficult to tie down obvious links of environmental exposures with morbidity and mortality [46]. Mercury (Hg) which is also toxic has no known function in human biochemistry and physiology. Inorganic forms of Hg cause spontaneous abortion, congenital malformation and gastrointestinal disorders. Poisoning by its organic forms like methyl mercury, formed in aquatic sediments through bacteria methylation found in fish muscles, is also associated with congenital malformation [47].

The results of earthworm biomarker enzymes analyzed showed a significant increase ($p < 0.05$) in the activities of alkaline and acid phosphatases in all the sites (S1, S2, S3, S4) when compared to the control (Sc) with ALP activity being significantly higher in S4 than other sampling sites. However, the reverse was the case for ACP whose activity was lower in S4 than other sampling sites. The same trend in the activity of ALP was observed in the formation of malondialdehyde with a significantly increased concentration ($p < 0.05$) in all the sites compared to the control. However, the highest malondialdehyde concentration was recorded in S4 (Figures VI and VIII).

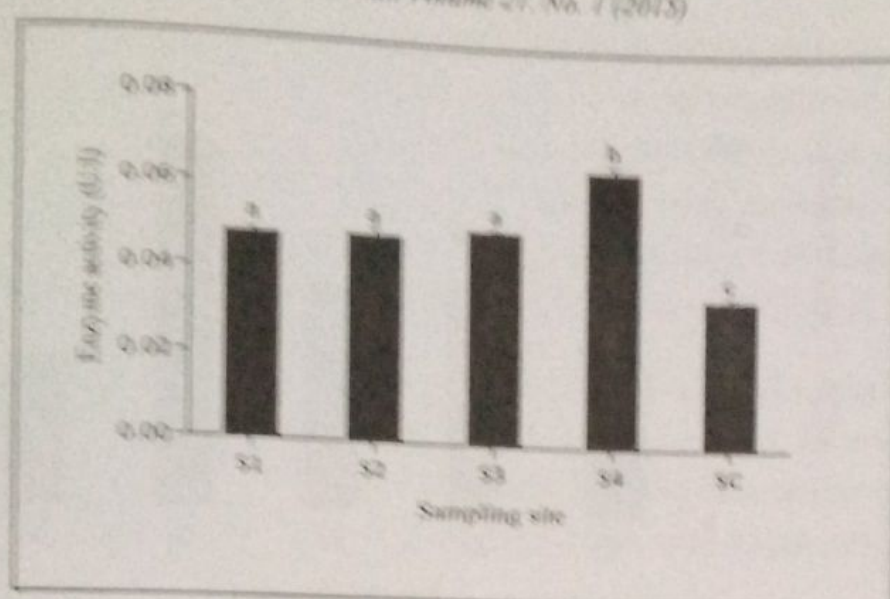


Figure VI: Alkaline phosphatase activity (IU/l) in earthworm from soil around Odo-Efo River, Ilorin, Kwara State

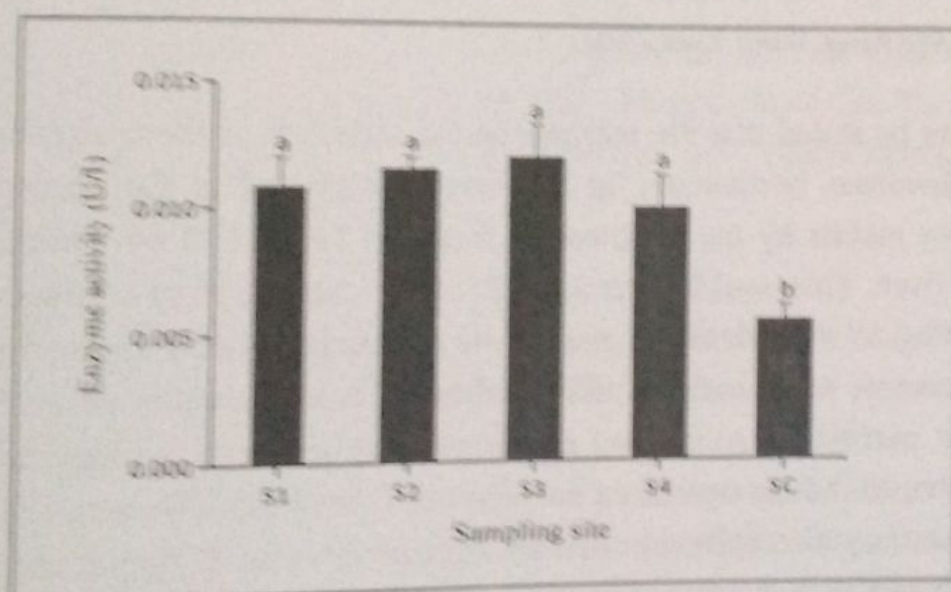


Figure VII: Acid Phosphatase activity (IU/l) in earthworm from soil around Odo-Efo River, Ilorin, Kwara State

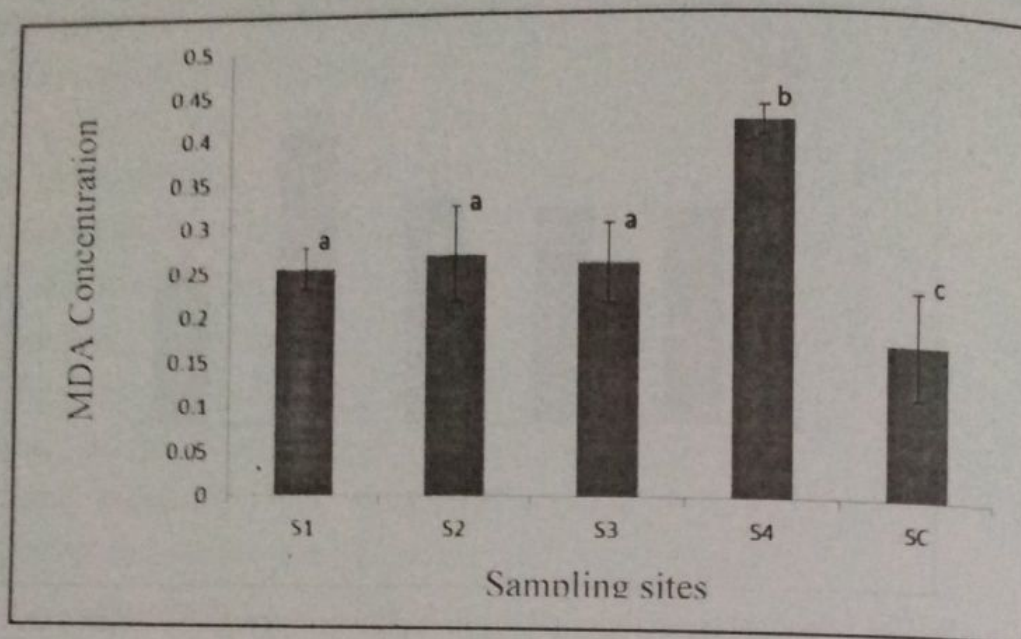


Figure VIII: Malondialdehyde Concentration ($\mu\text{mole/L}$) in earthworm from soil around Odo-Efo River, Ilorin, Kwara State

It can be stated that the increase in the activities of these enzymes found in earthworms, particularly in S4, maybe attributed to the absorption of the heavy metals by the earthworms found in the soil of S4 which is closer to the river. This could be as a result of the disruption of cellular membranes leading to the release of these two membrane-bound biomarker enzymes. Moreover, some indirect effects of metal contamination on earthworm, e.g. poor nutritional conditions of earthworms in metal contaminated soil are known to induce unwanted enzyme activities [26]. The result obtained here is contrary to results reported by Marzadori *et al.* [12] that the activities of ALP and ACP decreased with increasing concentrations of heavy metals and this agrees with the report of Karaca *et al.* [6] and Zhang *et al.* [25] who reported that enzyme activities are influenced in different ways by metals due to the different chemical activities of the enzymes in the soil system.

The significant increase in the concentration of malondialdehyde is an indication of rapid lipid peroxidation caused by oxidative stress in the earthworms resulting to the generation of reactive oxygen species (ROS). The exposure of the earthworms to heavy metals in the contaminated soil

may have facilitated the generation of ROS as heavy metals are highly persistent, toxic and can potentially induce severe oxidative stress and stimulate the production of these reactive oxygen species that can damage cells [48-53]. Cu and Cd in addition to binding with aromatic amino acid residues in enzyme molecules, can also cause oxidative damage to protein by the induction of oxidative stress with production of ROS [54].

According to Gallego *et al.* [55] an increased level of lipid peroxidation may occur because exposure to heavy metals usually results in conversion of unsaturated fatty acids into small fragments of aldehydes which can strongly affect the functionality of biological membranes and eventually cause cell death.

Conclusion

The results from this study revealed that the effluents from the industry may contain heavy metals responsible for the high soil content of Cd, Pb, Hg and Cu which may have long term effects on the soil characteristics and, subsequently, on the food chain.

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