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Effects of Heavy Metals on Soil Enzymatic Activities in the Ishiagu Mining Area of Ebonyi State-Nigeria.

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Abstract: *Effects of heavy metal pollution on the enzymatic activities of soils were investigated in the Ishiagu mining area of Ebonyi State, Nigeria. Soil dehydrogenase, polyphenol oxidase, hydrogen peroxidase, alkaline and acid phosphatases and urease were measured to evaluate the effects of heavy metals mining operations on soil biochemical characteristics. Results showed that the soil pH ranged from 5.04 to 6.56 while the heavy metals were of the ranges: Pb (13,754-29,491 mg/kg), Zn (1,151-2,778 mg/kg), Cd (17.65-27.71 mg/kg), Cu (9.59-39.91 mg/kg) and total heavy metals (014,932.24-32,336.62 mg/kg). Soil heavy metals concentrations significantly decreased with the increase of distance from the mining pit while soil pH increased with the increase of the distance from the mining pit. Analysis of the soil enzyme activities indicated a significant positive correlation at P≤ 0.05 between soil enzyme activities and soil pH. On the contrary, the activities of dehydrogenase, polyphenol oxidase, hydrogen peroxidase, alkaline and acid phosphatases and urease showed significant negative correlation at P≤ 0.05 with the heavy metal contents except for Zn against dehydrogenase activity and Cd against hydrogen preoxidase and urase activities that were though negative but statistically not significantly correlated at P≤ 0.05. This showed that the activities of the enzymes analysed could be used as sensitive indicators of heavy metals contamination. The results in general indicated that the mining operations at Ishiagu, Ebonyi State affect the soil quality due to heavy metal contamination.*

Key words*: Enzyme activities, heavy metals, Ishiagu, soil, pollution.*

I. Introduction

Contamination and subsequent pollution of the environment by toxic heavy metals has become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem.
Soil does not only provide a place for plants, animals and microbial life, but also a natural reservoir for metals. Its heavy metal concentration is associated with biogeochemical cycle, parent material, mineralogy, soil age, organic matter, particle size distribution, soil pH, redox concentration, oxidation state and microbial activities [1];[2].,[3];];[4].

Aside these natural processes, anthropogenic activities, such as agricultural practices, industrial activities, military activities and waste disposal methods tend to increase the concentrations of heavy metals and other trace elements in the soil [5];[6]. These anthropogenic sources of metal loading interact with the preexisting natural sources of metal that originated from metal-rich parent material [7]. Such conditions resulting to excessive accumulation of heavy metals in the soil are frequently found in areas with metal mining operations like that of Ishiagu.

Excessive amount of heavy metals affects soil biological properties and may change its basic physicochemical properties. [8] showed that microbial respiration was reduced in soil contaminated with heavy metals. Also, [9] reported that cadmium and zinc led to a reduction in soil respiration.
Moderation of many other biological processes of the soil such as the count and species diversity of macro-and microorganisms and changes in enzymatic activities resulting from contamination with heavy metals has been well documented [10]; [11]; [12]; [13] for various anthrogenic sources of heavy metals but not in Ishiagu mining area.

The effects of heavy metals mining operations on soil enzymatic activities in Ishiagu have not been documented. To understand if soil functions or biochemical characteristics are affected by long term metals mining operations, soil enzymatic activities in Ishiagu heavy metals mining area were evaluated in this study.

II. Materials and method

2.1. Sampling sites

The sampling site is located in Ishiagu in Ivo Local Government Area of Ebonyi State, Nigeria. The area lies between latitude 5^0 51′ and 5^0 59′ N and Longitude 7^0 24′ and 7^0 40′ E and is typically of tropical

climatic conditions with guinea savanna features, covering an area of over 8000 square Kilometers. The industrial activity in the area is predominated by metal mining, which has been going on for over twenty-five (25) years with its attendant environmental pollution. Quarrying activities (rock blasting) is also enormous in the area. However the local dwellers are mainly farmers, producing mainly staple crops.

2.1.1 Sample collection and analysis

This study was conducted between the months of July and August 2007. Four sampling points designated as 0 meter, 5 meters, 10 meters and 30 meters were mapped out from a randomly selected excavated site. A farmland, about 500 meters away from the excavated site was used as the control. Three (3) replicate soil samples were randomly collected from each of the sampling points at a depth of 15cm and pooled together to obtain a homogenous unity sample. The soil samples were transported in plastic bags to the department of biochemistry laboratories of Abia State University, Uturu, where the soil enzymatic activities were analysed. Heavy metal analysis was done at a commercial analytical laboratory in Port Harcourt, Rivers state, Nigeria. The soil pH was determined *ex situ* from 1:2.5 fresh soil/water ratios, using the glass electrode pH meter as described by [14].

2.1.2 Analysis of heavy metals

The heavy metals contents of the soil samples were determined according to the procedure of [15]. 0.5g of air-dried soil sample was weighed into a digestion flask to which was added 10ml of aqua riga (3HCl: $1HN0_3$). The mixture was shaken and heated on a hot plate until near dryness. The digest was allowed to cool and 20ml of deionized water was added to it and stirred. This was filtered through a whatman No. 42 filter paper into a 50mL vol. flask with rinsing using deionized water and made up to the mark with deionized water. A blank digestion was also carried out using the same procedure. The test sample filtrates were run in an atomic absorption spectrophotometer against the blank using the appropriate cathode lamp and wavelength. Lead was determined at 285nm, Zinc at 213nm, Cadmium at 227nm and Copper at 324nm.

2.1.3 Analysis of soil enzymatic activities

Soil dehydrogenase activity was determined by the method of [16] as modified by [17] using 0.25% triphenyl tetrazolium chloride (TTC) solution per 5g of dry soil sample. TTC is converted to triphenyl formazan (TPF) that is detected using a spectrophotometer at 485mm after incubation for 6 hours at 37^0C. Polyphenol oxidase activity was determined based on the formation of purpurogallin from pyrogallic acid amended soil sample after 3 hours incubation at 30^0C which absorbance was measured at 450mm as outlined by [17] in a method modified from [18].

Acid phosphatase and alkaline phosphatase activities were determined according to the procedure described by [19] and [18]. 2g of dry soil sample were incubated with 5ml of 5mM p-nitro phenylphosphate substrate at 20^0C for 1 hr. The soil sample for acid phosphatase activity was brought to pH of 4.9 while that of alkaline phosphatase activity was brought to pH of 10.0 with the substrate buffer. 0.1ml each of 0.5M $CaCl_2$ and 0.5M NaOH were added to 2ml of the supernatant, which was transferred to a clean sterile tube after centrifugation at 3000xg for 3min to halt the enzymatic activity and facilitate colour development. Prior to spectrophotometric analysis at 410nm, each sample of the supernatant was diluted with 8mL of distilled/deionized water.

Soil urease activity was determined by the method of [20]and [21] based on the NH_3-N formation in the urea-amended soil sample after 5 hours incubation at 37^0C. 5g of dry soil sample were amended with 1mL of 60mM urea solution incubated with 5ml of distilled water at 37^0C and intermittently shake for 5 hours. 2mL of phenylmercury acetate solution in 2M KCl was added to stop the reaction. The mixture was centrifuged and the concentration of the NH_3-N was determined spectrophotometrically and the soil urease activity was expressed as mg NH_3-N g-1 dry soil $5h^{-1}$

Hydrogen peroxidase activity was determined by the $KMn0_4$ titration method according to [22].

2.1.4 Data analysis

Statistical analysis was done using Pearson's simple correlation coefficient analysis. The population correlation coefficients were compared for significance using the hypothesis test for population correlation coefficient ($P \leq 0.05$) [23].

III. Result

The pH and the heavy metal content of the soil from the Ishiagu mining area are shown in table 1. The heavy metal content decreased with distance away from the pit. The pH of the soil ranged from 5.04 to 6.56 decreasing with the increase of the distance from the pit. Pb was the most abundant of the four heavy metals assessed with values ranging from 568 mg/kg to 29,491 mg/kg. Compared to the control, the total heavy metal

content was 29.2 times greater at the pit region and 13.5 times greater at distance 30 meters away from the pit. The pH of the soil was negatively correlated with the heavy metal content (Table 3). There were significant positive correlation between Pb, Zn, Cd, Cu and the total heavy metals at $P \leq 0.05$. Soil heavy metals concentrations significantly decreased with the increase of the distance from the pit.

Table 2 shows the enzyme activities of the soil from the Ishiagu mining area. The soil enzyme activities decreased with the decrease of distance from the pit. In comparison to the control, there was a reduction in dehydrogenase activity by 36.34%, polyphenol oxidase by 20.42%, hydrogen peroxidase by 10.45%, alkaline phosphatase by 17.02%, acid phosphatase by 21.15% and urease by 9.32% at 30 meters away from the pit. There were significant positve correlation between the activities of soil enzymes and the soil pH. On the contrary, there were significant negative correlation between the activities of soil enzymes analyzed and the heavy metal contents of the soil. Data analyses showed a significant negative linear relationship between the heavy metal contents and the activities of the soil enzymes at $P \leq 0.05$ except for Zn against dehydrogenase activity and Cd against hydrogen peroxidase and urease activities where the correlation were though negative but not statistically significant.

IV. Discussion

Soil enzymes have been proposed as useful indicators of soil quality. They play an important role in chemical changes involving soil nutrients. Dehydrogenase plays an essential role in the initial stages of the oxidation of soil organic matter by transferring hydrogen and electrons from substrates to acceptors [3]. Hydrogen peroxidase is associated with aerobic microbial activities, and decomposes hydrogen peroxide into molecular oxygen and water, thus alleviating its toxicity to organisms. Polyphenol oxidase is associated with carbon cycle and is important to the formation of humic substances, whereas phosphatases and urease are involved in the biochemical cycles of phosphorus and nitrogen respectively [10]; [17]; [24].

The finding of this research conducted showed that at the current pollution level, the heavy metals in the soil of Ishiagu mining area affects the soil quality as they exhibit inhibitory effect on the soil enzyme activities. [10] and [11] observed similar results in heavy metal polluted soils. However, [7] reported that heavy metal input to agricultural soil does not always inhibit the soil enzyme activities. This could however be attributed to the levels of the heavy metal input or contamination in line with the reports of [25] and [26] who observed that enzyme could be stimulated when the soil heavy metals only slightly exceed natural values but can be inhibited under the influence of excessive heavy metal concentrations. Thus, the mining operations at Ishiagu from the results could be said to have polluted the soils with excessive heavy metals.

There were strong significant negative correlation coefficients between the soil heavy metal contents and the activities of soil enzymes analyzed, except for Zn against dehydrogenase activity and Cd against hydrogen peroxidase and urease activities, which were statistically non-significant ($P \leq 0.05$). This shows that the levels of heavy metals present in the soil were strongly antagonist to the activities of the soil dehydrogenase, polyphenol oxidase, hydrogen peroxidase, acid and alkaline phosphatases and urease. The results also showed a negative linear relationship between the heavy metals and the activities of soil enzymes implicating that the higher the concentrations of heavy metals, the lower the activities of soil enzymes.

Contrary to this negative linear relationship obtained, [17] reported an increase in the activities of soil dehydrogenase, hydrogen peroxidase and polyphenol oxidase with increasing concentrations of the pollutant. The differences may be attributed to the differences in the pollutants since [17] worked on the effect of petroleum-containing wastewater that is a carbon source, after all. Petroleum aliphatic and polycyclic hydrocarbon can act as sources of carbon and energy for the growth of soil microorganisms [27], hence the increase in the soil enzyme activities.

The soil pH observed in this study was generally acidic and showed significant negative correlation ($P \leq 0.05$) with the heavy metal contents and significant positively correlation ($P \leq 0.05$) with the soil enzyme activities except for pH against Cd where the correlation were negative but statistically not significant.

It is possible that the low soil pH may have resulted from the eventual transformation of soil sulphur input from the atmospheric sulphur and metal sulphide ores into H_2SO_4, which can acidify soil and water. [28] opined that this might be one of the mechanisms of lowering pH by heavy metals. It is generally expected that low soil pH elevates the solubility and speciation of heavy metals by releasing from the soil particles heavy metals to the solution, resulting in an enhancement of the toxicity of heavy metals on soil bacteria and plants [29]; [30].

Thus, the reduction in the enzyme activities as observed from this work could be ascribed to the indirect effect of the heavy metals on the number of soil microorganisms and its direct effect on the soil enzymes. [31] and [32] have reported that metal ion may inhibit enzyme reactions by complexing the substrate, reacting with the protein active groups of the enzyme or with the enzyme substrate complex.

The soil dehydrogenase activities was the least tolerant to the heavy metal pollution with 86.46% activity reduction at 0 meter and 36.34% reduction at 30 meters away from the pit relative to the control soil.

This shows that the activities of soil enzymes especially soil dehydrogenase activities are sensitive indicators of heavy metals. The correlation coefficients (table 3) between the different heavy metals and the different enzyme activities showed that there is a strong linear relationship between the various individual heavy metals analyzed and the different soil enzyme activities. However, the strength of the relationships did not follow a definite order among the different enzyme activities. This shows that each heavy metal affects a particular enzyme differently and that the actual reduction in activity of a particular enzyme observed from this research could be the joint effect of the total heavy metal content of the soil. [33] reported that heavy metals reduced the activity of soil dehydrogenase by 10-90% depending on the rate and type of metal involved. The effect of the various individual heavy metals and their combined effect on the different soil enzymes therefore deserve a close study. Also, the results clearly showed that the current pollution level in Ishiagu mining area is excessive and therefore appropriate remediation measures is recommended to avert the multiple effects of heavy metals poisoning in the ecosystem.

V. Conclusion

Investigation on the effects of heavy metals pollution on the soil enzymatic activities in the Ishiagu mining area showed that the heavy metal contents of the soil in the area were exceedingly higher than normal and as such has reached pollution levels. This is indicated by their strong antagonistic effect on the soil enzymatic activities. Soil dehydrogenase activity was the most affected by the heavy metal pollution. Thus the activities of the soil enzyme especially those of the soil dehydrogenase could be used as sensitive biological indicators of heavy metals pollution. The pH of the soil in Ishiagu mining area was found to have played an important role in enhancing the toxicity of the heavy metals. In general, the heavy metals mining operations in Ishiagu area of Ebonyi State, Nigeria affects the soil quality as the soils are contaminated with heavy metals.

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Table 1: pH and concentration of heavy metals in soil from the Ishiagu mining area.

Soil sample distance from the pit (meters)	PH	Pb (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Total heavy metals (mg/kg)
0	5.04	29,491	2,778	27.71	39.91	32,336.62
5	5.74	20,106	1,725	20.37	31.85	21,883.22
10	6.37	17,687	1,157	18.94	17.31	18,880.25
30	6.56	13,754	1,151	17.65	9.59	14,932.24
Control	6.86	568	534	2.86	3.65	1,108.51

NB**:** Figures represent mean (n=3)

Table 2: Soil enzyme activities in soil from the Ishiagu mining area.

Soil sample distance from the pit (meters)	Dehydrogenase (mg $g^{-1}6h^{-1}$)	Polyphenol oxidase (mg $g^{-1}3h^{-1}$)	Hydrogen peroxidase (mlg^{-1})	Alkaline Phosphatase (mg $g^{-1}h^{-1}$)	Acid Phosphatase (mg $g^{-1}h^{-1}$)	Urease (mg $g^{-1}6h^{-1}$)
0	0.60	0.82	0.75	0.75	0.42	0.54
5	1.21	1.87	1.47	1.32	0.91	1.10
10	1.63	1.92	2.01	1.47	1.21	2.61
30	2.82	2.26	2.57	1.56	1.64	3.31
Control	4.43	2.84	2.87	1.88	2.08	3.65

NB: Figures represent mean (n=3)

Table 3: correlation coefficients between pH, heavy metal content and soil enzyme activities in soil from the Ishiagu mining area.

NB: * Implies non significantly different at $P \leq 0.05$

Variables	pH	Pb	Zn	Cd	Cu	THM	Deh	PPO	HPx	ALP	ACP	Urease
pH	1.000											
Pb	-0.917	1.000										
Zn	-0.986	0.936	1.000									
Cd	-0.854*	0.984	0.888	1.000								
Cu	-0.983	0.920	0.948	0.854*	1.000							
THM	-0.926	0.999	0.945	0.981	0.926	1.000						
Deh	0.880	-0.974	-0.869*	-0.955	-0.923	-0.971	1.000					
PPO	0.951	-0.967	-0.973	-0.923	-0.921	-0.972	0.912	1.000				
HPx	0.987	-0.936	-0.963	-0.871*	-0.991	-0.942	0.927	0.957	1.000			

ALP	0.973	-0.956	-0.993	-0.914	-0.937	-0.963	0.892	0.993	0.963	1.000		
ACP	0.965	-0.974	-0.951	-0.928	-0.979	-0.977	0.971	0.965	0.989	0.961	1.000	
urease	0.976	-0.889	-0.930	-0.819*	-0.997	-0.896	0.899	0.891	0.982	0.913	0.962	1.000

Deh = Dehydrogenase
ALP = Alkaline Phosphatase
ACP = Acid Phosphatase
THM=Total Heavy Metals
PPO=Polyphenol Oxidase
HPx=Hydrogen Peroxidase