

POST OPERATIONAL EFFECTS OF HEAVY METAL MINING ON SOIL QUALITY IN ISHIAGU, EBONYI STATE

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ABSTRACT: The post-operational effects of Pb and Zn Mining on soil quality was evaluated in Ishiagu, South East Nigeria using some physicochemical and biological indices. Soil samples from the excavated pits, waste heaps, heap side, between pits and control were analyzed. The soil pH was lowest at pit, (5.6), followed by waste heaps (6.0) while control had 7.4. Temperature was not affected. Organic matter, PO₄, SO₄ and NO₃ were lowest at pit, followed by waste heap, and heap side. The space between pits had values close to the control (only 20-30% lower). Heavy metals concentrations were 307.21 – 31.27mg/g and 196.72 – 39.15mg/g for Pb and Zn respectively. Highest values were at the pit and lowest were in the control. All soil enzymatic activities correlated negatively with Heavy metal concentration as the higher the Heavy metal values, the lower the enzymatic activities. The most affected enzyme was dehydrogenase while the least was Acid phosphatase. Hydrogen peroxidase, Phenol oxidase, urease and Alkaline phosphatase followed the same negative correlation with Heavy metal concentrations just as the various bacterial groups estimated. The most affected organisms were the Nitrifying bacteria while the least affected were heterotrophic bacteria. Others were the total coliforms, Phosphate – solubilizing bacteria. Results obtained call for extensive remediation, if the soil should be used for agricultural purposes.

Keywords: Enzymes, Physicochemical Parameters, Heavy Metals organisms, effect.

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INTRODUCTION

Man's quest for industrial development and comfort has resulted in several modifications of the delicate environmental balances of the ecosystem. The level of modification is dependent on the type and amount of the contaminant discharged in to the environment and time effect (Nwaugo *et al.*, 2007a, Oliveira and Pampulha, 2006). Generally, continuous discharge of wastes into an environment results in accumulation of the waste in the given environment. Consequently the effects become more pronounced.

Metal mining is one of man's oldest industrial activities. This leads to contamination of the soil which serves as the sink for the unextracted fractions. While the trace elements have little or no effects, the heavy ones adversely affect all living things (Lee *et al.*, 2002, Fagade and Adetutu, 1999). Nwaugo *et al.* (2007b), agreed with Li *et al.*, (2005) that the affects of soil pollution is spread to other areas by flooding, or rain water run offs; thereby affecting large expanse of land. It becomes important that soil pollution should be controlled, bearing in mind the important role soil plays in human life.

Heavy metal mining is common in Nigeria, but in most cases in very crude manner. Wastes generated in the process are neither treated nor appropriately discharged, resulting in environmental degradation. The excavation for those metals leaves large ponds and heaps of wastes in the wake of such mining activities. Regulations guiding these activities are either not there or not applied. Even when the mining activities are over, the area is simply abandoned for new areas without efforts to remediate the effects. This is the case of the lead and zinc mining area of Ishiagu, Ebonyi State, Nigeria. This work therefore was designed to access the level of soil pollution of the Pb and Zn Mining area of Ishiagu, Ebonyi State using some physicochemical parameters and bioindicators.

MATERIALS AND METHODS

Ishiagu is a rural community in Ebonyi State, near the northern boundary with Abia State. It lies within the typical Guinea Savannah region of Nigeria. Amicol and JOAMINESS Nigeria Ltd are the major companies mining the Pb and Zn in the area. These companies excavate for the metals and abandon the wastes generated in heaps, leaving the corresponding ponds behind. Indigenes of the area scavenge for the same metals in the abandoned helps thereby scattering these wastes.

SAMPLE COLLECTION

Soil samples were collected from five (5) areas. These areas were (1) the pits (2) spaces between the pits (3) the heaps (4) space down the heaps (5) and control which was an area away from the mining zone but within the same community. For each area, soil samples were collected from three spots and pooled together to give the major composite sample used in analysis. Sampling was done using the Sharpeck grab method and the collected soil samples were put into clean sterile containers before taking to the laboratory for analysis. Analysis of biological indices was done within 1-2 hours of collection (bioindicators) and 24-48 hours for physicochemical and heavy metals parameters. Preservation of samples was done by refrigeration.

Determination of Some Soil Physicochemical Parameters

The soil pH and temperature was determined at the site using the JENWAY Multipurpose tester (HANAA 1910 model). Organic matter was determined by the loss of ignition method involving the furnace (MAC 2000) as described by Lee *et al.* (2002). Soil Nitrate Phosphate and sulphate were determined by the AOAC (1998) method while the metal contents were estimated using the Atomic Absorption Spectrophotometric (AAS) (HACH/D2/2010 Spectrophotometer) after digestion.

SOIL ENZYMATIC ACTIVITIES

The enzymes whose activities were accessed include dehydrogenase, hydrogen peroxidase, and phosphatases (Acid and alkaline). The dehydrogenase was estimated according to Cassida *et al.*, (1964) as modified by Li *et al.* (2005). This was done using 10ml of 0.25% aqueous solution of Triphenyl Tetrazolium Chloride on 5.0g of soil. After incubation at 30°C for 6 hours, the Triphenyl formazon formed was extracted with methanol and the absorbance measured at 485nm. The result was expressed as $\text{TPF}^{-1} \text{ dry soil}^{-1} \text{ h}^{-1}$. Soil hydrogen peroxidase activity was estimated by the titrometric method of Alef and Nannipieri (1995) using KMnO_4 and expressing the result as $\text{mlg}^{-1} \text{ h}^{-1}$. Both acid and alkaline phosphatases activities were determined according to Tabatabai (1997) involving the incubation of p-nitrophenyl phosphate with 5.0g of the soil of 37°C at acidic (I) and alkaline (II) pH levels. Urease and phenol oxidase activities were determined according to Nannipieri *et al.* (1997).

MICROBIOLOGICAL ANALYSIS

Population of various bacterial groups were done using different culture media. The total heterotrophic bacterial count (THBC) was determined using Tryptone Soy Agar, total coliform count (TCC), by the use of McConkey Agar and nitrifying bacterial count (NBC) by using modified Mineral Salt Agar. The phosphate solubilizing bacterial count (PSBC) was done using Phosphate solubilizing Agar (US Patent 6638730). The bioloads were determined after a ten-fold serial dilution and spread plate inoculation as described by Chessbrough (2001).

STATISTICAL ANALYSIS RESULTS

Results obtained in this work were subjected to statistical analysis to test their significance. Tests used were correlation analysis and standard deviation.

RESULTS

Results obtained in this work showed that temperature was not significantly different at the various sampling areas while pH was highest (alkaline) away from the mining areas. The pit, heaps, and near the heaps had low organic matter, ranging from 2.61 to 3.94 mg/g while between the pits had 5.72 and control 7.2 mg/g. The PO_4 and NO_3 had ranges of 2.02 – 5.31 and 1.72 – 4.93 mg/g respectively with the lowest at the pit and the highest at the control. A similar trend was observed with SO_4 with a range of 14.37 – 37.71 mg/g (Table 1).

DISCUSSION

Analysis of the results obtained in the physicochemical parameters measured showed considerable effects of the mining activities on the soil. Temperature was not significantly affected but there were considerable differences in the other factor accessed. Organic matter, nitrate, and sulphate were lowest at the pit, followed by the waste heap but highest at control. The heavy metal concentrations were in the reverse order, highest at pit but lowest at control. Oliveria and Pampulha (2006) and Babich and Stokzky (1988) stated that low pH reduces metal solubilization and speciation which was observed in this work. High Pb and Zn were observed at the pit and waste heaps with pH values. This might have affected the organisms involved in the mineralization and production of nitrate, sulphate and phosphate in the soil hence their low levels. Mantellin and Touraine (2004) reported that low, pH and high heavy metal concentrations reduce plant nutrients in the soil which includes nitrate, sulphate and phosphate hence their low levels as observed in this work. The heavy metal levels observed in the soil, except the control and space between pits, were above the acceptable limits (CEC, Directive, 1984) hence would have caused the effects observed. Similarly, Peleczar *et al.* (2001) agreed that the plant nutrients are more available in the top soil, especially 1-20cm of the soil and decreases with depth into the soil. The mining pits are quite deep and the waste heaps contained soil from below 5 to 10m deep hence could be low in these substances. This is because the excavation brings up the deeper portions of the soil to the surface.

Enzymatic activities observed showed negative correlation with the heavy metal concentrations of the soil. Nwaugo *et al.* (2007a) Chander *et al.* (2000) agreed with Kupperman and Caricero (1997) that heavy metals affected soil biogeochemical activities by inhibiting enzymatic reactions or affecting the survival and proliferation of soil organisms which produce the enzymes. This could have occurred in this work as the soil enzymatic activities positively correlated with the bioloads of the various soil bacterial groups estimated. Generally, the lowest soil enzymatic activities were observed in the soil sample with highest heavy metal concentrations and lowest bioloads and the reverse held for low heavy metal contents and high bioloads in the control. However, the various enzymes were affected at different rates.

The most affected enzyme was dehydrogenase, whose activities only 18.67% in the pit compared to the control. Dehydrogenase is produced by all organisms and is intracellular (Li *et al.* 2005). It therefore follows that the higher the microbial load, the higher the enzymatic (dehydrogenase) activity which was the case. Li *et al.* (2005) agreed that organic matter activates the activities of hydrogen peroxidase. Nwaugo *et al.* (2007a & b) reported that some enzymes are inhibited by high heavy metal concentration. The observation of low hydrogen peroxidase activity in soil with low organic matter could be attributed to the high heavy metal concentration. Similarly phenol oxidase which is important in the mineralization of compounds with aromatic rings was low in low organic matter soil. High aromatic rings compounds are common in soil with high humus and organic matter contents. It therefore follows that there was not enough substrate for the enzyme in the heavy metal contaminated soil, hence the low activity observed.

Urease is said to be susceptible to many types of disturbances (Li *et al.*, 2005, Kandelar & Gerber, 1988) but its activities are said to be inhibited by high nitrogenous compounds. In this case, its activity is even low in low organic matter soil with heavy metal concentration and low microbial bioload. This shows that the enzyme is produced by micro-organisms which were inhibited by the heavy metals. These organisms were no more there to produce the urease for any reaction.

Alkaline phosphatase was more affected than acid phosphatase. While the soil with highest metal concentration had 52.31% of control soil value in the acid phosphatase, it was only 37.32% for alkaline phosphatase showing higher suppression of alkaline phosphatase. This could be attributed to the soil pH which was more acidic than alkaline. Wyzskowska and Kucharski (2000) and Nwaugo *et al.* (2007a) had earlier observed such situation. This could easily reduce phosphate content in the soil.

Generally, the positive correlation of soil enzymatic activities with bacterial load confirms further that soil organisms produce most of the enzymes whose activities were estimated. Of all the groups of soil organisms evaluated the most affected was nitrifying bacteria while the least was the THBC. The Nitrifying bacteria are quite sensitive to any form of distortion of ecological factors, hence are adversely affected by the effects of the heavy metal mining. Like the NB, the coliforms and PSB are specialized groups. The THBC is the sum of all culturable organisms (bacteria) in the soil. It therefore follows that some of the specialized bacterial groups are equally among the THBC.

In conclusion, this work therefore calls for extensive rehabilitation of the soil in the Pb and Zn mining zone of Ishiagu, if the land will be converted to farm land.

REFERENCE

- Alef, K. and Nannipieri, P. (1995). *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London.
- APHA (1998) *Standard Methods for the examination of water and waste water*. American Public Health Association, 18th Edition. Washington, D.C.
- Babich, H. and Stotzy G. (1980). Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. *CRC Crit. Rev. Microbiol* 8:99-145
- Cassida, L.E., Klein, J.D. and Santoro, D. (1964). Soil dehydrogenase activity. *Soil Sc*, 98:371-374
- CEC Directive (1986). Commission of the European Communities Council Directive on the protection of the environment and in particular of the soil, when sewage sludge is used in agriculture. *Official J. Eur. Comm.* L181 6
- Chander, K., Dyckmans, Y., Joergensen, R.G., Meyer, B. and Raubuch, M. (2000). Different sources of heavy metals and their long term effects on soil microbial properties. *Biol. Fertil. Soil* 34:241-247
- Chessbrough, M. (2001). *Laboratory Manual for Tropical countries*. Vol. II. Microbiology. *Tropical Health Technology*. ELBS, London.
- Fagade, O.E. and Adetutu, E.M. (1999). Lead solubilization and Accumulation by two strains of *Pseudomonas* species obtained from a battery manufacturing factory's effluent. *Nig. J. Microbiol* 13:39-46.
- Kandelar, E. and Gerber, H. (1988). Short term assay of Urease activity using colorimetric determination of ammonium *Biol Fertil. Soils* 6:68-72.
- Kuperman R. G. and Carreiro, M.M. (1997). Soil Heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. *Soil Biol. Biochem* 29:179-190
- Lee, I., Kim, K. Chang, Y. Bac, B., Kini H.H. and Baek K. (2002). Heavy metal concentrations and enzyme activities in soil from a contaminated Korean Shooting Range. *J. Biose. Biomin.* 94(5). 406-411.
- Li, H., Zhang, Y., Zhang, C.G. and Chen G.X. (2005). Effect of Petroleum containing waste water. Irrigation on Bacterial diversities and enzymatic activities in a Paddy soil irrigation area. *J. Environ Qual.*, 34:1073-1080.
- Mantellin, S. and Tauraine, B. (2004). Plant growth-promoting bacteria and nitrate availability: Impacts on root development and nitrate uptake. *J. Expt. Bot.* 55(394) 27-34.
- Nannipieri, P.B., Creccanti, A., Cervelli, S. and Matarese, E. (1980) Extraction of phosphatase, Urease, protease, organic carbon, and nitrogen from soil. *Soil Sc. Soc. Am. J.* 44:1011-1016.
- Nwaugo, V.O., Onyegba, R.A., Akubugwo, E.I. and Ugbogu, O.C. (2007a). Soil bacterial flora and enzymatic activities in heavy metal (Pb and Zn) contaminated soil of Ishiagu, Ebonyi State. *Estud. Biol.* 66 (In Press)
- Nwaugo, V.O., Onyegba, R.A., Azu, N. and Nwaorie, O. (2007b). Petroleum Produced water induced changes in bacterial quality and soil enzymatic activities in a farmland in Egbema, Southern Nigeria. *Estud. Biol.* 66 (In Press).
- Oliveira A. and Pampulha, M.E. (2006). Effects of long-term Heavy metal contamination on soil microbial characteristics. *J. Biosc. Bioeng.* 102(3):157-161.
- Pascal, J.A, Hernandez, C., Garcia and Ayuso, M. (1998). Enzymes activities in arid soil – amended with urban organic wastes. Laboratory experiments. *Bioresouruce Technol.* 64:131-138
- Pelczar, M.I., Chan, E.C.S. and Kriegel, I (2001). *MicrobiologyY; Applications and practices*. 6th edition. McGraw-Hill Inc. London.
- Tabatabai, M.A. (1997). Soil Enzymes, in page A.L Millers, R.H, & Kenny, D.R (Eds). *Methods of soil Analysis parts: Chemical and microbiological properties*. Soil Science. Society of American Madison. W.I.
- Tabatabai, M.A. and Bremaer, J.M. (1969). Use of P-nitrophenyl Phosphate for assay of soil Phostase activity. *Soil Biol Biochem* 1:307-312.
- Wyszkowska, J. and Kuchariski J. (2000). Biochemical Properties of soil contaminated by petrol. *Pol. J. Environ. Stud* 9(6): 479-485.