

Research paper

Evaluation of the concentration of selected heavy metals and the effects on soil enzymatic activities in an abandoned cement factory Nigercem Nkalagu and the environs

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ABSTRACT

This study investigated the concentrations of heavy metals, the activities of some selected enzymes and the pH of the soil surface (1-10 cm) and depth (11-20 cm) within the vicinity of an abandoned cement factory Nigercem Nkalagu, and further away 1km from the factory centre. The results indicate that the pH and heavy metals gradually decreased with increase in distance from the cement factory centre with the exception of Fe, Zn and Pb which was the reverse. The concentrations were also higher in the depths. The enzymatic activities increased away from cement factory centre and decreased with depth. There was a significant difference ($p \leq 0.05$) between most of the sampling points and the control (1 km) for all the parameters studied, except Cd and Cr. The enzymatic activities correlated positively with Fe, Zn and Pb and negatively with the other parameters. The inverse correlation between most of these heavy metals, the pH, and the activities of the soil enzymes are indicative that pollution caused by cement production still exhibit a significant effect on the enzymatic activities and this may invariably affect the quality of the soil of this area.

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Key words: cement, pollution, heavy metals, enzymes, soil.

1.0 INTRODUCTION

The environment is the natural surrounding of an organism which includes air, water and land; they contain certain amount of elements and compounds in a given proportion by nature and are considered polluted when there is an imbalance in the natural composition (FEPA, 1991). The soil

plays a vital role in life of organisms, not only for anchorage and source of nutrients but also as a sink to many industrial wastes most of which are hazardous (Prescot *et al.*, 2001). The biochemical processes of the soil are usually carried out by enzymes which are primarily derived from micro-organisms but can also originate from plants and animals (Paul and Clark, 1996). Enzymes are usually sensitive to changes in soil properties due to presence of pollutants from anthropogenic activities and therefore can be used as measure of soil quality (Gazso, 2001). Cement production is one of the major anthropogenic sources of soil pollution. It is characterized by particulate air pollutants usually dust (El-Daly, 1994). Dust from limestone is highly alkaline with pH varying between 6.0 and 12.0 (Arslan and Boybay, 1990). Cement dust also contains metals that have direct toxic effects on the environment. They include Mg, Al, Si, Pb, Cu, Fe, Cd, Cr etc (Gbadebo and Bankole, 2007). Some of these metals at very low concentrations are essential components of enzymes, pigments and structural proteins, but on a high concentration alters the soil environment and adversely affect soil biotic properties such as microbial biomass and enzymatic activities (McCarthy *et al.*, 2007).

Micro-organisms in the soil are responsible for nitrogen fixation, assimilation and degradation of organic residues to release nutrients. Heavy metals released from cement pollution retained in the soil interfere with these key biochemical processes and affect soil quality. This implies that soil fertility is closely linked to microbial flora and fauna (Prescot *et al.*, 2001), (Mathe-Gaspar *et al.*, 2005). In assessing soil microbial properties, culturable organisms are used. This method is deficient as it estimates culturable organism while the unculturable ones are underestimated (Li *et al.*, 2005).

Therefore the use of enzyme activities becomes important in the estimation of soil quality since it evaluates both the extracellular and intracellular activities (Wyszkowska, and Kucharski, 2003). Toxic effect of heavy metals and other pollutants from cement production on microbes manifests in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling, impaired enzyme synthesis. Toxicity to enzymes is due to their ability to induce oxidative stress, high affinity for the functional groups of protein. They may also displace constituent metals of proteins (Petr *et al.*, 2000). It was also believed that an additive or synergistic effect is responsible for enhanced toxicity of metals (Nwuche, and Ugoji, 2008). Soil pH and type of soil greatly influences the solubility availability and toxicity of metals. High pH of cement dust also affects the activities of soil microbial enzymes (Utgikar *et al.*, 2003). The aim of this study is to determine the level of heavy metals from cement production in the soil of the abandoned cement industry and the effects on soil enzyme activities and hence estimate the suitability of the soil for plant production.

2.0 Materials and methods

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62 2.1 Site description

63 NIGERCEM Factory is situated at Nkalagu in Ishielu Local Government Area of Ebonyi State, Nigeria.
64 The geographical coordinates are $6^{\circ}29^1\text{N}$ and $7^{\circ}46^1\text{E}$. The area is typical of tropical climatic
65 conditions with guinea savannah features. Cement production and limestone quarrying activities
66 was on in this area for about 42 years. The factory has now been abandoned for close to a decade.
67 The rural dwellers in this area are mainly farmers with cassava, rice and yam as major crops.

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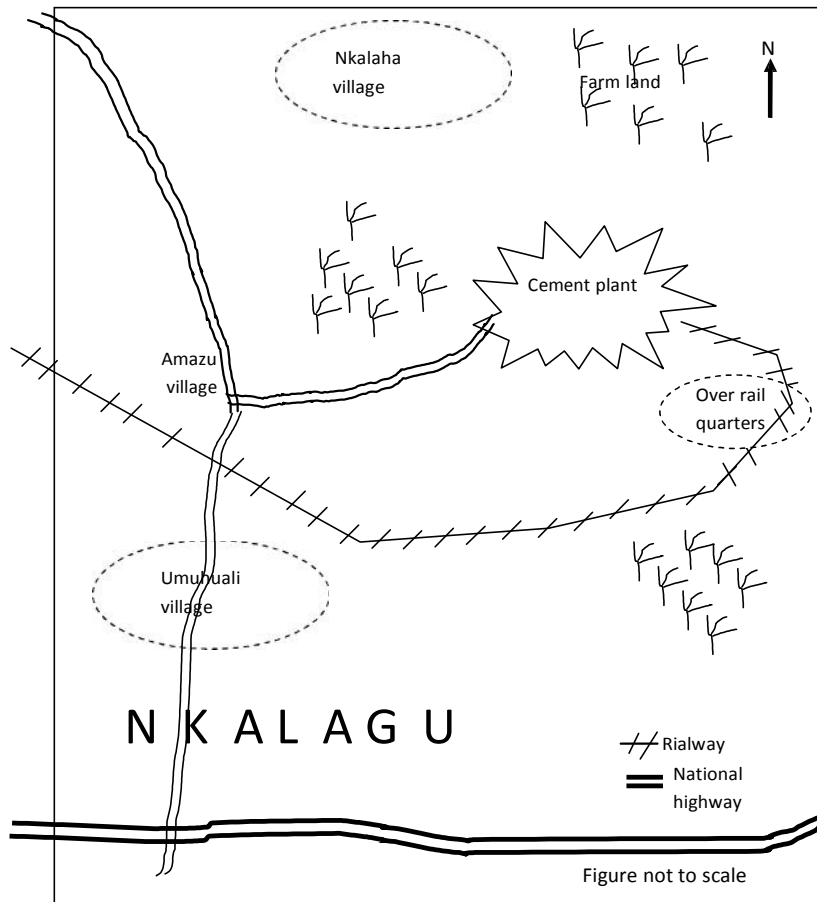


Fig 1: Location map of Nigercem cement factory, Nkalagu Eastern Nigeria

74 2.2 Soil sampling

75 Soil samples were collected in August, 2011 at different distances from the cement factory:
76 factory centre, factory edge, 100m from the edge, and 200m from the edge. The control sample was
77 collected at about 1km from the cement factory. The collection of samples was done with a plastic
78 soil auger. At each sampling point, three (3) Samples were collected at random at two (2) depths, 1-
79 10cm (surface) and 11-20cm (depth), and were pooled together to make one composite sample.

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80 2.2.1 Preparation of soil samples for analysis

81 Soil samples were air dried to a constant weight and crushed with a wooden roller, sieved with 2mm
82 sieve and stored in a soil sac for the pH, heavy metals and enzymatic activities determination.

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83 2.3 Chemical and enzymatic analysis

84 The digested samples were spectrophotometrically analyzed for heavy metals (Mn, Si, Pb, Cu, Fe, Zn,
85 Cd, and Cr) according to AOAC (2005). The following enzyme activities were determined: total
86 dehydrogenase according to Alef, (1995), β -glucosidase according to Eivazi, and Tabatabai, (1988),
87 acid and alkaline phosphatases according to Tabatabai, (1982), urease according to Kandeler and
88 Gerber, (1988), and arylsulphatase according to Alef and Nannipieri, (1995), pH was determined
89 according to Nayek *et al.*, (1996), moisture content according to APHA (1998).

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90 **2.4 Statistical analysis:** Statistical analysis was performed using the analysis of variance (ANOVA).

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91 The data represented means calculated from three replicates. A least significant difference test was
92 employed for comparison of the changes and values at $p \leq 0.05$ are said to be significant.

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93 3.0 Results

94 The tables below show the result of the chemical analysis while those of the enzymatic activities are
95 represented in figures 2 to 7. It was observed that among the heavy metals, Fe, Pb and Zn increased
96 with increase in distance from the cement factory for both surfaces and depth. Mn, Si and Cu were
97 the reverse. Cd and Cr were slightly higher at the factory center and edge but maintain almost the
98 same level in the rest of the samples. In table 3, the pH and temperature decreased with increase in
99 distance from the factory centre, but slightly higher at the depth, while the moisture content
100 increased in that direction.

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101 The soil enzyme activities generally increased with increase in distance from the centre of
102 the factory with little exception on alkaline phosphatase which was not linear. The enzymatic
103 activities were also higher at the surface when compared to the depth. Total dehydrogenase has the

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104 highest activity while the least was recorded for urease. There were significant differences between
105 the level of the parameters in most of the samples and the control except for Cd and Cr as indicated
106 in the result. The enzymatic activities correlated negatively with the pH and most of the heavy
107 metals studied.

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109 **Table 1: Heavy Metals Concentrations (mgkg⁻¹) Soil Surface (1-10cm)**

Heavy metals	Centre	Edge	100m	200m	Control (1km)
Mn	0.80±0.36 ^a	0.60±0.10 ^a	0.05±0.26 ^a	0.42±0.02	0.41±0.05
Si	2.40±0.40 ^a	3.10±0.00 ^a	0.80±0.26 ^a	0.30±0.00	0.30±0.17
Pb	8.20±1.60 ^a	10.10±0.43 ^a	12.90±0.81	13.60±0.35	41.20±0.35
Cd	0.70±0.03	0.70±0.36	0.06±0.03	0.06±0.03	0.60±0.02
Cr	0.70±0.03	0.70±0.36	0.06±0.25	0.06±0.04	0.06±0.03
Fe	10.700.36 ^a	10.60±0.53 ^a	15.40±0.53 ^a	16.30±0.26 ^a	17.30±0.10
Zn	12.10±0.46 ^a	14.30±0.30 ^a	18.30±0.46 ^a	20.30±0.26 ^a	21.70±0.30
Cu	11.30±0.44 ^a	11.00±0.26 ^a	10.60±0.40	10.60±0.20	10.40±0.20

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111 Values are mean ± standard deviation of three values. ^a = significantly higher or lower (p≤0.05)

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123 **Table 2: Heavy Metals Concentrations (mg/kg⁻¹) Soil Depth (11-20cm)**

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Heavy metals	Centre	Edge	100m	200m	Control (1km)
Mn	0.80±0.17 ^a	0.60±0.00 ^a	0.41±0.06 ^a	0.43±0.04	0.40±0.20
Si	2.20±0.36 ^a	1.80±0.52 ^a	0.70±0.28 ^a	0.30±0.10	0.30±0.10
Pb	8.30±0.26 ^a	10.30±0.30 ^a	13.80±0.60	13.80±0.69	14.10±0.53
Cd	0.80±0.53	0.70±0.20	0.06±0.20	0.06±0.00	0.06±0.20
Cr	0.70±0.26	0.70±0.10	0.06±0.05	0.06±0.00	0.06±0.17
Fe	10.60±0.35 ^a	11.20±0.42 ^a	15.90±0.10	16.70±0.44	17.40±0.61
Zn	13.10±0.26 ^a	14.90±0.36 ^a	18.80±0.35 ^a	20.70±0.61	21.80±0.20
Cu	13.60±0.21 ^a	13.40±0.00 ^a	13.10±0.26	13.20±0.43	13.10±0.10

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127 Values are mean ± standard deviation of three values. ^a = significantly higher or lower (p≤0.05).

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134 **Table 3: Soil physicochemical properties**

	Centre		Edge		100m		200m		Control(1km)	
	S	D	S	D	S	D	S	D	S	D
pH	10.30 ±0.10 ^a	10.50 ± 0.42 ^a	9.80 ±0.20 ^a	9.90 ±0.10 ^a	8.40 ±0.32 ^a	8.40 ±0.20 ^a	7.20 ± 0.20	7.30 ± 0.30 ^a	6.80 ± 0.20	6.60 ± 0.20
Temperature °C	30.30 ±0.26 ^a	32.10 ±0.10 ^a	30.10 ± 0.01 ^a	31.40 ±0.17 ^a	29.20 ± 0.17	29.10 ±0.17 ^a	29.10 ± 0.10	28.70 ± 0.20 ^a	28.50 ± 0.44	27.50 ± 0.26
Moisture %	12.20 ± 0.10 ^a	15.20 ± 0.20 ^a	13.10 ± 0.10 ^a	16.31 ±0.05 ^a	16.70 ±0.26 ^a	14.70 ±0.30 ^a	20.10 ±0.26 ^a	19.30 ± 0.17	21.70 ± 0.10	20.40 ± 0.30

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136 Values are mean ± standard deviation of three values. S = surface, D = depth, ^a = significantly higher
 137 or lower (p≤0.05).

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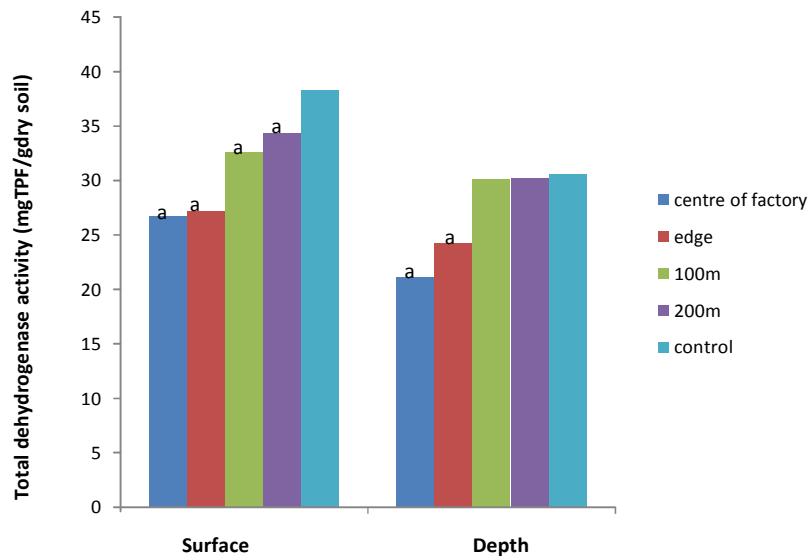


Fig 2: Total dehydrogenase activity (mgTPF/gdry soil) of the factory

a = significantly higher or lower than control ($p \leq 0.05$)

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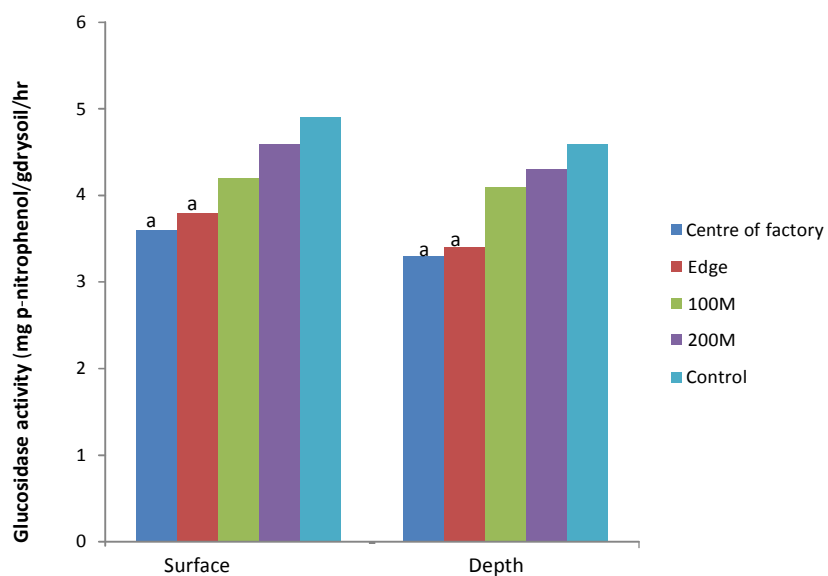
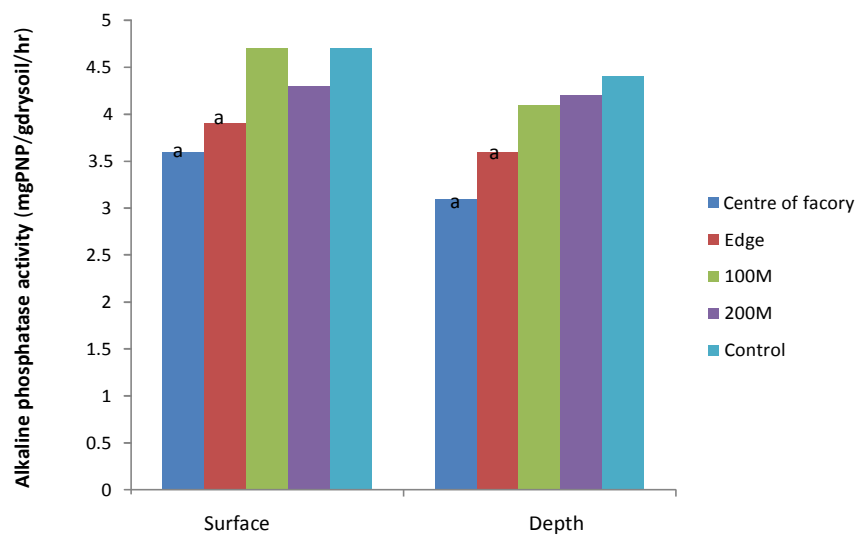


Fig 3: β -Glucosidase activity mgPNP/gdrysoil/hr

a = significantly higher or lower than control ($p \leq 0.05$)

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167 Fig 4: Alkaline phosphatase activity (mgPNP/gdrysoil/hr)

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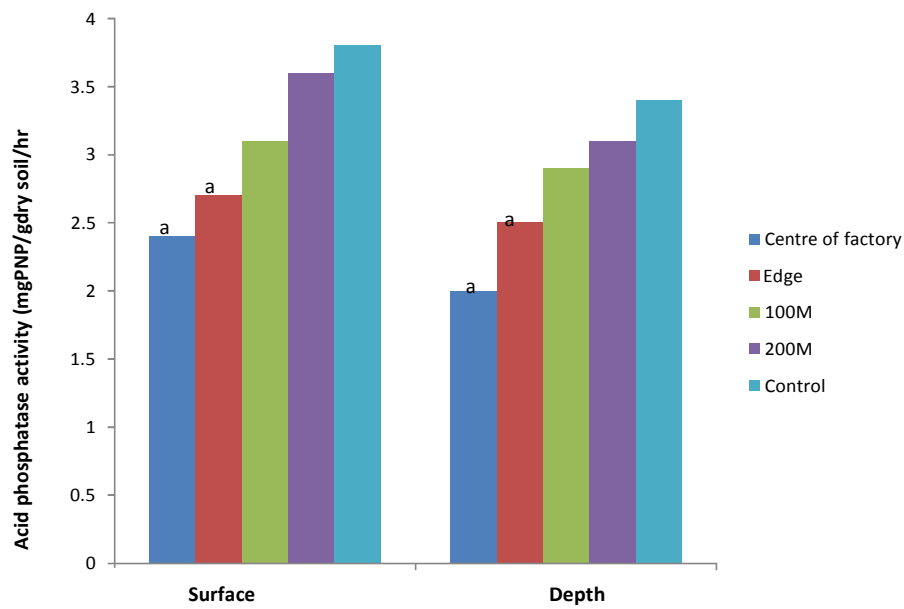


Fig 5: Acid phosphatase activity (mgPNP/gdrysoil/hr)

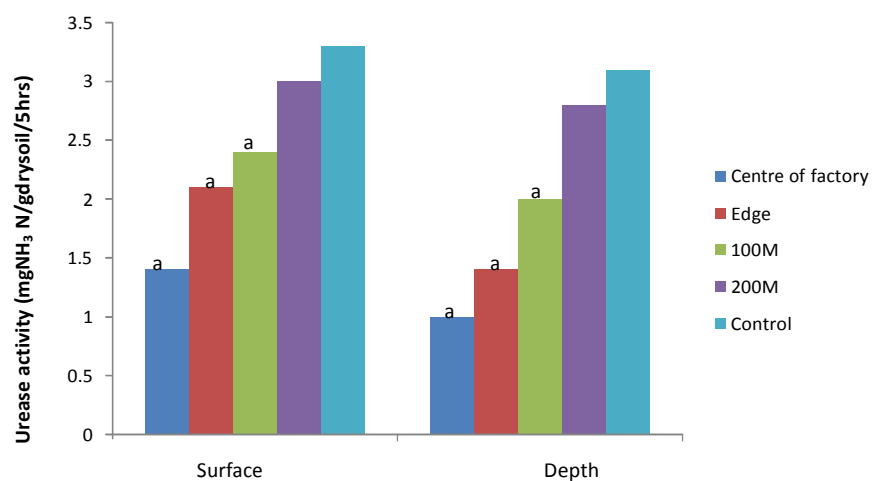
a = significantly higher or lower than control ($p \leq 0.05$)

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194 Fig 6: Urease activities (mgNH₃-N/gdry soil/5hrs) of the factory soil a

195 = significantly higher or lower than control ($p \leq 0.05$)

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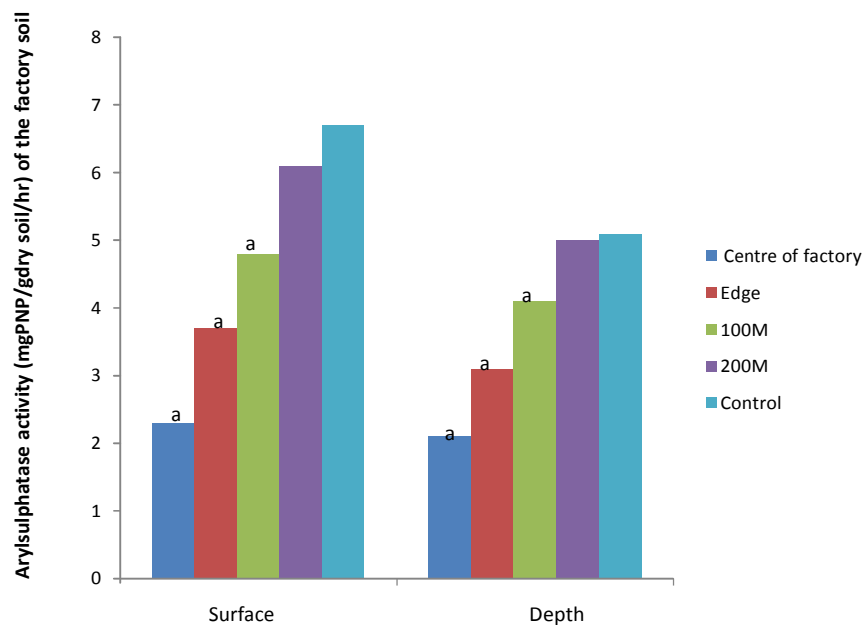
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205 **Fig 7: Arylsulphatase activity (mgPNP/gdry soil/hr) of factory soil**

206 **a = significant higher or lower than control ($p \leq 0.05$)**

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215 4.0 Discussion

216 The result revealed that Fe, Zn, Pb were significantly higher in the control than in the other
 217 sampling points in the order centre < edge < 100 m < 200 m < control. This suggests that there might
 218 be other anthropogenic sources of these metals other than the cement factory. Cd and Cr
 219 maintained almost the same level at all the distances and control with little exceptions. Si, Cu, Mn Cr
 220 and Cd correlated positively which is an indication that they are of the same source. The values of
 221 the metals are generally higher at the depths as recorded in our result. This may be attributed to no
 222 production activities in the cement factory over a long period and hence factors such as surface run-
 223 off, leaching, sedimentation, and bioaccumulation of these metals by some plants come into play
 224 thereby making them less available on the top soil. The pH of the soil ranges between 10.30 ± 0.10
 225 (at centre) to 6.80 ± 0.20 (at control) indicates that the factory soil is still alkaline. It was also
 226 observed that enzyme activities increased with increase in distance from the cement factory. This
 227 may be due to pollution from cement dust particles and increase in pH as shown in our result. The
 228 decrease in enzymatic activities with depth could also be attributed to high PH, heavy metal
 229 accumulation in the depth due to sedimentation, or perhaps due to aerobic condition in the surface
 230 which supports microbial activities than the underlying soils or the combination of these factors. This is
 231 concordant with the works of Wright and Reddy, (2001). Soil enzymes are primarily derived from
 232 micro-organisms but can also originate from plants and animals (Franzluebbers, 2004). Hence,
 233 factors affecting microbial activities will invariably affect soil microbial enzymes. Dehydrogenase has
 234 the highest enzymatic activities while the lowest value was recorded for urease. This may be as a
 235 result of higher sensitivity of urease to alkaline pH as similarly reported by (Marzadori, 1996). Heavy
 236 metals have been reported to inhibit enzymes singly or in combination by synergism (Marzadori,
 237 1996). Cr, Cd, and Pb, has been shown to inhibit dehydrogenase enzymes in the soil while Cr, Cu, Cd,
 238 Zn, Pb strongly inhibit urease. Other enzymes such as the phophatases, glucosidases, and
 239 arysulphatases are also susceptible to heavy metal stress (Parisa *et al*, 2008). Zn, Pb, and Fe have
 240 positive correlation with the enzymes while others were the reverse. The concentrations of the
 241 heavy metals observed were generally low. It seems likely that the levels of metal content typically
 242 found in soil do not generally explain variability of biological activities in the soil. Therefore pH and
 243 other soil parameters might have contributed immensely. This is in agreement with the data of
 244 Wyszowska *et al.*, (2006), who reported that dehydrogenase and phosphatase activities in mining
 245 soil which had developed from metal-rich parent materials, were sometimes higher than that
 246 detected in corresponding reference soils with low metal content.

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5.0 Conclusion

This study showed a high level of heavy metals and alkaline pH within the vicinity of the abandoned cement factory which still exhibits a significant negative effect on the soil enzymes, although, there may be contribution from pedogenic sources. However, it was observed that heavy metals such as Fe, Zn, Pb, have positive correlation with the enzymatic activities, while others were the reverse. Generally, the changes in elemental composition of the soil due to cement production still have significant influence on the soil enzymatic activities. All the heavy metals have higher concentration in the 10 cm depth which is within the reach of plant roots and this may have an effect on the natives who are already using this site as an agricultural farm. We suggest further studies of the chronic toxicity of these heavy metals on human subjects from this area.

References

- FEPA (1991). Guidelines and standards for environmental pollution control in Nigeria. Federal Environmental Protection Agency. Government Notice, Federal Republic of Nigeria.
- Prescot, L. M., Klein, D. A. and Harley, J. P. (2001) Microbiology. 5th edn. McGraw Hill, USA.
- Paul, E. A. and Clark, F. E. (1996). Soil Microbiology and Biochemistry. (Textbook devoted to the classical study of soil microbiology and its role in the environment.)
- Gazso, L. G. (2001). The key microbial processes in the removal of toxic metals and radionuclides from the environment (a review). *Central Europ J. of occup. Environ and Med.* **7(3)**:178-185.
- El-Daly, A. F. (1994). The status of cement industry environmental pollution and measures. In: proceedings of the National conference on pollution from Industrial installations. Working Force and Training Alexandria –Egypt, 3-6 December (1994).
- Arslan, M. and BoyBay, M. (1990). A study on the characterization of dust fall. *Atmos. Environ.* **24**: 267-271.
- Gbadebo, A. M. and Bankole, O. D. (2007). Analysis of Potential Toxic Metals in Airborne Cement Dust around Sagamu, Southwestern Nigeria. *J. of Appl. Sci.* **7(1)**: 5-40.
- McCarthy, G. W., Siebielec, G., Stuczynski, T. I. (2003). Response of soil microbiological activities to cadmium, lead, and zinc salt amendments. *J. of Environ. Qual.* **32**: 1346-1355.
- Mathe-Gaspar, G., Mathe, P., Szabo, L., Orgovanyi, B., Uzinger, N. and Anton, A. (2005). After effect of heavy metal pollution in a brown forest soil. *Acta Biologica Szegediensis* **49**(1-2): 71-72.

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- 281 10. Li, H., Zhang, C. G. and Chen, G. X.. (2005). Effect of petroleum containing waste water
282 irrigation on bacterial diversities and enzymatic activities in a paddy soil irrigation area. J. of
283 Environ. Qual **34**:1073-1080.
- 284 11. Wyszowska, J. and Kucharski, J. (2003). Biochemical and physicochemical properties of soil
285 contaminated with heavy metals. Zesz. Prob. Nauk Rol. In Polish J. S. of environ. Stud.
286 **492**:241.
- 287 12. Petr, B., Carsten, I D. W., Jiri, G., Frantisek, N. and Frantisek, Z. (2000). Influence of cadmium
288 and mercury on activities of lignolytic enzymes and degradation of polycyclic aromatic
289 hydrocarbon by *Pleurotus ostreatus*. Appl. And Environ. Microbiol. **66**(6): 2471-2478.
- 290 13. Nwuche, C. O. and Ugoji, E. O. (2008). Effects of heavy metal pollution on the soil microbial
291 activity. Int. J. of Environ. Sci and Technol. **5**(3): 409-414.
- 292 14. Utgikar, V. P., Tabak, H. H., Haines, J R. and Govind, R. (2003). Quantification of toxic
293 inhibitory impact of copper and zinc on mixed cultures of sulphate reducing bacteria.
294 Biotechnol. And Bioeng. **82**(3):306-312.
- 295 15. AOAC (2005). Official Methods of Analysis. Association of Analytical Chemistry 18th edn.
- 296 16. APHA (1998). Standard Methods for examination of water and waste water. American Public
297 Health Association. American Water Works Association and water Pollution control
298 Federation. 20th edn. Washington DC, USA.
- 299 17. Alef, K. (1995). Dehydrogenase activity. Methods in applied soil microbiology and
300 biochemistry. Alef, K. and Nannipieri, P. (eds.). Academic press, London. 228-231.
- 301 18. Eivazi, F. and Tabatabai, M. A. (1988). Gucosidases and galactosidases in soils. Soil Biol. And
302 Biochem. **20**:601-606.
- 303 19. Tabatabai, M, A. (1982). Soil enzymes. In: Methods of soil analysis, Part 2. Agronomy
304 monography, Page, A. L. (ed). American Society of Agronomy, Madison. **9**:903-904.
- 305 20. Kandeler, E. and Gerber, H. (1988). Short term assay of soil urease activity using colorimetric
306 determination of ammonia. Boil. Fert. Soils **6**:68-72.
- 307 21. Alef, K and Nannipieri, P. (1995). Methods in Applied Soil Microbiology and Biochemistry.
308 Academic press, New York.
- 309 22. Nayek, D. C., Sen, T. K., Chamuah, G. S. and Seghal, J.C C. (1996). Nature of soil acidity in
310 some soils of Mainpur. *Journal of Society of Soil Science* **44**: 209-212.
- 311 23. Wright, A. L. and Reddy, K R. (2001). Phosphorus loading effects on extracelullar enzyme
312 activity in everglades wetland soils. Soil Sci. Soc. of America J. **65**: 588-595.
- 313 24. Franzluebbers, A. J. (2004). Soil Biology. United States Department of Agriculture (USDA),
314 Agricultural Research Service, Watkinsville.

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- 315 25. Marzadori, C., Ciaviatta, C., Montecchio, D. and Gessa, C. (1996). Effects of Lead pollution on
316 different soil enzyme activities. *Boil. Fert. Soils* **22**:53-58.
- 317 26. Parisa, S., Hasan, S. V. and Ahmad, H. (2008). Evaluation of some paddy soil properties on
318 urease enzyme activity. Conference on international Research on Food Security. National
319 Resources Management and Rural Development, Iran.
- 320 27. Wyszowska, J., Kurcharski, J and Lajszner, W. (2006). The effects of copper on soil
321 biochemical properties and its interaction with other heavy metals. *Polish J. of Environ.*
322 *studies* **15**(6):927-934.
323