Soil Enzymes Assessment Around Amega Cement Factory in Nigeria.



Oluseye Olalekan Oludoye^{*&}** and Lanrewaju Amos Ogunyebi***



*International Program in Hazardous Substance and Environmental Management (Interdisciplinary Program), Graduate School, Chulalongkorn University, Bangkok, 10330, Thailand. **Centre of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok, 10330, Thailand. ***Environmental Biology Laboratory, Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria. Corresponding Author: Oluseye Oludoye, e-mail: soludoye@yahoo.com

Abstract

High soil quality is important for agricultural activities but pollution from cement industries is of great threat to sustainable agriculture in Nigeria. The aim of this study is to assess the effect of heavy metals in cement dust on the soil enzymes activities.

This study investigated the activities of some selected soil enzymes, the microbial communities' population, the concentrations of some heavy metals, some selected soil macronutrients and the physico-chemical properties (pH, moisture, and temperature) of the soil surface (0-15 cm), within the vicinity of a cement factory in Nigeria.

The pH, total organic nitrogen (TON), total organic carbon (TOC) and heavy metals decreased with increasing distance from the cement factory. The microbial population around the factory was $(34.00 \pm 2.64 \text{ CFU g}^{-1})$ and $(92.00 \pm 1.00 \text{ CFU g}^{-1})$ at 100 m and the control site respectively. All the soil heavy metals contents analyzed are less than the permissible limit except Cd. Moreover, the enzyme activities increased away from cement factory except alkaline phosphatase that decreases with increase in distance. The enzymes activities (except alkaline phosphatase) correlated negatively with the content of all heavy metals, the pH, the TON, the TOC and temperature but positively correlated with moisture, phosphorous and microbial populations. The inverse correlations between most of the heavy metals, the pH and soil enzymes activities could be a result of the pollution. This means that the cement production exhibits a significant effect on the enzyme activities.

Key Words: anthropogenic impact, bioaccumulation and biomagnification, geomagnification, soil enzymes, xenobiotics.

Introduction

The biophysical environment contains definite amount of elements and compounds in a given proportion by nature and is considered polluted when there is an imbalance in the natural composition of all these compounds. The toxic metals, from the xenobiotics, entering the ecosystem may lead to geoaccumulation, bioaccumulation and biomagnifications in organisms in the environment (Ogunkunle and Fatoba, 2014).

The xenobiotics can be released intentionally into the soil or dispersed by wind and rain to a far distance from the source of pollution. The concentrations of xenobiotics in soils are associated with biological and geochemical cycles and are influenced by anthropogenic activities, such as transport, waste disposal, industrialization, social and agricultural activities. They have an effect on environmental pollution and the global ecosystem.

One industry that emits pollutants in the form of dust and gases which find their way into the soil is the cement industry (Addo, 2013). Dust from cement and other factories leads to considerable change in pH and accumulation of metals in the soil which may affect both the composition and physiological processes of microorganisms leading to a reduction in microbial biomass and enzyme activities (Utobo and Tewari, 2014).

Soil enzymes are a group of enzymes that inhabits the soil and are continuously playing an important role in maintaining the soil biological, physical and chemical properties, fertility, and soil health (Das and Varma, 2011). All soils contain a group of enzymes that determine the soil metabolic processes (Baldrian, 2009) which in turn, depend on its physical, chemical, microbiological, and biochemical properties.

Some of the soil enzymes including amylase, b-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, and urease are released from plants and animals, organic compounds, and microorganisms in the soil. Soil enzymes are the mediators and catalysts of important soil functions and have a crucial role in carbon (β -glucosidase and β -galactosidase), nitrogen (urease), phosphorus (phosphatase), and sulphur (sulphatase) cycle (Karaca *et al.*, 2011).

The importance of soil as an ecosystem cannot be underestimated. It serves as reservoir to a lot of nutrients and minerals which are essential for plant growth. However, soils around Lafarge cement factory in Sagamu are under threat of serious degradation as a result of cement dust deposition. It is therefore important to access the level of degradation for the purpose of conservation of biodiversity and protection of ecosystem around the factory.

Also, to the best of our understanding, no work till date has reported the use of soil enzymes around Lafarge cement factory. Therefore, it is very important to assess the level of soil pollution around the study area using the enzyme activities, because the knowledge can be used as bioindicator of ecosystem perturbation, agricultural practices and xenobiotic pollution.

The aim of this study is to assess the effect of heavy metals in cement dust on soil enzymes activities around Lafarge cement factory in Sagamu, Sagamu Local Government Area, Ogun state, Nigeria.

Materials and Methods The Study Area

The study was carried out in the Lafarge cement factory which is situated in Sagamu, southwest Nigeria (6°50' 7°00' N; 3°45' 4°00' E). The factory is located 72 km southeast of Ibadan (largest city in West Africa), 32 km west of Ijebu Ode, 63 km southeast of Abeokuta and 67 km northwest of Lagos. The factory was established, commissioned and became fully operational in 1978 with the present production capacity of 900 000 tonnes/year (Lafarge Cement WAPCO PLC, 2011). The area is characterized by high annual temperature, high rainfall, high evapotranspiration and high relative humidity which make it to be classified as humid tropical region (Ogunkunle and Fatoba, 2014). The soil type of Shagamu is Ferralitic and Ferruginous (Ilalokhoin *et al.*, 2013). The climate is classified as humid tropical climatic zone and controlled by the Tropical Maritime and Tropical Continental air masses (Ilalokhoin *et al.*, 2013). The mean annual rainfall of Shagamu for 2015 was 1 100 mm (NIMET, 2015).



Figure 1. Google map of Lafarge factory

Collection of Samples

Sampling was carried out in the wet seasons of 2015 in the area surrounding the factory excluding the northern part of the factory because of the presence of swamps. At each sampling location, three subsamples were collected at the depth of 0 - 15 cm within 10 m radius of a point using a soil auger. The samples were bulked together to obtain a composite sample and recorded against the coordinate position. A total of 15 composite topsoil samples were collected in the west, the south and the east parts at 100 m, 200 m, 300 m, 500 m and 1000 m distance from the

factory respectively. The coordinates at every sampling points were taken by a hand-held GPS (Garmin 2H).

Control samples were collected at a distance of about 15 km from the cement factory where there was no record of cement dust pollution. The soil samples collected were placed in polyethylene bags, properly labeled and transported to the laboratory. Before chemical analysis in the laboratory, the soil samples were air–dried, sieved through a 2–mm sieve mesh to remove coarse material and debris, and pulverized into powder. The soil was transferred to the storage room and was stored at 30 $^{\circ}$ C until the time of analysis. Microbial and enzyme analysis were done within 24 to 48 h.

Soil Physico-Chemical Analysis

The soil pH was measured in a soil water suspension (1 : 2, soil : water) using glass electrode and temperature was also measured by soil thermometer (Frederick *et al.*, 2014) while the moisture content was determined gravimetrically after drying at 105 ^oC according to AOAC (2005). The following soil nutrients were selected and analyzed as follows: total organic carbon was determined based on modified Walkley-Black chromic acid wet oxidation method according to Sato *et al.* (2014). Total phosphorus (TP) was determined according to AOAC (2005). Total nitrogen was determined by Kjeldahl method according to AOAC (2005).

Heavy Metal Analysis in Soil

The soil samples (air-dried and digested) were analyzed for heavy metals content using Peking Elmer Model 403 atomic absorption spectrophotometer (AAS) according to AOAC (2005) after nitric acid digestion of the soil. The heavy metals studied were copper (Cu), cobalt (Co), lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni) and zinc (Zn). In addition, calcium (Ca) and aluminum (Al) content of the soil samples were also analyzed because they are one of the main constituent elements in the cement.

Enzymatic Analysis and Microbial Population in Soil

Enzyme substrates were purchased from Sigma-Aldrich Inc., England. Enzyme activities were measured according increase of resultant or decrease of substrate by colorimetric determination methods. Soil urease, acid phosphatase, alkaline phosphatase and dehydrogenase were determined according to Ofoegbu *et al.* (2013). β -glucosidase activity was determined according to Ramamoorthy (2015) and Zhang (2010). Soil invertase activity was determined according to the method described by Frankenberger Jr. and Johanson (1983) cited by Effron *et al.* (2004). Cellulase activity was measured with a method adapted from the one described by Alef and Nannipieri (1995). The method employed for determining soil amylase activity was essentially based on starch hydrolysis according to Khan *et al.* (2007).

Also, the number of microbial population was determined by the method of serial dilutions (Kulandaivel *et al.*, 2015). Soil samples were subjected to serial dilution and spread on

nutrient agar plates and potato agar plates, incubated for 48 h to grow the microbial colonies proper. Colony forming units were counted by colony counter.

Statistical Analysis

Data were subjected to two-way Analysis of Variance (ANOVA) and Dunnett's multiple comparison tests at $p \le 0.05$ probability level using Statistical Package for the Social Sciences (SPSS) version 20. Spearman correlation analysis was also employed to determine the relationships existing among the heavy metals content, the pH, the microbial population, enzyme activities and soil nutrient contents.

Results and Discussion

The results from the Analysis of Variance (ANOVA) showed that there were significance mean differences at (p < 0.05) between the soil samples taken at various distances and the control sample for all the enzyme activities, heavy metals; the soil nutrients except total organic nitrogen (f = 0.18), the soil physiochemical properties except pH values and lastly significant for all the microbial population constituents.Fang *et al.* (2017) analyzed the activity of a number of enzymes, in three types of land (farmland, woodland, and grassland) to evaluate soil pollution by heavy metals (Pb, Zn, and Cd). Their results showed that urease was the most sensitive enzyme to heavy metal pollution in the farmland. This is in agreement with our results where urease had the lowest enzyme activities (at 100 m). Caravaca *et al.* (2017) also reported that Urease activity was only decreased in the zone nearest to cement factory; and glucosidase activities declined significantly with decreasing distance to the cement factory in comparison with that in the unaffected zone which is in agreement with our work. Furthermore, Chen *et al.* (2017) reported that the soil moisture, contents of silt and total nitrogen were the most important variables responsible for soil extracellular enzyme activity variation.

Enzyme activities and the physicochemical properties of the soil

There was significant difference (p < 0.05) in soil enzymatic activities with respect to the distance from the factory except alkaline phosphatase. This implies that the enzymatic inhibition could originate from the factory (cement dust). The highest activity was recorded for invertase $(517.33 \pm 2.08 \ \mu g \ g^{-1})$ at the control site while the lowest activity was recorded for urease (0.14 $\pm 0.03 \ \mu g \ g^{-1}$) at 100 m distance respectively. The enzyme inhibition could be attributed to the binding of the heavy metals at the active site of the enzymes which could subsequently inhibit their catalysis. According to Poli et al. (2009) heavy metals is a strong enzyme inhibitors.

The pH range between 6.62 ± 0.10 (at the control) to 10.77 ± 0.81 (at 100 m) indicated that the factory soil was alkaline. The alkalinity was due to deposition and liming by calcium ion (Ca2+) from cement dust. Invertase had the highest enzymatic activity ($517.33 \pm 2.08 \ \mu g \ g-1$) at pH of 6.62 ± 0.10 (at the control) while the lowest value was recorded for urease ($0.14 \pm 0.03 \ \mu g \ g-1$) at pH of 10.77 ± 0.81 . This may be as a result of higher sensitivity of urease to alkaline pH as similarly reported by Frederick et al. (2014). Generally, the lower enzymes activities could be

due high pH around the factory as most of these enzymes could only function optimally at lower pH except alkaline phosphatase.

Enzyme	Distance - radius (m)						
(µg g-1)	100 200		300	500	1000	15000	
URS	0.14 ± 0.03	0.21±0.04	0.30±0.06	0.37±0.06	0.48±0.08	0.97±0.01	
ALP	68.67±4.16	52.67±5.03	57.67±4.04	54.33±2.08	32.67±1.57	26.67±0.58	
ACP	101.33±3.0	114.67±4.16	134.33±5.51	145.00±4.00	163.00±5.0	177.67±1.5	
	6				0	3	
BGLU	2.47±0.38	5.17±0.25	6.74±0.31	7.83±0.25	9.27±0.32	11.23±0.25	
DHA	1.60±0.3	2.27±0.60	3.0±0.79	3.63±0.75	4.5±1.10	7.77±0.15	
INVS	120.67±22.	156.33±20.4	212.33±36.6	268.33±69.4	349±66.26	517.33±2.0	
	75	0	7	0		8	
CLS	17.67±3.06	23.00±4.58	28.67±4.50	33.33±3.51	40.67±3.51	65±2.64	
AMY	4.71±0.95	6.21±1.73	7.49±1.48	8.76±1.19	10.78±0.58	12.17±0.12	

Table 1. *The relationship between the enzyme activity and the sampling distance from different locations*

Values are mean \pm standard error of triplicate values.

KEY: Urease (URS) (μ g N-urea $g^{-1}_{dry soil} 5h^{-1}$), Alkaline phosphatase (ALP) (μ gp-nitrofenol g^{-1}_{dry} soil h^{-1}), Acid phosphatase (ACP) (μ gp-nitrofenol $g^{-1}_{dry soil} h^{-1}$), B-glucosidase (BGLU) (μ gp-nitrofenol $g^{-1}_{dry soil} h^{-1}$), Dehydrogenase (DHA) (μ gtriphenylformazan $g^{-1}_{dry soil} 24h^{-1}$), Invertase (INVS) (μ g reducing sugar $g^{-1}_{dry soil} 24h^{-1}$), Cellulase (CLS) (μ g glucose $g^{-1}_{dry soil} 24h^{-1}$), Amylase (AMY)(μ g starch degraded $g^{-1}_{dry soil} 2.5h^{-1}$).

The study showed that the soil pH around factory was positively correlated for alkaline phosphatase while it was negatively correlated with all other enzymes, because the enzyme requires alkaline pH for optimal activity. This is in agreement with the work of Karaca *et al.* (2011) and Caravaca *et al.* (2017).

Also, there were positive correlations between the soil moisture content and the enzymes activities. This showed that moisture is required for optimum enzyme activity (through high microbial activities) in the soil. Also, high moisture has been predicted to support high microbial activities in the soil (Ahmed *et al.*, 2018).

There was significance difference (p < 0.05) between the mean values of the pH, soil moisture (except the temperature) and the enzyme activities at different sampling sites compared with the control samples.

The heavy metals, Calcium, Aluminum and the enzyme activities

The heavy metal concentrations decrease with increasing distance from the factory. The lowest concentration was observed at the control soil sample (15000 m) except zinc. The concentration of zinc at the control site (15.32 mg kg⁻¹) was higher than the concentrations of Zn around the factory. There were statistically significant variations in the concentrations of Cu, Pb, Mn, Cd, Ni, Co, Zn, Al and Ca at some distances, when compared to the control samples (p < 0.05). This implied that heavy metals are from the cement dust pollution. Moreover, Ca has the highest concentration at all sampling points which are also all statistically significant to the control and could be the main contributor to soil pollution in the environment. This is because Ca is an important element in the cement kiln process.

Dunnett's Multiple Comparison tests also revealed further that for Cu and Mn, there were no significant differences in the mean soil samples at 500 m, 1000 m compared to the control. Cadmium showed significant differences at different locations except at 1000 m only. We reported previously that Zn showed no significant difference at 200 m, 300 m, 500 m and 1000 m compared to the control and Al at 1000 m to the control samples (Oludoye and Ogunyebi, 2017).

There were also negative correlations between the enzyme activities and heavy metal concentrations around the factory except the alkaline phosphatase that was positively correlated. Heavy metals have been reported to inhibit enzymes singly or in combination by synergism (Frederick *et al.*, 2014). Therefore, this implied that some of these metals might inhibit the activities of all the enzymes studied except alkaline phosphatase activities which are also in accordance with the work of Das and Varma (2011).

In order to determine the magnitude of the pollution, the heavy metals content was compared with Nigeria Federal Environmental Protection Agency (FEPA) maximum permissible concentration (MPC) for soil. It was observed that the concentrations of all the heavy metals except cadmium are within the MPC of FEPA. The soil Cd concentration is higher than the MPC at all distances except at the control. This shows that Cd concentration is very high around the cement factory. Cadmium is one of the trace elements found in raw mill and coal which are used in the formation of clinker (the most important constituent of cement) (Cipurkovic *et al.*, 2014). This high Cd concentration is unhealthy for the environment and human health because there are some farming activities currently going on in the vicinity of the cement factory. Cadmium could subsequently enter the food chains through the crops from the farms.

Heavy metals	WHO limit (mg/kg)	FEPA limit (mg/kg)
Zn	50	300-400
Cu	36	70-80
Pb	85	1.6
Cd	0.8	3.0
Mn	Not fixed	Not fixed

 Table 2. FEPA and WHO maximum permissible concentrations for soil

Ni	35	18
Со	Not fixed	20

However, it is not only cement production that could release Cd into the environment but geologic processes (ferratillisation) could also contribute to the high concentration around the factory. It was also reported that high soil pH caused by limestone deposition (a component of cement) could immobilize Cd in the soil (Yun and Yu, 2015). Furthermore, Kim (2014) reported that Cd is maximally adsorbed at high pH and high temperature which significantly affected the adsorption capacity in alkaline soil.

Heavy	Average distance - radius (m) from east, west and south part of the factory							
metal	100	200	300	500	1000	15000		
(mg kg ⁻¹)								
Cu	6.52 ± 0.80	5.69 ± 0.50	4.62 ± 0.79	3.71 ±0.58	3.04±0.53	2.16 ± 0.02		
Pb	1.34 ± 0.06	1.24 ± 0.07	1.14 ± 0.09	1.06 ±0.08	0.96±0.09	$\textbf{0.27} \pm \textbf{0.01}$		
Cd	9.27 ± 0.42	8.63 ± 0.55	7.58 ± 0.44	6.60 ±0.59	5.7±0.42	2.56 ± 0.09		
Mn	9.87 ± 0.19	9.25 ± 0.24	8.42 ± 0.49	8.00 ±0.36	7.38±0.27	6.77 ± 0.04		
Ni	3.52 ± 0.09	3.30 ± 0.25	3.16 ±0.21	2.97 ±0.17	2.86±0.16	$\textbf{2.09} \pm \textbf{0.07}$		
Со	16.97 ± 0.72	16.22 ± 0.61	15.05 ± 0.42	14.14 ±0.54	13.74±0.81	10.48 ± 0.10		
Zn	17.25 ± 0.21	16.68 ± 0.26	16.15 ± 0.37	15.47 ±0.70	14.71±0.54	15.32 ± 0.25		

Table 3. The mean value of heavy metal concentrations in the soil around the factory

Values are mean ± standard error of triplicate values

Table 4. The mean value of Ca and Al concentrations in the soil around the factory

Metal	Average distance - radius (m) from east, west and south part of the factory							
$(\mathrm{mg}\mathrm{kg}^{-1})$	100 200 300 500 1000 15000							
Ca	201.05±12.0	160.92±	144.02±11.5	133.80±9.8	97.06±1.53	84.13 ± 0.26		
		11.08	8	6				
Al	4.15 ± 0.06	3.65 ± 0.34	3.17±0.32	2.53±0.45	2.13±0.17	1.81 ± 0.03		

The soil nutrients analysis

There was decrease in the concentration of available phosphorus at various distances which showed a significant mean difference to the control at 15000 m. This can attributed to the precipitation of phosphorus into calcium phosphate at high pH (Cao *et al.*, 2007) while there was gradual decrease in the total organic carbon and nitrogen content of the soil from the factory to the control site, respectively.

Dunnett's Multiple Comparison tests showed that for there were no significant differences between the means for all the soil samples taken at varying distances and the control sample, for organic nitrogen. In addition, there was no significant mean difference at 300 m, 500 m and 1000 m for total organic carbon.

Distance (m)	Phosphorous (part per million)	Nitrogen (mg kg ⁻¹)	Carbon (%)
100	21.43 ± 2.59	178.00 ± 4.00	14.27 ± 1.02
200	24.57 ± 2.61	164.00 ± 4.00	12.53 ± 2.06
300	27.60 ± 3.53	144.00 ± 3.00	9.44 ± 0.70
500	29.47 ± 3.19	133.00 ± 3.00	7.59 ± 0.78
1000	31.03 ± 2.06	110.00 ± 2.00	6.72 ± 0.44
Control	58.30 ± 0.18	98.00 ± 4.00	6.30 ± 0.20

 Table 5. The mean values of selected soil nutrients with respect to distance

Values are mean \pm standard error of triplicate values.

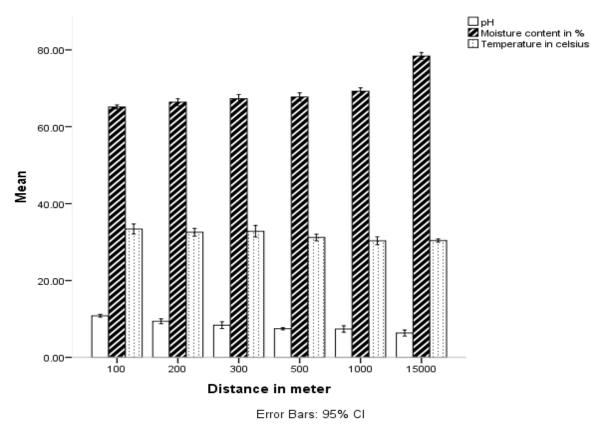


Figure 2. The relationship between the pH, moisture, and temperature with respect to distance

Soil microbial population

The microbial communities of the soil studied were significantly affected as a result of cement dust deposition. The result showed that the microbial communities of the control were statistically significantly higher (p < 0.05) than the polluted soil around the factory, which is in agreement with the work of Kulandaivel *et al.* (2015). Also, Caracava *et al.* (2017) reported that the prolonged exposure of soil to cement dust could result in shifts in the soil microbial function. This could also be attributed to the decrease in microbial biomass due to reduced enzymes activity.Spearman correlation analysis revealed that there was positive correlation between the microbial communities and the enzyme activities. This implied that enzymes are primarily derived from microorganisms but can also originate from plants and animals. Hence, factors affecting microbial activities will invariably affect soil enzymes. Moreover, the microbial count is also negatively correlated with the soil pH. This could be due to the fact that some of the microbes could not survive in alkaline conditions as this could affect the osmotic balance of their cells and consequently, cause reduction in their cell biomass.

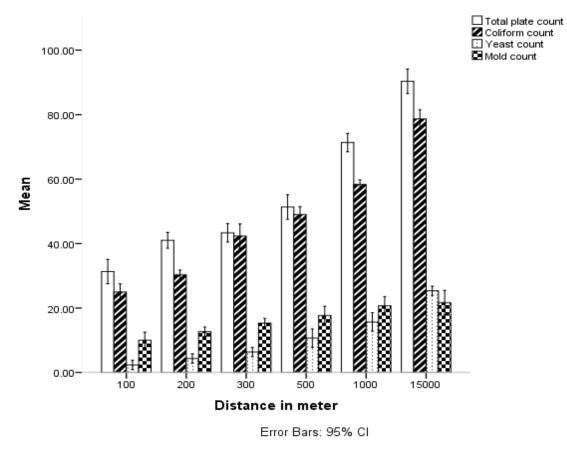


Figure 3. The relationship of microbial population from different soil samples with respect to distance from the factory.

	Cu	Pb	Cd	Mn	Ni	Co	Zn	Ca
Urease	-0.888	-0.900*	-0.918*	-0.892*	-0.994**	-0.984**	-0.669**	-0.863*
ALP	0.893*	0.856	0.910*	0.903**	0.888^*	0.877^{**}	0.824^{*}	0.894**
АСР	-0.997*	-0.875*	-0.997	-0.999**	-0.928**	-0.951**	-0.921**	-0.992**
BGLU	-0.978*	-0.880**	-0.980**	-0.973	-0.985**	-0.943**	-0.899**	-0.989**
DHA	-0.950**	-0.970*	-0.972**	-0.952**	-0.988	-0.990**	-0.791**	-0.935**
Invertase	-0.950**	-0.970**	-0.972**	-0.952**	-0.988**	-0.990	-0.791**	-0.935**
Cellulase	-0.922**	-0.990*	-0.945**	-0.923**	-0.997**	-0.992**	-0.728	-0.900**
Amylase	-0.992*	-0.884**	-0.996**	-0.993**	-0.935**	-0.951**	-0.917**	-0.990

Table 6. The Spearman's correlation coefficient among the enzyme activities and the heavymetal concentrations

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Conclusion

All the heavy metals content of soil around the cement factory are below the MPC of FEPA except cadmium. The high Cd content could have emanated from the factory cement dust because cadmium is one of the trace elements in the fuel that is used for kiln production. Also, soil geologic processes and high pH around the factory could also result in Cd immobilization in the soil. Moreover, there is low nutrients content (phosphorus), low microbial population and high pH within the vicinity of the cement factory. All these factors had a significant adverse effect on and are inversely correlated with the soil enzymes. This indicates the ability of soil enzymes early soil pollution signal (or indicator) despite the permissible level of heavy metals (except cadmium).

The heavy metals content (especially cadmium) in the top soil (0-15 cm) is within the reach of plant roots and could have negative effects on the natives who are already using the sites around the factory as agricultural farms for planting food crops such as sugar cane, cassava, banana and maize respectively.

Therefore, there should be comprehensive ecological risk assessment of the soil ecosystem around the factory. In addition, human health risk assessment should be carried out to determine carcinogenic and non-carcinogenic risk through dietary exposure to some of these heavy metals (especially cadmium). This would help prevent the pollutants (heavy metals) from entering the food chain and help reduce the exposure of the inhabitants and the farmers. Also, farming activities should be totally discouraged around the factory.

Reference

Addo, M.A., Darko, E.O., Gordon, C., Nyarko, B.J.B., Gbadago, J.K., Nyarko, E., Affum, H.A., Botwe, B.O., 2012. Evaluation of heavy metals contamination of soil and vegetation in the vicinity of a cement factory in the Volta region, Ghana. *International Journal of Science and Technology* 2(1): 40–50.

Adesanwo, O. O., Ige, D. V., Thibault, L., Flaten D., Akinremi, W. 2013. Comparison of Colorimetric and ICP Methods of Phosphorus Determination in Soil Extracts. *Communications in Soil Science and Plant Analysis* 44(21): 3061-3075. Doi: https://doi.org/10.1080/00103624.2013.832771.

Ahmed, M. A., Sanaullah, M., Blagodatskaya, E., Mason-Jones, K., Jawad, H., Kuzyakov, Y., Dippold, M. A. 2018. Soil microorganisms exhibit enzymatic and priming response to root mucilage under drought. *Soil Biology & Biochemistry* 116: 410-418. Doi: https://doi.org/ 10.1016/j.soilbio.2017.10.041.

Alef, K., Nannipieri, P. 1995. Cellulase activity. In: Alef P (ed) Methods in applied soil microbiology and biochemistry. Academic Press, London, pp 382–384.

AOAC. 2005. Official methods of analysis. Association of official analytical chemists: Agricultural Chemicals; Contaminants; Drugs. 18th Ed. Association of Official Analytical Chemists Inc. Arlington, Virginia, USA.

Baldrian, P., 2009. Microbial enzyme-catalyzed processes in soils and their analysis. *Plant and Soil Environment* 55 (9): 370–378.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* 72(1-2): 248–254. Doi: <u>https://doi.org/10.1016/0003-2697(76)90527-3</u>.

Cao,X., Harris, W. G., Josan, M. S. and Nair,V. D., 2007. Inhibition of calcium phosphate precipitation under environmentally-relevant conditions. *Science of the Total Environment*. 383(1-3): 205-215.

Chen, H., Luo, P., Wen, L., Yang, L. Q., Wang, K. L., Li, D. J. 2017. Determinants of soil extracellular enzyme activity in a karst region, southwest China. *European Journal of Soil Biology* 80: 69-76. Doi: <u>https://doi.org/10.1016/j.ejsobi.2017.05.001</u>.

Das, S.K., Varma, A., 2011. Role of enzymes in maintaining soil health. In: In: Soil Enzymology, Soil Biology 22. Shukla, G., Varma, A. (Eds.). Springer-Verlag, Berlin Heidelberg, pp.25-42.

Cipurkovic, A., Trumic, I., Hodžic, Z. Selimbašic, V. and Djozic, A. (2014). Distribution of heavy metals in Portland cement production process. 5(6): 252-259.

Effron, D., de la Horra, A.M., Defrieri, R.L., Fontanive, V., Palma, P.M., 2004. Effect of cadmium, copper, and lead on different enzyme activities in a native forest soil. *Communication of Soil Science and Plant Analysis* 35(9-10): 1309–1321. Doi: <u>https://doi.org/10.1081/CSS-120037548</u>.

Egejuru, L.O., Akubugwo, E.I., Ude, V.C., Ugbogu, O.C., Ugbogu, A.E., 2014. Evaluation of physicochemical properties, microbial loads and enzymes activity studies of agrochemicals on the Imo-River basin farm in Imo State Nigeria. *International Journal of Current Microbiology and Applied Sciences* 3(2): 776-786.

Fang, L. C., Liu, Y. Q., Tian, H. X., Chen, H. S., Wang, Y. Q., Huang, M. 2017. Proper land use for heavy metal-polluted soil based on enzyme activity analysis around a Pb-Zn mine in Feng County, China. *Environmental Science and Pollution Research* 24(36): 28152-28164.Doi: <u>https://doi.org/10.1007/s11356-017-0308-4</u>.

Frankenberger Jr., W. T., Johanson, J. B., 1983. Method of measuring invertase activity in soils. *Plant and Soil* 74: 301-311.

Frederick, O.O., Iroha, A.E., Oswald, E.C., 2014. Evaluation of the concentration of selected heavy metals and the effects on soil enzymatic activities in an Abandoned Cement Factory in NigercemNkalagu and its environs. *International Journal of Biochemistry Research and Review* 4(1): 16-27. Doi: <u>https://doi.org/10.9734/IJBCRR/2014/5019</u>.

Ilalokhoin, P.O., Otaru, A.J., Odigure, J.O., Abdulkareem, A.S., Okafor, J.O., 2013. Environmental impact assessment of a proposed cement plant in southwestern Nigeria. *IOSRJournal of Environmental Science, Toxicology and Food Technology* 3(5): 83–99.

Karaca, A., Cema, C.C., Turgay, O.C., Kizilkaya, R., 2011. Soil enzymes as indicator of soil quality. In: Soil Enzymology, Soil Biology 22. Shukla, G., Varma, A. (Eds.). Springer-Verlag, Berlin Heidelberg, pp.119-148.

Khan, S., Cao, Q., Hesham, A.E., Xia, Y., He, J., 2007. Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. *Journal of Environmental Science* 19(7): 834–840. Doi: https://doi.org/10.1016/S1001-0742(07)60139-9

Kim, M. (2014). A study on the adsorption characteristics of cadmium and zinc onto acidic and alkaline soils. *Environmental Earth Sciences*. 72, (10): 3981–3990.

Kulandaivel, S., Nagarajan, S., Priyanga, A., Saravanapandian, R., Thangarani, A., 2015. Effect of cement dust pollution on microbial properties and alkaline phosphatase enzyme activity in soil. *International Journal of Current Microbiology and Applied Science4*(2): 641-649.

Lafarge Cement WAPCO PLC. 2011. Lafarge WAPCO: Supporting Nigeria's development, Annual Report–Lafarge Cement WAPCO PLC. pp. 2–4.

Nayak, D.C., Sen, T.K., Chamuah, G.S., Sehgal, J.L., 1996. Nature of soil acidity in some soils of Mainpur. *Journal of Indian Society of Soil Science* 44(2): 209-212.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biological Chemistry* 153: 375–380.

NIMET. 2015. Seasonal rainfall prediction and socio–economic implications for Nigeria. Federal Government of Nigeria, Nigerian Meteorological Agency (NIMET), Abuja, Nigeria, Available at [access date : 30.11.2017]: <u>http://nimet.gov.ng/sites/default/files/publications/2012-seasonal-rainfall-prediction.pdf</u>.

Ofoegbu, C.J., Akubugwo, E.I., Dike, C.C., Maduka, H.C.C., Ugwu, C.E., Obasi, N.A., 2013. Effects of heavy metals on soil enzymatic activities in the Ishiagu mining area of Ebonyi State-Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Science* 5(6): 66-71.

Ogunkunle, C.O., Fatoba, P.O., 2014. Contamination and spatial distribution of heavy metals in topsoil surrounding a mega cement factory. *Atmospheric Pollution Research* 5(2): 270–282. Doi: <u>https://doi.org/10.5094/APR.2014.033</u>.

Oludoye, O. O. and Ogunyebi, L. A., 2017. Nutrients Assessment of Tropical Soils Around A MegaCement Factory In Southwest Nigeria. Journal of Ecological Engineering. 18(2): 18-25.

Poli, A., Salerno, A., Laezza, G., di Donato, P. and Dumontet, S., 2009. Heavy metal resistance of some thermophiles: potential use of alpha-amylase

from *Anoxybacillusamylolyticus* as a microbial enzymatic bioassay. *Research in Microbiology*. 160: 99-106.

Ramamoorthy, D., 2015. Assessment of heavy metal pollution and its impacts on soil physical, chemical properties and β -glucosidase activities in agricultural lands, Puducherry region. Project Report. Department of Science, Technology and Environment Government of Puducherry. 45pp

Sato, J.H., de Figueiredo, C.C., Marchão, R.L., Madari, B.E., Benedito, L.E.C., Busato, J.G., de Souza, D.M. 2014. Methods of soil organic carbon determination in Brazilian savannah soils. *Scientia Agricola* 71(4): 302-308. Doi: <u>http://dx.doi.org/10.1590/0103-9016-2013-0306</u>.

Utobo, E.B., Tewari, L., 2014. Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecology and Environmental Research* 13(1): 147-169. Doi: <u>https://doi.org/10.15666/aeer/1301_147169</u>.

Wainwright, M., Duddridge, J. E., Killham, K., 1981. Assay of a-amylase in soil and river sediments: its use to determine the effects of heavy metals on starch degradation. *Enzyme and Microbial Technology* 4: 32-34.

Wang, Y.P., Shi, J.Y., Wang, H., Lin, Q., Chen, X.C., Chen, Y.X., 2007. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. *Ecotoxicology and Environmental Safety* 67(1): 75–81. Doi: https://doi.org/10.1016/j.ecoenv.2006.03.007.

Wright, A.L., Reddy, K.R., 2001. Heterotrophic microbial activity in northern Everglades wetland soils. *Soil Science Society of America Journal* 65(6): 1856–1864. Doi: https://doi.org/10.2136/sssaj2001.1856.

Yun, S. and Yu, C., 2015. Immobilization of Cd, Zn, and Pb from Soil Treated by Limestone with Variation of pH Using a Column Test. *Journal of Chemistry*. <u>http://dx.doi.org/10.1155/2015/641415</u>.

Zhang, X., Li, F., Liu, T., Xu, C., Duan, D., Peng, C., Zhu, S., Shi, J., 2013. The variations in the soil enzyme activity, protein expression, microbial biomass, and community structure of soil contaminated by heavy metals. *ISRN Soil Science* Article ID 803150. Doi: http://dx.doi.org/10.1155/2013/803150.