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Microbial and Heavy Metal Determination of Contaminated Soil Using *Melissa officinalis* L

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Abstract

In Nigeria and other part of the world, heavy metal pollutions are becoming increasingly common. Heavy metals are natural elements of the environment. This research was designed to remediate a heavy metal polluted environment of Angwa Kawo (AK) in Rafi Local Government Area, Niger State, Nigeria with *M. officinalis* L. Microbial loads of the soil was monitored and the bacterial counts ranges from 1.0±1.0 to 4.0±0.57 x10^5 cfu/g while the fungal counts has 0.33±0.33 to 2.0±0.57 x10^2 cfu/g. Physicochemical properties (Organic carbon, pH, Total nitrogen, Phosphorous, Organic matter, moisture, trace elements, Electrical conductivity, Exchangeable acidity and cationic exchange) of the soil were done using standard methods. The plant (*M. officinalis* L) mopped up heavy metals (Cd, As, Pb) and their concentration varied from 0.007 to 0.33 mg/kg, As (0.09 to 4.39 mg/ kg) and Pb (0.07 to 10.35 mg/kg) respectively. The concentration of Cd in the residual soil varied from 0.026 to 0.58 mg/kg, As from 0.32 to 5.48 mg/kg, Pb from 5.88 to 12.37 mg/kg. Soil remediation was further confirmed using scanning electron microscopy (SEM) analyses, which revealed structural and morphological changes of the soil. *Melissa offinalis* L proved to have the potential to remediate heavy metal polluted soil as revealed in this study.

Keywords

Pollution, Heavy Metal, Microbial, Soil, Environment

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1. Introduction

Pollution is one of the most important problems around the world today in which thousands to millions of world inhabitants suffer health problems related to industry, mining and atmospheric pollutants [1]. Amongst many anthropogenic activities, mining has been identified with the potential of impacting negatively on the quality of the environment [2; 3]. Mining causes the destruction of natural ecosystems by altering soil, vegetative covers and covering of organisms beneath excavation sites. Aside the physical habitat destruction with accompanying loss of biodiversity resources, the accumulation of pollutants in different media have been recorded around mining sites [4]. Therefore, mining sites portend great toxicological challenges for the surrounding ecosystems and on human health [5].

Heavy metal toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems associated with mining. The unwanted release of environmental contaminants predisposed by mining activities had reached an alarming proportion that deserves attention [4]. Anthropogenic activities such as artisanal mining in

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developing countries like Nigeria, have exposed the environment to serious hazards by the generation and uncontrolled discharge of enormous amounts of toxic aqueous wastes containing toxic heavy metals, as well as various organic pollutants, which impact adversely on human health and the ecosystem [6]. Some communities in Niger State such as Shikira community in Rafi Local Government Area (RLGA), where there are large gold deposits and through a series of mining activities have their environment heavily polluted with lead (Pb) and other metals [7]. This lead poison caused the death of 28 children in the year 2015 as reported by the Federal Ministry of Health FMH, Nigeria (FMH, 2015). Therefore, this study is aimed to remediate the heavy metal polluted soil using *Melissa officinalis* L.

2. Materials and Methods

2.1. Study Site

The study site was Angwan Kawo is situated on the eastern flanks of Kagara town, the headquarters of Rafi Local Government Area (RLGA) of Niger State, Nigeria (Figure 1). Niger State is located between longitude 3° 30′ E and 7° 30′ E and latitude 8° 10′ N and 10° 30′ N. The site was selected based on the incidence of lead poisoning that was reported in May, 2015 due to artisanal mining activities [8; 7]

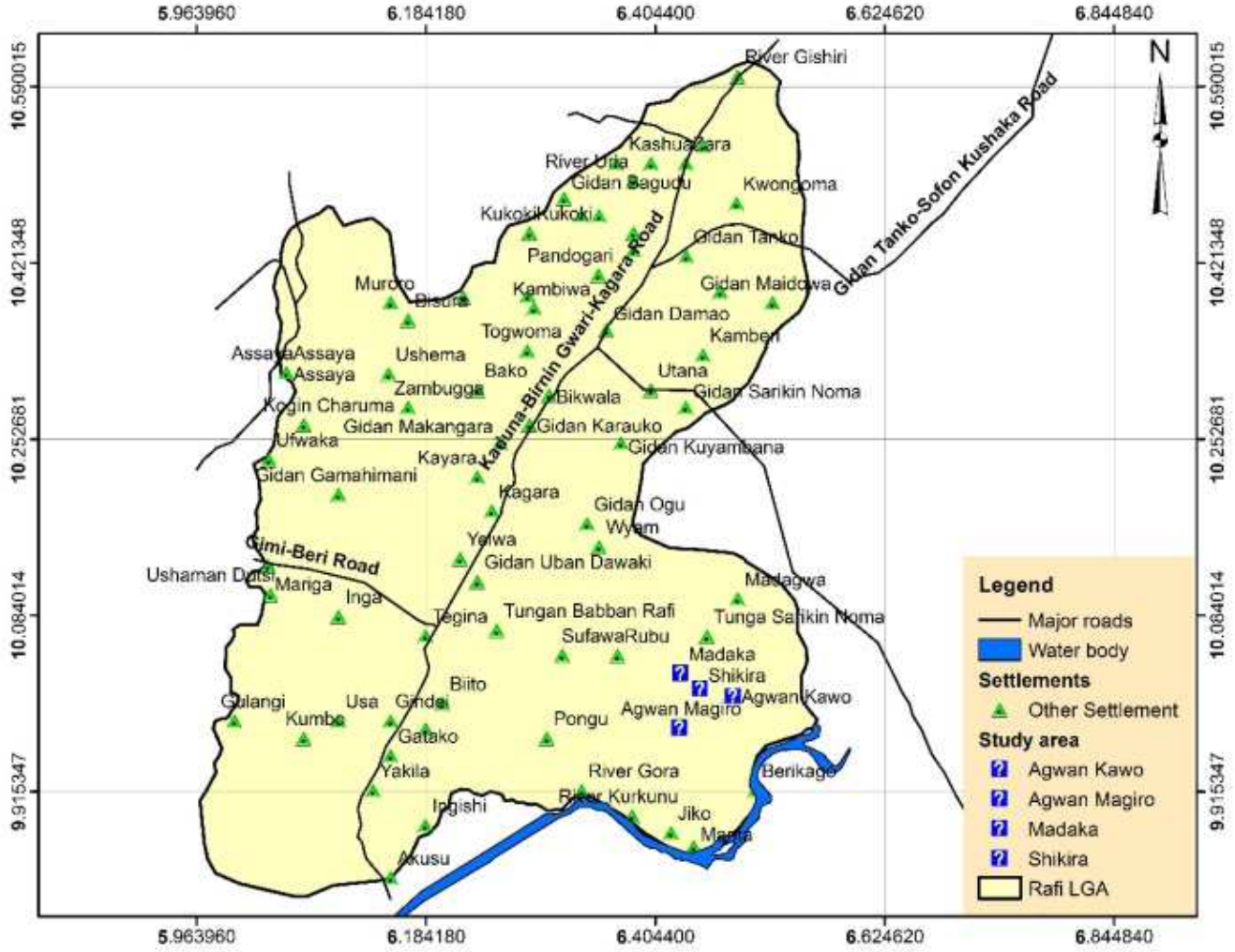

Figure 1. A. Map of Nigeria showing Niger State; B. The study area (Madaka district, Shikira community) Rafi LGA, Niger State, Nigeria.

2.2. Collection and Processing of Samples

Plants and Heavy metal polluted soil for this experiment were collected from the mining sites of Angwan Kawo, Rafi Local Government Area, Niger State, Nigeria, from a depth of 0-15 cm with clean stainless steel shovel and transported in polythene bags to Federal University of Technology, Minna, for analysis.

2.3. Physicochemical Properties

The physicochemical properties of the soil were determined using standard methods as described by different authors.

Soil pH was determined using the method of Eckerts and Sims [9], organic carbon and the exchangeable cations were determined by the method of Walkley and Black [10] and Agbenin [11]. The method of Black [12] and Agbenin [11] were used to determine the total nitrogen while the soil particle size was done using the methods of Bouyoucos [13], available phosphorus was determined by the method of Nordberg et al. [14]

2.4. Microbial Analysis of Contaminated Soil Samples

One gram (1 g) of soil sample was aseptically introduced into 9 mL of distilled water in a test tube, shaken and serially diluted. One milliliters (1 mL) of the serially diluted sample was introduced into Petri dishes and Nutrient agar (NA) and Sabouraud dextrose agar (SDA) was added using the pour plate method [15], mixed thoroughly for the enumeration of bacteria and fungi respectively. The NA was allowed to solidify and was incubated at 37°C for 24 hours while the SDA was incubated at room temperature (28±2°C) for 3-5 days after which the colonies were counted and expressed as colony forming units per gram (cfu/g) of soil.

2.5. Experimental Design and Setup

The study was a pot experiment. Polluted soils were collected and transported from Angwa Kawo of RLGA and the experimental layout was conducted at the biological garden of Federal University of Technology, Minna. The setup was a complete randomized design and the treatments was replicated three times. The experimental pots were filled with 5 kg polluted soils. Seedlings of *M. officinalis* L planted to each pot containing the polluted soil for the phytoremediation. The set-up was done and monitored. The physicochemical properties and the microbial counts of the soil were done.

2.6. Determination of Heavy Metal in the Harvested Plants

After harvesting, plant shoots and roots were separated from soil, carefully washed with tap water, and followed by distilled water until all dirt's were removed. All samples were air-dried at the Microbiology Laboratory, Federal University of Technology, Minna, for seven days. The samples were oven-dried at 600°C until constant weights were obtained. The dried plant parts were ground to powder using a horizontal grinder [16]. The dried samples were digested with a mixture (3:1) of concentrated nitric acid and hydrofluoric acid in microwave assisted Kjeldahl digestion. Six milliliters (6 mL) of nitric acid and 2 mL of hydrofluoric acid were added into each microwave extraction vessels with 0.8 g of plant sample. The vessels were capped and heated in a microwave unit at 800 W to a temperature of 190°C for 20 min with a pressure of 25 bars. The digested samples were diluted to 50 mL and subjected to analysis of the metals by atomic absorption spectrophotometer using flame atomization. Results were expressed on dry weight basis of each component [16].

3. Results and Discussion

3.1. Physicochemical Characteristics of the Polluted Soil and Remediated Soil

The physicochemical characteristics of polluted soils from Angwa Kawo were presented in Table 1 These were compared with physicochemical properties of the same soils after seven months of remediation. The pH the polluted soil ranged from 4.98 to 7.08.

Table 1. Physicochemical properties of polluted soil of Shikira community.

Parameters	AKBR	AKAR
pH	4.98	7.08
Nitrogen (%)	0.01	0.70
Phosphorus	26.11	35.21
Organic Matter (%)	0.73	4.84
Organic Carbon (%)	0.27	6.40
Moisture (%)	6.02	11.37
Sand (%)	44.24	43.20
Silt (%)	30.28	30.81
Clay (%)	25.48	22.10
Na^+ (Cmol/kg)	0.34	0.56
K^+ (Cmol/kg)	0.28	0.31
Mg^{2+} (Cmol/kg)	2.91	2.87
Ca^{2+} (Cmol/kg)	6.64	6.78
Electrical Conductivity (µ/cm)	55	282
Exchangeable Acidity (Cmol/kg)	0.27	2.03
Cation Exchange Capacity (Cmol/kg)	9.40	9.76

KEY: AKBR: Angwa Kawo soil before remediation, AKAR: Angwa Kawo soil after remediation, Mg/kg: milligram per kilogram cmol/kg: centimoles of charge per kilogram

Though these values were generally within the range for soil pH established by FEPA [17]. The pH of the soil after the remediation (7.08) for AKAR and (6.33) for AMAR were higher than the pH of both soils before remediation probably, due to the impacts of the remediation processes with the organic manure, the plant and plant growth promoting bacteria (PGPB) playing a greater role to influence the soil pH. Soil pH plays major role in the sorption of metals; it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation and solubility of organic matter, as well as surface charge of Fe, Mn, and Al-oxides, organic matter, and clay edges [18]. These indicate that metal uptake is influenced by soil factors including pH, organic matter, and cation

exchange capacity as well as plant species, cultivation, and age. The mobility and availability of heavy metals in soil are generally low, especially when soil is high in pH, clay, and organic matter [19].

3.2. Microbial Counts of the Polluted Soil During the Study

Examinations of microbial loads of the soil were done (Figure 2). It was observed that fungal counts had the lowest value ranging from 0.00±0.00 to 2.33±0.33 (x10^2 cfu/g).

Microorganisms counts were scanty in this heavy metal polluted soil and may be due to heavy metal toxicity, such is, breaking fatal enzymatic functions, react as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein of the organisms [20]. The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Cr and Cd are capable of inducing oxidative damage and denaturation of microorganisms as well as weakening the bioremediation capacity of microbes. Cr (III) may change the structure and activity of enzymes by reacting with their carboxyl and thiol groups [21].

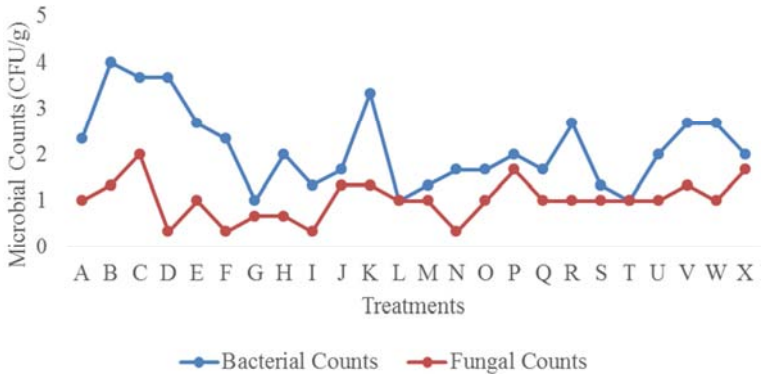

Figure 2. Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the First Month (April) of the Study.

Key: A=Soil (5kg) + M. *officinalis* L, B= Soil (5kg) + *M. officinalis* L + PGPB, C= Soil (5kg) +*M. officinalis* L + CDV+ PGPB, D= Soil (5kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5kg) + *M. officinalis* L + CDV, F= Soil (5kg) + *M. officinalis* L + GMV, G= Soil (5kg) + *S. acuta,* H= Soil (5kg) + *S. acuta* + PGPB, I= Soil (5kg) + *S. acuta* + CDV+ PGPB, J= Soil (5kg) + *S. acuta* + GMV+ PGPB, K= Soil (5kg) + *S. acuta* + CDV, L= Soil (5kg) + *S. acuta* + GMV, M= Soil (5kg) + M. *officinalis* L, N= Soil (5kg) + *M. officinalis* L + PGPB, O= Soil (5kg) + *M. officinalis* L + CDV+ PGPB, P= Soil (5kg) + *M. officinalis* L + GMV+ PGPB, Q= Soil (5kg) + *M. officinalis* L + CDV, R= Soil (5kg) + *M. officinalis* L + GMV, S= Soil (5kg) + *S. acuta,* T= Soil (5kg) + *S. acuta* + PGPB, U= Soil (5kg) + *S. acuta* + CDV+ PGPB, V= Soil (5kg) + *S. acuta* + GMV+ PGPB, W= Soil (5kg) + *S. acuta* + CDV, X= Soil (5kg) + *S. acuta* + GMV

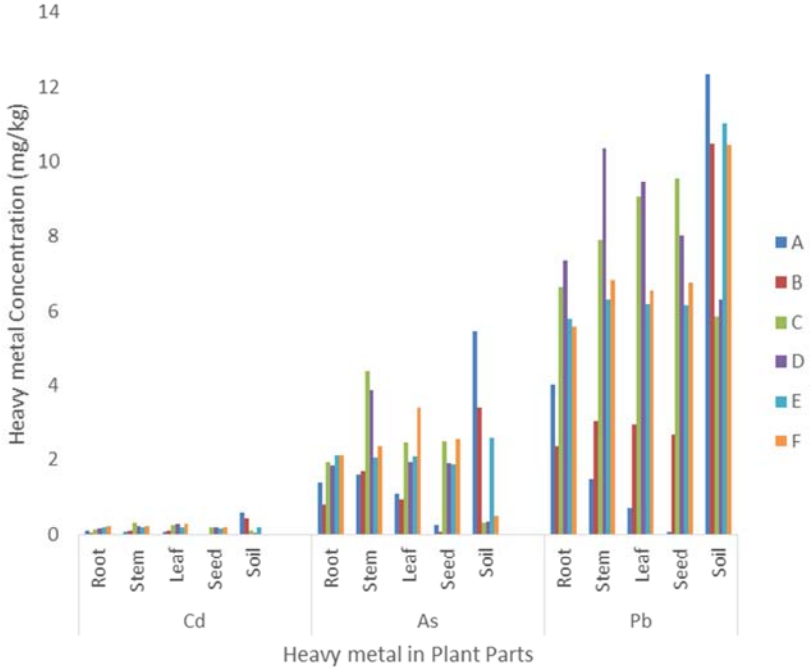

Figure 3. Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *M. officinalis* L Grown on Angwan Kawo Soil.

A=Soil (5kg) + M. *officinalis* L, B= Soil (5kg) + *M. officinalis* L + PGPB, C= Soil (5kg) + *M. officinalis* L + CDV+ PGPB, D= Soil (5kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5kg) + *M. officinalis* L + CDV, F= Soil (5kg) + *M. officinalis* L + GMV

3.3. Heavy Metals in *M. officinalis* L Planted on Angwan Kawo (AK) Soil

The expression of the mopped up heavy metals by *M. officinalis* L used for the remediation of AK polluted soil is represented in Figure 3.

The plant (*M. officinalis* L) parts mopped up heavy metals (Cd, As, Pb) from the soil in its root, stem, leaf and seed. For the whole plant parts, concentration of Cd, As and Pb varied from 0.007 - 0.33 mg/kg, 0.09 - 4.39 mg/ kg and 0.07 - 10.35 mg/kg respectively. The concentration of Cd in the residual soil ranged from 0.026 - 0.58 mg/kg, 0.32 - 5.48 mg/kg for As and 5.88 - 12.37 mg/kg for Pb (Figure 3). However, the concentration of Cd was highest (0.33 mg/kg) in the stem part of the plant and the lowest (0.007) was recorded at the seed part, 4.39 mg/kg was recorded as the highest As concentration at the stem part of the plant while the seed part recorded the lowest of 0.09 mg/kg. Pb concentration at the plant part was highest (10.35 mg/kg) also at the stem part and had its lowest concentration of 0.07 mg/kg at the seed part. Based on different concentrations of heavy metals in the polluted soil before remediation, Pb had the highest metal concentration (12.37 mg/kg) while Cd (0.026 mg/kg) was the lowest (Figure 3). Generally, the recovered bioavailable metal contents of the soil in this location (AK) were low compared to the total metals and this could be linked to the pH of the soil and the mineralogy of the soil [22]. Reports has indicated that fluctuating pH of soil reduces metal mobility [23]. Halim *et al*. [24] stated that bioavailability of metals could be increased when these metals form soluble complexes with organic matter content of the soil.

3.4. SEM Micrographs of Polluted and Remediated Soil

The severity of remediation was further validated by the structural morphological changes observed using SEM (Figures 4 and 5). After seven month of remediation process, All the polluted soil before the remediation by *M. officinalis L.* (Figure 4) exhibited a smooth large compact structural surface which is an indication of metal pollution whereas the remediated soil (Figure 5) exhibited small rough structural surfaces validating the remediation of the soil by the two plants. The SEM micrographs of the soil from Angwan Kawo shows more clarity of remediation and showed fine breakage of the soil structure. Various pores/pits and irregularities formed as a result of remediation activity (Figure 5). These surface changes observed in the SEM micrographs indicated changes the soil structure of the remediated soil with the plant.

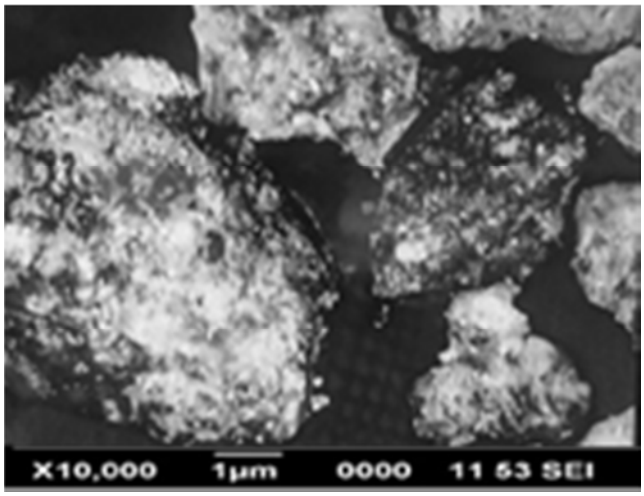

Figure 4. SEM Micrographs of the Polluted Soil of Angwan Kawo Before Remediation with *M. officinalis* L.

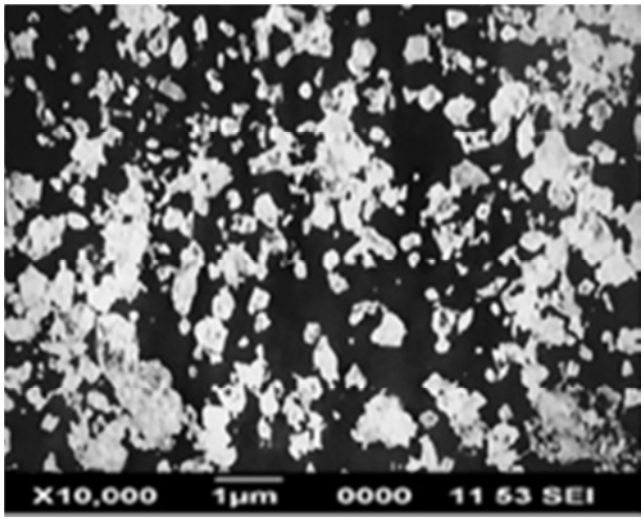

Figure 5. SEM Morphological Appearance of the Remediated Soil of Angwan Kawo with *M. officinalis* L After Remediation.

4. Conclusion

Phytoremediation is a promising green technology that can be used to remediate heavy metal contaminated soils. In developing countries like Nigeria, this technology can provide low-cost solution to remediate contaminated area, especially abandoned industrial sites (mines and landfills). This study has revealed that the native plant *M. officinalis* L. around the polluted mining site of Angwan Kawo settlement have great potential for phytoextraction and phytostabilization of all metals under study. The results of this study can be used for management and decontamination of Angwan Kawo soil polluted with heavy metals using plant species *M. officinalis* L.

Declaration

The data will be made available on request.

Funding Source

By authors.

Authors Contribution

Author SAA anchored the field study. Author UJJI designed the layout while author OPA and JDB managed the literature and edited the manuscript.

References

[1] Martinez T., Lartigue J., Avilaperez P., Navarrete M., Zarazua G., Lopez C., Cabrera L., Nadal M., Schuhmacher M. and Domingo J. L. (2004). Metal pollution of soils and vegetation in a petrochemical industry. *Science of Total Environment*, 321: 59-69.

[2] Donkor, A. K., Bonzongo, J. C. J., Nartey, V. K., and Adotey, D. K. (2005). Heavy metals in sediments of the gold mining impacted Pra River basin, Ghana, West Africa. *Soil and Sediment Contamination*, 14 (6): 479-503.

[3] Aransiola, S. A., Ijah, U. J. J., Abioye, O. P., and Victor-Ekwebelem, M. O. (2021). ANAMMOX in Wastewater Treatment. In: Maddela N. R., García Cruzatty L. C., Chakraborty S. (eds) Advances in the Domain of Environmental Biotechnology. Environmental and Microbial Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-15-8999-7_15

[4] Abiya, S. E., Odiyi, B. O., Ologundudu, F. A., Akinnifesi, O. J., and Akadiri, S. (2019). Assessment of Heavy Metal Pollution in a Gold Mining Site in Southwestern Nigeria. *Biomedical Journal of Scientific and Technical Research,* 2 (2), 22-36.

[5] Franco, H. M., Vásquez, M. M. S., Patiño-Siciliano, A., and Dendooven, L. (2010). Heavy metals concentration in plants growing on mine tailings in Central Mexico. *Bioresources Technology,* 101 (11), 3864- 3869.

[6] Nuhu, A. A., Sallau, M. S. and Majiya, M. H. (2014). Heavy Metal Pollution: The Environmental Impact of Artisanal Gold Mining on Bagega Village of Zamfara State, Nigeria. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 5 (6), 306-313.

[7] Ikhumetse, A. A., Abioye, O. P. and Aransiola, S. A. (2019). Biosorption Potential of Bacteria on Lead and Chromium in Groundwater Obtained from Mining Community. *Acta Scientific Microbiology*, 2 (6), 123-137.

[8] Federal Ministry of Health, FMH, Nigeria (FMH, 2015). Lead poison led to death of 28 children in the year 2015.

[9] Eckert, D. and Sims, J. T. (1995). Recommended soil pH and lime requirement tests. http://ag.udel.edu/extension/information/prod_agric/chap3-95.htm

[10] Walkley, A. and Black, I. A. (1934). An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sciences*, 63, 251-263.

[11] Agbenin, J. O. (1995). Laboratory manual foe soil and plant analysis (selected methods and data analysis). Faculty of Agriculture/ Institute of Agricultural Research, ABU, Zaria, pp 7-71.

[12] Black, C. A. (1965). Method of soil analysis, Agronomy No. 9 part 2, Amer. Soc. Agronomy, Madison, Wisconsin Blaylock MJ and Huang JW (2000). Phytoextraction of metals: In phytoremediation of toxic metals. Using plants to clean up the environment. Ed. I Raskin, B. D Ensley, NY Wiley. pp. 53-70.

[13] Bouyoucos, G. J. (1962). Hydrometer method improved for making particles size analysis of soil. *Agronomy Journal*, 53, 464-465.

[14] Nordberg, G., Nogawa, K., Nordberg, M., and Friberg, L. (2007). Cadmium. In: Handbook on toxicology of metals. Nordberg G, Fowler B, Nordberg M, Friberg, L editors New York: Academic Press, pp. 65-78.

[15] Harrigan, W. F. and McCance, M. E. (9760). Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, pp. 1-115.

[16] Kai, W., Jie, Z., Zhiqiang, Z., Huagang, H., Tingqiang, L., Zhenli, H., Xiaoe, Y., and Ashok, A. (2012). Chicken manure vermicompost (PMVC) can improve phytoremediation of Cd and PAHs co-contaminated soil by *Sedum alfredii*. *J Soils Sediments*, 12, 1089–109.

[17] Federal environmental protection agency (FEPA), (1991). Guidelines and standards for Industrial effluent, gaseous emissions and hazardous waste management in Nigeria, 1991.

[18] Ameh, E. G., Omatola, O. D., and Akinde, S. B. (2019). Phytoremediation of toxic metal polluted soil: screening for new indigenous accumulator and translocator plant species, northern Anambra Basin, *Nigeria, Environmental Earth Sciences*. 78: 345.

[19] Rosselli, W., Keller, C., and Boschi, K. (2003). Phytoextraction capacity of trees growing on a metal contaminated soil, *Plant and Soil*, 256 (2) 265–272.

[20] Gauthier, P. T., Norwood, W. P., Prepas, E. E., and Pyle, G. G. (2014). Metal–PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes. *Aquatic Toxicology*, 154, 253–269. https://doi.org/10.1016/j.aquatox. 2014.05.026

[21] Tarekegn, M. M., Salilih, F. Z., and Ishetu, A. I. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment, *Cogent Food and Agriculture,* 6 (1), 1783174.

[22] Bani, A., Echevarria, G., Sulce, S., Mullai, A., and Morel, J. L (2007). In-situ phytorextraction of Ni by native populations of *A. murale* on an ultramafic site (Albania), 293: 79-89.

[23] Smejkalova, M., Mikanova, O., and Boruka, L. (2003). Effects of heavy metal concentrations on biological activity of soil micro-organisms. *Plant Soil Environ*, 49: 321-326.

[24] Halim, M., Conte, P., and Piccolo, A. (2003). Potential availability of heavy metals in phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere*, 52: 265-275.