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Research Paper

Effect of Bioremediation Technology on Heavy Metals in Soil Contaminated with Organophosphate Pesticide.

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Abstract
Knowledge of soil heavy metal concentration is very important for assessing the purity and quality of the soil in an environment. The concentrations of of seven(7) heavy metal (SHM), i.e Ni^{2+}, Cu^{2+}, Pb^{2+}, Cr^{3+}, Zn^{2+}, Cd^{2+}, Fe^{2+} were identified and quantified using the PED-XRF Spectrometry, from the near-surface soils (0–15 cm) from ATBU School Farm in Bauchi state using X-ray fluorescence (XRF) spectroscopy analysis. The aim of this study was to determine the degree of soil contamination by Ni^{2+}, Cu^{2+}, Pb^{2+}, Cr^{3+}, Zn^{2+}, Cd^{2+}, Fe^{2+} and the extent of conversion using microorganism and fortified cattle dung s in biotransformation of heavy metals into nontoxic forms. A laboratory study with the objective of evaluating and comparing the effects of bioattenuation(A1), biostimulation(A2), bioaugumentation(A3) and hybrid of bioaugumentation and biostimulation(A4) was performed. This work showed that the concentrations of heavy metals in wooden Microcosm were respectively: in theA1, the concentration of Ni was 3.72 mg/kg, Cu 13.21 mg/kg, Pb 5.32 mg/kg ,Cr 102.3 mg/kg, Zn 81.88 mg/kg, Cd 3.05 mg/kg and Fe 105.3 mg/kg ; in theA2, Ni was 7.0 mg/kg, Cu 22.4 mg/kg, Pb 15.6 mg/kg ,Cr 109.8 mg/kg, Zn 89.4 mg/kg, Cd 8.7 mg/kg and Fe 54.64 mg/kg; in the A3 Ni was 3.5 mg/kg, Cu 14.7 mg/kg, Pb 7.8 mg/kg ,Cr 97.0 mg/kg, Zn 67.0 mg/kg, Cd 10.0 mg/kg and Fe 107.3 mg/kg and in the A4, Ni was 7.5 mg/kg, Cu 27.3 mg/kg, Pb 20.4 mg/kg ,Cr 75.0 mg/kg, Zn 67.7 mg/kg, Cd 8.0 mg/kg and Fe 85.3 mg/kg. In addition, A4 had the highest metal removal rate (7.5%) and A1 had the lowest metal removal rate (1.26%).

Keywords: *Heavy metals, X-ray fluorescence (XRF), soil pollution, soil sample wooden Microcosm*

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I. Introduction

Heavy metals are part of the soil, but their high levels are considered toxic. Sources of the heavy metal pollution are industrial activities such as mining, metal smelting, production of different oils, fertilization, pesticide use, house waste, etc (Marilda *et al*., 2015; Kabata and Pendias, 1989). The presence of metals in groundwater and soils can pose a significant threat to human health and ecological systems. The chemical form of the contaminant metal influences its solubility, mobility, and toxicity in ground-water systems. Heavy metals are almost everywhere in the environment, as a result of both anthropogenic and natural activities, and humans are exposed to them through various pathways (Kodom *et al*., 2012 ; Khan *et al*., 2007; Wilson and Pyatt, 2007). Even though heavy metals such as Fe, Ca, Cu, Mg, Mn, Zn, Co, Ni, Mo, and other trace elements are essential for proper functioning of biological systems, their deficiency or excess could lead to a number of disorders as well. Excessive accumulation of heavy metals in soils may not only result in soil pollution or contamination, but can also lead to elevated heavy metal (HM) uptake by plants, and thus affect food quality and safety (Kodom *et al*., 2012; Muchuweti *et al*., 2006). HM accumulation in soils and plants is of increasing concern due to the potential human health risks (Singh *et al*., 2010), which eventually lead to food chain contamination. This food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body (Kodom *et al*., 2012; Khan *et al*., 2007). Conventional methods to remediate heavy metals contaminated site are excavation and solidification/ stabilization, these technologies have certain limitations which includes; cost-effectiveness limitations, generation of hazardous by-products or inefficiency (Bahi *et al*., 2012). On

the other hand, biological methods potentially solve these drawbacks since they are easy to operate, do not produce secondary pollution. Heavy metals having relatively high density are toxic at low concentration (Iram *et al.*, 2013). Microorganisms and plants are usually used for the removal of heavy metals. Process of involvement of microorganisms to reduce pollutant concentration is known as bioremediation which is a natural process and its importance of biodiversity (above or below the ground) is increasingly considered for clean-up of metal contaminated and polluted ecosystem. This study investigates qualitatively and quantitatively the concentrations of heavy metals present in the near-surface (0–15 cm) soil of the ATBU school farm artificially contaminated with OPP using X-ray fluorescence spectrometry to determine whether or not the respective concentrations exceed their threshold limit value (TLV). The (threshold limit values) TLVs of the heavy metals used in this paper were adopted from the European Union (EU) standards.

II. Material and Methods

Sample Collection and Characterization

An un-impacted soil samples with little or no history of pre and post treatment of pesticide from Agricultural and Bio-resources Engineering Farms (Soil and Water Field), Abubakar Tafawa Balewa University (ATBU), Bauchi State were collected from the surface layer of the vadose zone 15 to 30 cm below the land surface. The soil samples were air-dried, homogenized, passed through a 2 mm (pore size) sieve to enhance proper mixing and extract consisting mainly of stones and dead plant debris discarded, the sample was then stored in a polyethylene bag at room temperature of 29^0C. The sieved soil was contaminated artificially with Organophosphate Pesticide to a pollutant level of 50,000ppm and its moisture content level was increased from 1.37 to 15 using distilled water. The soil matrix was properly mixed at ambient temperature (25-30^0C). Each of the contaminated soil (5kg) was stacked into four wooden boxes lined with polyethylene bags internally to prevent the leaching as shown in plate 1. Each box had dimension of 10cm height X 30cm length X 30cm width with soil layer 1.27cm deep. Various treatment options were prepared according to Table 1. Samples were taken bi weekly for PED-XRF analysis for a period of 8weeks.

Table 1: Composition of Various Treatment Options.

Microcosm	Sample	Sample Mass(g)	Treatment
1	A_1	5000	Sample + No Heat + H_2O
2	A_2	5000	Sample + No Heat + Fortified Cow Dung+ H_2O
3	A_3	5000	Sample + Heat + S_1&S_2+ H_2O
4	A_4	5000	Sample + Heat + S_1&S_2+ Fortified Cow Dung + H_2O

Key Note:

S_1= *Bacillus subtilis*

S_2= *Pseudomonas aerouginosa*

Wooden microcosm under Organic manure treatment had a C: N: P ratio of 100:10:1

Plate 1: wooden microcosm stacked with different treatment options as presented in Table 1

Sample Preparation and Analysis

The samples were air-dried in open air for seven days, to ensure proper drainage and to decrease the moisture content below 20%. This is due to the fact that moisture content above 20% could interfere with the XRF analysis and also alter the soil matrix for which the XRF spectrometer has been calibrated (Kodom *et al.*, 2010; EPA/ROC, 1998) with respect to solid (powdered) samples. To achieve this goal, the samples were prepared for drying by breaking them down into aggregates and spreading them evenly on polyethylene sheets or plywood trays in open air, and ensuring with great circumspection that there was no sample cross-

contamination or contamination from any external source (s). In order to reduce soil matrix effects, the soil samples were thoroughly homogenized and sieved to fine particle sizes of about 75 µm with Retsch aluminium test-sieves with vibratory electronic sieve shaker. Generally, XRF probes better only at a depth of 100 µm or less for most sample matrices (Kodom *et al.,* 2012 ;Guthrie, 2007). Therefore, the fine powdery particles that passed through the 75 µm sieve were re-packaged and carefully stored for further investigation. As described by Kodom *et al*. (2010), pulverization of the soil samples in general includes grinding, mixing, or milling into a finer loose powder state. Since XRF spectrometers only analyze a sample's surface layer, which must be representative of the whole sample, each sample was carefully and homogeneously prepared into pellets with smooth surfaces of equal density. This was achieved by milling or pulverizing the loose powdered samples (75 µm) to further reduce the particle size to about 60 µm and below. Before the milling or mixing procedure, 0.9 g of powdery binder (Hoechst wax) containing cellulose, starch, polyvinyl alcohol, or other organics (Kodom *et al.,* 2012 ; Buhrke *et al*., 1998) was weighed into 4 g of each sample using an electronic balance. The resulting mixture (sample and binding material), having a total mass of 4.9 g, was put into deformable aluminum cups (screw-top grinding jars) for the process of thorough milling and homogenization using the RETSCH Mixer Mills (MM 301), which also aided the further pulverization of the sample. By using a SPECAC hydraulic press with a maximum pressure limit of 15 tons (or 15000 kg), each pulverized sample was manually pressed into pellets with uniform diameter (32 mm) and thickness (3 mm), as discussed by Kodom *et al*. (2010).

XRF Instrumentation and Elemental Analyses.

The concentrations of the heavy metals were determined using a polarized energy dispersive X-ray fluorescence (PED-XRF) spectrometer. Specifically, the Spectro X-LAB 2000 PED-XRF spectrometer equipped with a Rh anode X-ray tube, and a 0.5 mm Be side window was employed. This Spectro X-Lab 2000 XRF equipment has a carousel (circular rotating position sample changer) placed inside a sample chamber that has the capacity to hold a maximum of 20 sample holder disks of 32 mm diameter for sequential sample analyses. The XRF is a very sensitive technique; hence great caution was taken to avoid contamination of the pellets ahead of the analysis. Poor handling of the samples could seriously affect the results of the analysis due to high sensitivity of the spectrometer, which was sensitive enough to detect fingerprints on the pellet's surface layer (Kodom *et al*., 2010; Brouwer, 2006). Taking into consideration these cautious measures, the 15 sample holder disks (sample cups) were all filled with the pellets by holding the sample pellets by the edges (not the surface layer). In the sample chamber, the 15 samples were characterized by allocating them with sample numbers for easy identification.

A computer-based multi-channel analyzer, which contained menu-based SPECTRO X-LAB Pro. Software Package (Turboquant), was set for controlling the spectrometer functions, spectral analysis, collecting and storage of data, as well as data evaluation. The samples were therefore analyzed using a pre-set method, made up of a series of tasks, which acquires several spectra for each sample, one spectrum per target (Kodom *et al.,* 2012 ; Guthrie, 2007). The instrumentation used was a SPECTRO X-LAB 2000 equipped with a 400 W Rh end window tube and a Si (Li) detector with a resolution of 148 eV. The irradiation chamber could be operated under vacuum or by using a gas purge either with nitrogen or helium. The automatic sample changer could be equipped with 20 samples maximum (Kodom *et al.,* 2012 ; Schramm *et al*., 1999). Inside the spectrometer, the method selected the first task and all sample spectra were acquired one after the other. By rotating the target carousel and adjusting other parameters, the system moved on to the next task and acquired the next set of spectra lines. The net top positions of the spectra generally represented the various elements present, and the areas of the line spectra represented the intensity. Qualitatively, the top positions of the spectra characterized the various elements in the sample, while quantitatively the net intensity of the peaks of the spectra were converted into concentrations (Kodom *et al.,* 2012 ; Brouwer, 2006). The PEDXRF systems depend on semiconductor-type detectors, which receive the entire emitted spectrum from the sample and decode it into a histogram of number of counts versus photon energy. The peak height for any element is directly related to the concentration of that element within the sampling volume (Kodom *et al.,* 2012 ; HORIBA, 2009). To identify the net intensity of the peaks of the spectra and convert them into concentrations, calibration standards with accurately known element concentrations are used to generate calibration curves (XRF peak intensity versus concentration). These curves are then used to calculate concentrations from observed spectra. The method works extremely well, and is recommended for best accuracy (Kodom *et al.,* 2012 ; HORIBA, 2009), since a good relationship exists between the peak height and the recorded concentrations. Peaks in the energy spectrum, once acquired, are subject to a large degree of massaging by the software in the connected computer. Sophisticated algorithms sense and quantitatively correct for high backgrounds due to Compton scattering from low atomic number matrices (Kodom *et al.,* 2012; Guthrie, 2007; Metz *et al.,* 1994). Due to the secondary targets used by the spectrometer, several energy spectra for each sample were acquired, one from each target. Since each target yields better sensitivity in one part of the spectrum, the information from the energy spectra is combined to quantitate each element being analyzed.

III. Results and Discussion.

Heavy Metals in the Soil.

From the elemental analysis, concentration of seven(7) heavy metal (SHM), i.e Ni^{2+}, Cu^{2+}, Pb^{2+}, Cr^{3+}, Zn^{2+}, Cd^{2+}, Fe^{2+} were identified and quantified using the PED-XRF Spectrometry, the result are discussed below. Based on the result obtained in Table 2, it was observed that the order of the mean of the heavy metal concentration in the un-amended sample (A1) followed the trend of $Fe^{2+}>Cr^{3+}>Zn^{2+}>Cu^{2+}>Pb^{2+}>Ni^{2+}>Cd^{2+}$. The sample treated with organic manure cow dung (A2), the order of the mean concentration of the heavy metal followed observed trend as, Cr^{3+},Zn^{2+},Fe^{2+},Cu^{2+},Pb^{2+},Cd^{2+},Ni^{2+}. In the third microcosms treated with exogenous microbes (A3), the mean concentration followed the order of $Fe^{2+}>Cr^{3+}>Zn^{2+}>Cu^{2+}>Pb^{2+}>Cd^{2+}>Ni^{2+}$. In the fourth set of microcosm treated with combined microbes (Bacteria) and Organic Manure (Cow Dung), the best treatment condition (A4) of the mean concentration of heavy metals followed the order of $Fe^{2+}>Cr^{3+}>Zn^{2+}>Cu^{2+}>Pb^{2+}>Cd^{2+}>Ni^{2+}$.

Table 2: - Initial and Residual Metal Contents for Various Treatment Option after Eight Weeks.

Heavy Metals	INITIALS	A1(mg/kg)	A2(mg/kg)	A3(mg/kg)	A4(mg/kg)
NI	3.75	3.72	7	3.5	7.5
CU	13.21	13.01	22.4	14.7	27.3
Pb	5.32	5.31	15.6	7.8	20.4
Cr	102.3	101.1	109.8	97	75
Zn	81.88	80	89.4	67	67.7
Cd	3.05	3	8.7	10	8
Fe	105.3	104.7	54.64	107.25	85.3

The nickel content in the various treatment options varies from 3.5mg/kg to 7.5mg/kg as shown in Figure 6; its average concentration is 5.5mg/kg. The target value for nickel is 35mg/kg as shown in Table 3, while the intervention value is 210mg/kg (HSE, 1994; WHO (1996); Marilda *et al.*, 2015)

Table 3: . Permissible Limits of Heavy Metals in Soil (Marilda *et al.*, 2015)

Metal	Target values(mg/kg)	Intervention values(mg/kg)
Cd	0.8	12
Cr	100	360
Cu	36	190
Pb	85	530
Ni	35	210
Zn	150	300
Fe	-	50000

*Target values are specified to indicate desirable maximum levels of elements in unpolluted soils **Intervention when remedial action is necessary; Source: WHO (1996).

Cr concentrations in the treatment options varied from 115mg/kg to 75mg/kg, its average concentration is 97.84mg/kg. The permissible limit for Cr is 100mg/kg. Cr content in all samples was less than the permissible limit as shown in Figure 3. Zinc concentrations in treatment options varied from 89.4 to 44.31mg/kg, its average concentration is 74.18mg/kg. The maximum intervention limit for zinc in the soil is 150-300mg/kg (CEC, 1986; Marilda *et al.*, 2015). Zn content in all samples fell lower than the permissible limit as shown in Figure 2. The concentration of Pb varied from 5.01mg/kg to 20.4mg/kg, its average concentration is 13.17mg/kg. The permissible limit for Pb is 85mg/kg as shown in Table 3 (HSE, 1994; Marilda *et al.*, 2015). Pb content in all samples fell within the standard values as shown in Figure 1. Cd concentrations in the treatment options varied from 2.97mg/kg to 10.0mg/kg, its average concentration is 6.54mg/kg. The permissible limit for Cd is 0.8mg/kg, while its intervention value is 12mg/kg. The average concentration of Cd was more than the permissible limit, but less than the intervention value as shown in Figure 4. Fe concentrations in the treatment options varied from 54.64mg/kg to 117.6mg/kg, its average concentration is 87.85mg/kg. The intervention limit for Fe is 50000mg/kg, while the average concentration of Fe is less than the intervention value as shown in Figure 7. Cu concentrations in the treatment options varied from 12.87mg/kg to 27.3mg/kg, its average concentration is 19.5mg/kg. The permissible limit for Cu is 36.0mg/kg, while its intervention value is 190mg/kg and the average concentration of Cu was less than the permissible limit as shown in Figure 5. The percentage concentrations of the heavy metal content before and after bioremediation process are presented in Figure 8. The total metal content before bioremediation processes was 314.81mg/kg, which was less than the maximum limit of 700ppm required for effective bioremediation(**Carmen**,2016). Thus, treatment option A4 had the highest percentage in decrease (7.5%) in Heavy metal concentration as compared with the following order A3 (2.4%), A2 (2.31%), A1 (1.26%). The increase in the metal content observed in some treatment options could probably be attributed to the nature of the parental material of the soils (minerals rocks and Organic matter) and intrusion of individual heavy metals ions through the addition of water as indicated by high

values than their respective initial values. Therefore, A4 remained the best treatment option offering the best heavy metal content concentration removal as compared to the initial sample.

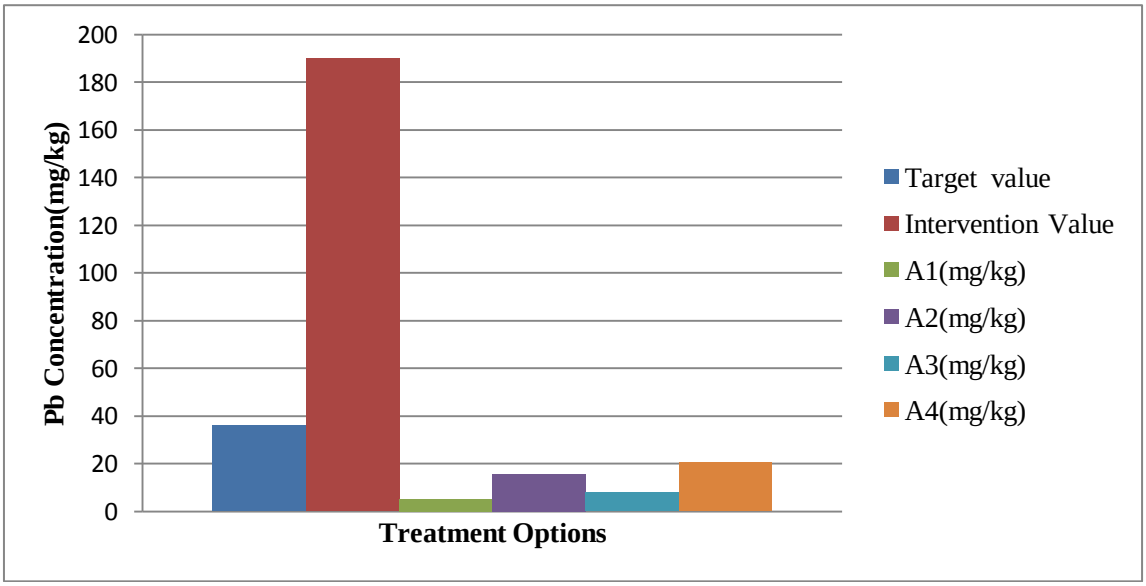

Figure 1: Measured mean Pb concentration at each treatment Options with Target and Intervention Levels

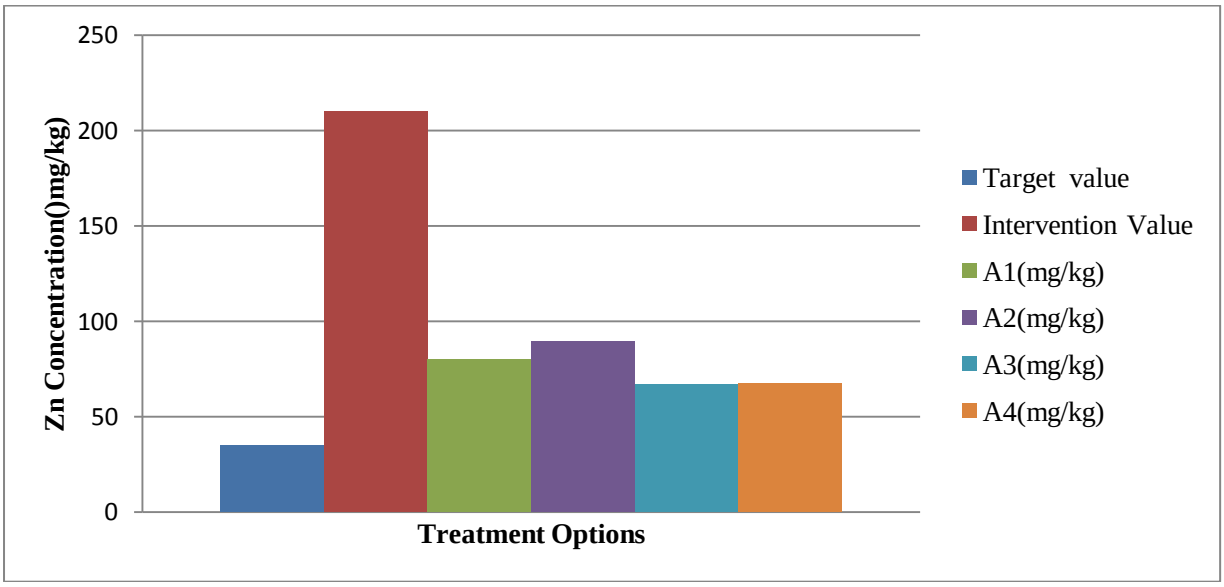

Figure 2: Measured mean Zn concentration at each treatment Options with Target Value and Intervention Levels

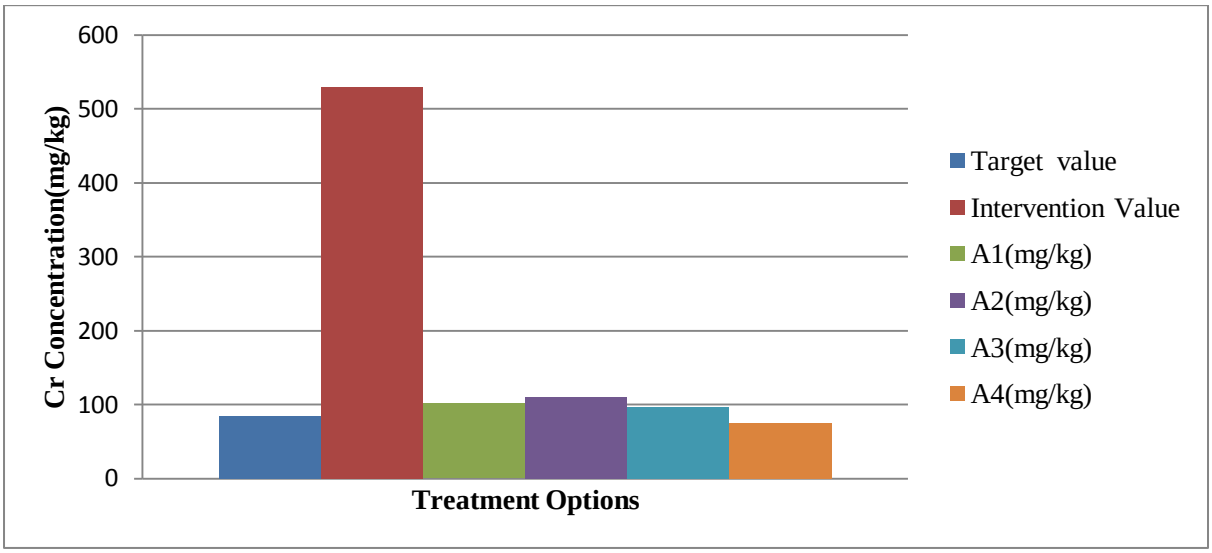

Figure 3: Measured mean Cr concentration at each treatment Options with Target and Intervention Value

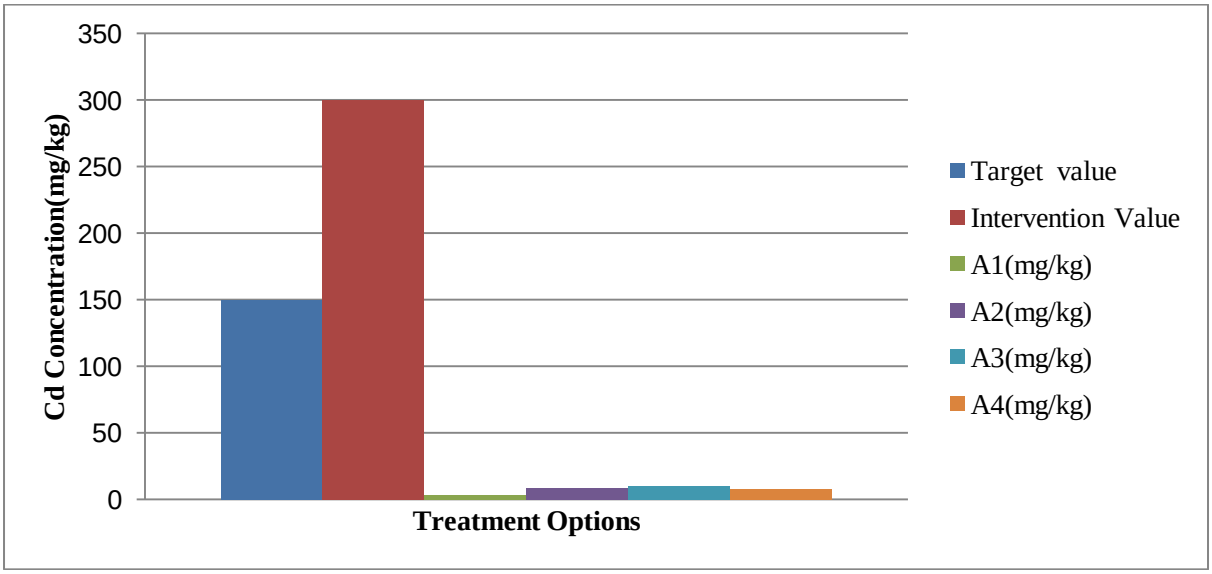

Figure 4: Measured mean Cd concentration at each treatment Options with Target and Intervention Levels

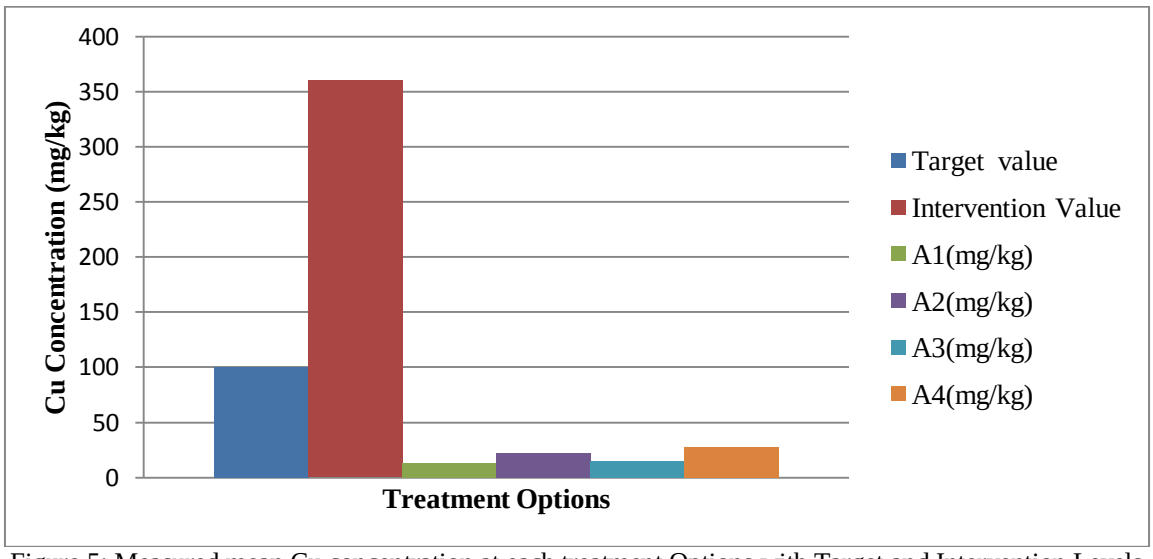

Figure 5: Measured mean Cu concentration at each treatment Options with Target and Intervention Levels

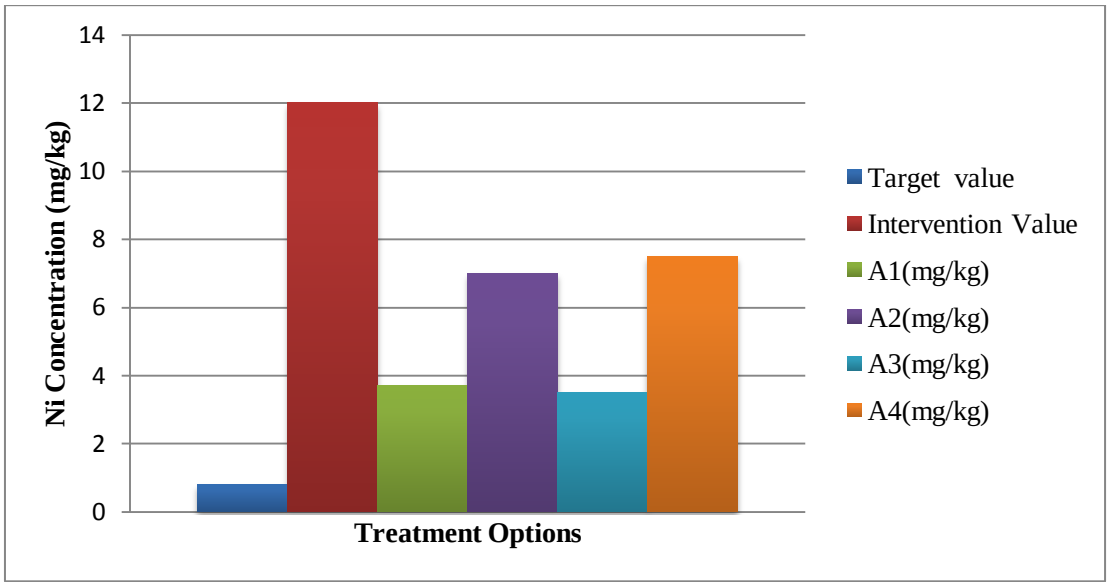

Figure 6: Measured mean Ni concentration at each treatment Options with Target value

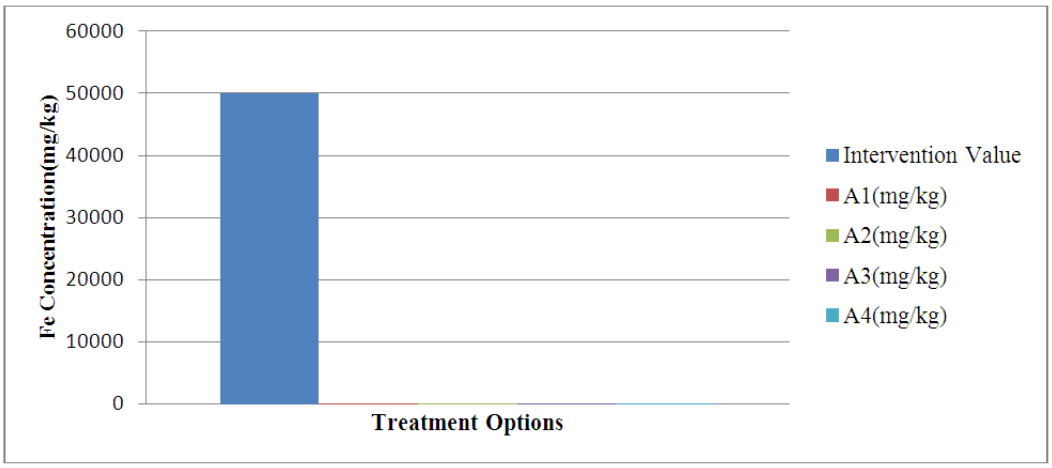

Figure 7: Measured mean Fe concentration at each treatment Options with Intervention Value

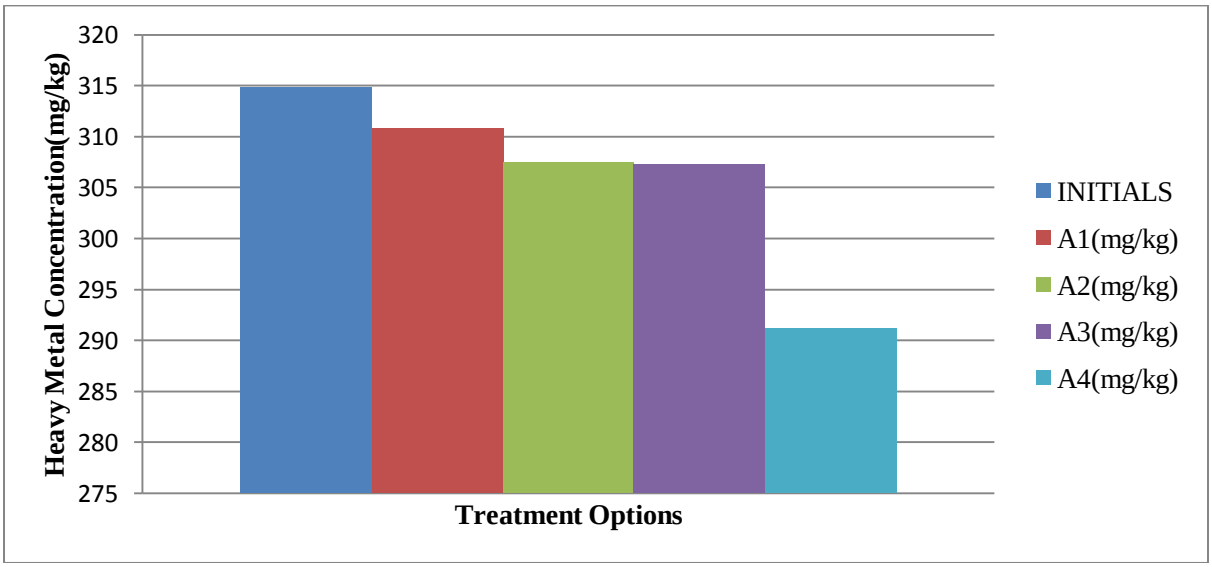

Figure 6: Extent of Percentage Reduction of Heavy Metal in Various Treatment Options.

IV. CONCLUSION

The work on Effect of Bioremediation Technology on Heavy Metals in Soil Contaminated with Organophosphate Pesticide was carried out successfully within the limits of experimental errors. The following conclusion can be drawn based on the findings from the work. The environmental and economic impacts of heavy metals pollution on soil are enormous; eliciting changes capable of affecting nutrient cycling that can cause serious damages to soil fertility and soil-born microorganism. In this present study, the result showed that 23g/l carbon source supplement at pH 5-8 enhanced the heavy metal bioremediation potentials of the hybrid of bioaugumentation and biostimulation treatment option (A4) exhibiting the highest degradation rate of heavy metal removal at 7.5% in contrast with the natural attenuation treatment option(A1) exhibiting the lowest degradation of heavy metal at a removal rate of 1.26%, therefore making the hybrid of bioaugumentation and biostimulation the best treatment option in this study. The overall results/analysis shows the effectiveness of the use of microbes in the remediation process can be optimized and utilized for the standardization of bioremediation strategies as well as establishment of biodegradation protocols. The over-reaching benefits could provide effective reclamation of natural ecosystem (environment) contaminated with Heavy Metals.

REFERENCES

[1]. Bahi, J. S., Radziah, O., Samsuri, A. W., Aminudin, H. and Fardin, S. (2012). Bioleaching of Heavy Metals from Mine Tailings by Aspergillus fumigatus.Bioremediation Journal, 16: 57-65.

[2]. Brouwer, N. P. 2006. Theory of XRF- Getting Acquainted with the Principles, 2nd ed., PANalytical B.V., 2, Almelo, the Netherlands, 8–25.

[3]. Buhrke, V. E., Jenkins, R., and Smith, D. K. 1998. A Practical Guide for the Preparation of Specimens for X-ray Fluorescence and X-ray Diffraction Analysis, John Wiley and Sons, Inc., New York, 1–7.

[4]. Carmen Cristorean, Valer Micle, Ioana M. Sur (2016). A critical analysis of ex-situ bioremediation technologies of hydrocarbon polluted soils

[5]. EPA/ROC. 1998. Environmental Information of Taiwan: ROC: Yearbook of Environmental Protection in Taiwan, Environmental Protection Agency (EPA), Taipei, Taiwan, 82–83.

[6]. Gosa Girma (2015). Microbial Bioremediation of some Heavy Metals in Soils: An updated review Journal of Resources Development and Management ISSN 2422-8397 An International Peer-reviewed Journal Vol.10, 2015

[7]. Guthrie, J. M. 2007. Overview of X-ray Fluorescence: University of Missouri Research Reactor – Columbia. Available at: http://www.missouri.edu/copyright.php, accessed December 1, 2009.

[8]. HORIBA Scientific. 2009. X-ray Fluorescence Analysis: Tutorial. Available at: http://www.horiba. com/fr/scientific/products/x-ray-fluorescence-analysis/tutorial/, accessed January 1, 2009.

[9]. Iram, S., Uzma, G., Rukh, S. and Ara, T. (2013) Bioremediation of Heavy Metals Using Isolates of Filamentous Fungus Aspergillus fumigatus Collected from Polluted Soil of Kasur, Pakistan, International Research Journal of Biological Sciences, 2: 66-73

[10]. Kabata-Pendias A, Pendias H.(1989). Trace Elements in Soils and Plants. Fourth Edition. Mir, Moscow; 152–186

[11]. Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., and Zhu, Y. G. 2007. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Poll 6, 1–7.

[12]. Kodom, K., Wiafe-Akenten, J., and Boamah, D. 2010. Soil heavy metal pollution along Subin River in Kumasi, Ghana; Using X-ray fluorescence (XRF) analysis. 20th International Congress on X-Ray Optics and Microanalysis, X-ray Optics and Microanalysis, Proceedings Book Series. AIP Conf. Proc. 1221, 101–108.

[13]. KODOM, K.; PREKO, K. AND BOAMAH, D. (2012) X-ray Fluorescence (XRF) Analysis of Soil Heavy Metal Pollution from an Industrial Area in Kumasi, Ghana Soil and Sediment Contamination, 21:1006–1021, ISSN: 1532-0383 print / 1549-7887 online DOI: 10.1080/15320383.2012.712073.

[14]. Marilda O; Aida B; and Belinda H.(2015). Heavy Metals and Ni Phytoextractionin in the Metallurgical Area Soils in Elbasa. Albanian j. agric. sci. 14 (4): 414-419

[15]. Metz, U., Hoffman, P., Weinbruch, S., and Ortner, H. N. 1994. A comparison of X-ray fluorescence spectrometric (XRF) techniques for the determination of metal traces, especially in plastics. Mikrochimica Acta 117, 95–108

[16]. Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., and Lester, J. N. 2006. Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: Implication for human health. Agric. Eco. and Environ. 112, 41–48.

[17]. Schramm, R., Heckel, J., and Molt, K. 1999. ED(P)XRF: Screening Analysis and Quantitative Analysis with Polarized X-rays, JCPDS-International Centre for Diffraction Data, Newtown Square, PA.

[18]. Singh, A., Sharma, R. K., Agrawal, M., and Marshall, F. M. 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. Tropical Ecology 51(2S). 375–387.

[19]. Wilson, B., and Pyatt, F. B. (2007). Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey. UK. Ecotoxi. and Environ. Safety 66, 224–231.

[20]. World Health Organization (WHO).(1996).) Permissible limits of heavy metals in soil and plants. Geneva, Switzerland