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EFFECT OF MICROBIAL INOCULATION AND POULTRY MANURE ON HEAVY METALS (Cd, Pb and Hg) CONTENT IN OIL CONTAMINATED SOIL IN CALABAR, NIGERIA

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ABSTRACT
Bioremediation technique is the safest and cost effective method of decontaminating crude oil polluted soils, because it does not leave any negative effect on the soil. The aim of this study was to evaluate microbial inoculation and poultry manure amendment on heavy metal concentration in crude oil contaminated soil. The experiment was laid out in a 5 x 4 factorial fitted into a completely randomized design. The first factor was 4 different genera of microbes (Bacillus subtilis, Pseudomonas aeruginosa, Aspergillusocbraceus and Penicilliumoxalicum) and uninoculated (control). The second factor was poultry manure at the rates of 0g/2kg soil (control), 5g/2kg, 7.5g/2kg, and 10g/2kg soil. A total of 20 treatment combinations and replicated three times was established. Although the concentrations of cadmium (Cd), Lead (Pb) and Mercury (Hg) were not above the acceptable limit in the contaminated soil, the influence of microbial inoculation and poultry manure combination significantly (P=.05) reduced the three heavy metals. Among the four microbes, Pseudomonas aeruginosa performed better in degrading the three heavy metals followed by Bacillus subtitles, but Aspergillusochraceus and Penicilliumoxalicum did not differ in their ability to reduce the heavy metals. The second factor, poultry manure alone reduced the three heavy metals better at 10t/ha than 5 and 7.5 t/ha, but statistically 10t/ha was not better than 7.5 t/ha at (P>.05). Among the treatments combination, Pseudomonas aeruginosa with 10t/ha poultry manure performed better than the other three in reducing the three heavy metals. Statistically Pseudomonas aeruginosa combined with 10t/ha poultry manure was not different from the combination with 7.5t/ha at (P<.05), in reducing the three heavy metals. Therefore, to safe cost of transportation and purchase of poultry manure, Pseudomonas aeruginosa in combination with 7.5t/ha poultry manure can effectively reduce heavy metals in oil polluted soils.
Keyword: *Brormediatiob Microbial inoculation, poultry manure, heavy metals, contaminated.*

INTRODUCTION
Petroleum –based products are the major source of energy for industries and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport and storage of petroleum and petroleum products, pollution caused by petroleum and its derivatives are the most prevalent problem in the Nigeria's Niger Delta environment since the exploration of petroleum started in 1958 (Okoh, 2003). Release of hydrocarbons into the environment whether accidentally or due to human activities are the main cause of water and soil pollution (Holleger et al., 1997). The impacts of hydrocarbon pollution of soils include loss in the productive capacity of soil with implication on living organisms and economically on the people in the pullulated areas, and consequently high poverty rate and unemployment. Other pollutants such as heavy metals are also commonly associated with petroleum industry*(Ijah,*

1992).The heavy metals found in exploitation and production wastes include Cr, Cd, Pb and Hg (EPA,1987, Ijah, 1993). Soil contaminated by heavy metals is consequently the most critical environmental problems as it poses significant impact to the human health as well as the ecosystem (EPA, 1987).Restorations of fertility to agricultural land previously polluted by oil is of great importance. Among the current technologies available for decontaminating crude oil-polluted soils, bioremediation appears to be receiving emphasis. This may be due to the fact that bioremediation techniques do not leave any negative effects on the soils (Ogbnonna and Ogbonna, 2005; Agunwamba et al., 2006). Bioremediation may be regaded as a clean-up technology that uses naturally occurring microorganisms to degrade hazardous substances into less toxic or non-toxic compounds (Atlas et al., 1994). It is the optimization of natural biodegradation in which microgorganisms chemically alter and break down organic molecules into other substances such as carbon dioxide, fatty acids, and water, in order to obtain energy and nutrients (Molles 2002). *Bio-augmentation* and *bio-stimulation* are two approaches of bioremediation geared toward enhancing and speeding up the process (Odokuma and Dickson 2003); Odu et al., 2005). Bio-augmentation involves the additions of external microbial population (endogenous or exogenous) to the polluted site. Bacteria are the most common bio-augmentation organisms (Okoh, 2003). Bio-stimulation involves the addition of appropriate microbial nutrients to a polluted site to increase nutrient and microbial activities of indigenous microbial flora (Ogboghode et al., 2004), Odu et al., 2005, Nwadinegwe and Ezeamaman 2007). This research was therefore designed to investigate the effect of microbial inoculation and poultry manure on heavy metals in oil contaminated soils.

MATERIALS AND METHODS

Study Area

This research was conducted in Calabar Nigeria.

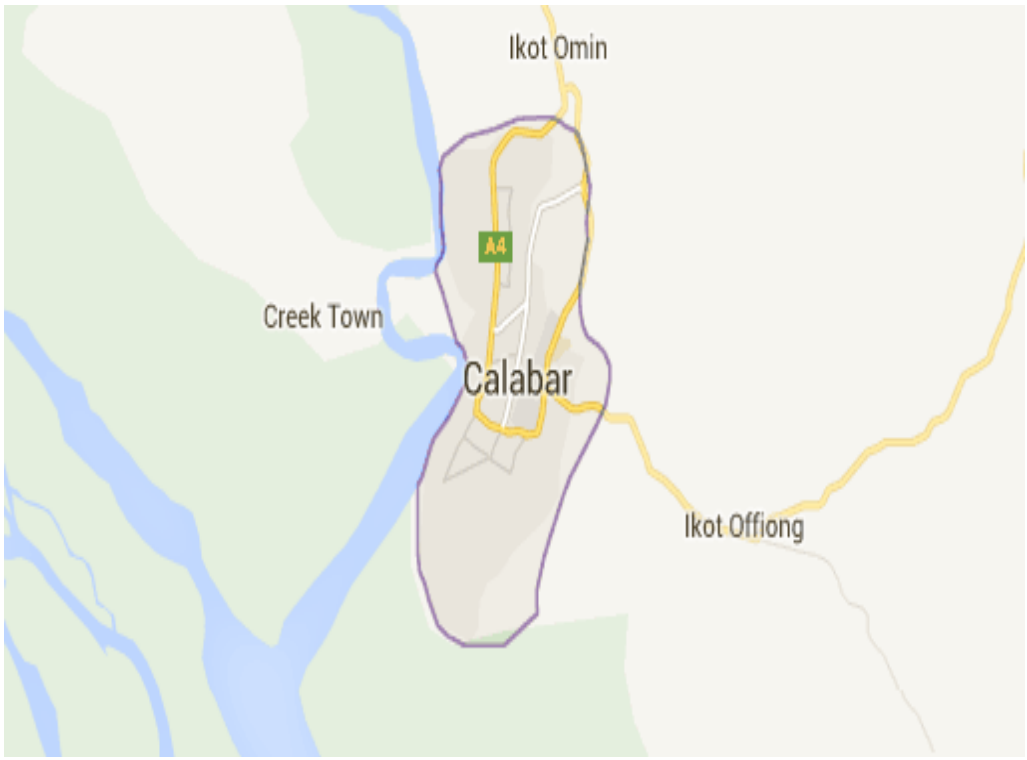

Fig 1: Map of the Study Area, Study Location

EXPERIMENTAL DESIGN

The experiment was laid out in a 5x4 factoral laid out in a completely randomized design. The first factor was made up of 4 different genera of soil microbes (*Bacillus subtilis, Pseudomonas aeruginosa, Aspergillusachraccus* and Penicilliumoxalicum) plus an uninoculated (control). This was combined with the second facto which is poultry manure at the levels of 0g/2kg soil (control), 5g/2kg soil, 7.5g/2kg soil and 10g/2kg soil twenty (20) treatment combinations each replicated three times to give a total of 60 experimental unit (pots).

Treatment Application

From the top soil that was collected at the Botanical garden, 2 kg was weighed into 60 perforated plastic buckets, each approximately 3 litres in capacity. To each bucket, 118ml of crude oil weighing 100g was added to give 5.00% (w/w) pollution level. Different application rates of poultry manure: 0g/2kg soil (0t/ha) as control, 5g/kg soil (5t/ha), 7.5g/2kg soil (7.5t/ha) and 10g/2kg soil (10t/ha) (Ibeawuchi et al. 2007), were applied to the soil two weeks after pollution. Each pot was inoculated with crude oil utilizing bacterial and fungal species. The inocula density for the bacterial species was $1.10x10^6$ cell/ml (Ogbonna et al. 2007)The fungal species was 3 agar plug ($1cm^3$) (Uzoamaka et al., 2009).

Soil Sampling

Initial soil sample was collected at the depth of 0-15 cm from the Botanical garden at 5 different points using soil auger. The 5 samples were mixed together to obtain a composite sample from the composite sample 4 kg was weighted out in duplicatein polythene bags labeled A and B for microbial and physico-chemical analyses. At the end of the experiment, some samples were taken from the three treatment combination and thoroughly mixed together to obtain a composite sample. A total of 20 composite samples were obtained for analysis.

Physico-Chemical Analysis

Soil samples were analysed following standard laboratory procedures.
Particle size analysis was done using theBouyoucous Hydrometer method (Bouyoucous 1951), soil pH was determined according to the method outlined by Udo and Ogunwale 1986).

Determination of Heavy Metals

For the heavy metals analysis, 5g air-dried soil was digested with a mixture containing hydroflunic acid and nitric acid. The residues was dissolved with hydrochloric acid, filtered and diluted with deionizer water. Then the concentrations of cadmium, lead and mercury were determined by atomic absorption spectrometry (AAS) (Udo and Ogunwale, 1986)

RESULTS AND DISCUSSION

The results in table 1 showed the physico-chemical properties of the soil of experimental site inoculated with microbes and poultry manure. The particle size distribution showed that sand fraction ranged from 82.5 to 85.7%, silt ranged from 7.9 to 10.8% and clay fraction ranged from 4.5 to 7.8% with the mean values of 84.2, 7.0 and 6.1% respectively. This gives rise to loamy sand textural class. The experimental soil reaction was between strongly acid to slightly acid condition with the pH range of 5.2 to 5.9 with the mean value of 5.5. The slight increase in pH of the soil observed was as a result of microbial inoculation and poultry manure adoption of which soil treated with Pseudomonas aeruginosa and 10t/ha poultry manure (PM) gave the highest pH (5.9) value followed by the soil inoculated with Bacillus subtitles and 10t/ha PM and uninocualted soil and 10t/ha PM. The reason for the low pH of the soil may probably be due to the organic acids produced by microbes as organic metabolitescould be responsible for the low pH range. This finding confirmed earlier reports by Ijah et al.,, (2008) and Ijah and Antai (2003) that organic manures' have the capacity to raise the pH of soil and that, poultry manure has buffering effect on crude oil polluted soils. There was a general increase in organic carbon content of the soil which ranged from 2.09% to 3.17% with the mean value of 2.69% compared to the control with the value of 1.75%. This increase in organic carbon in the treated soil was

expected since crude oil is a compound of hydrogen and carbon only and also calcium carbonate used in the formulation of poultry feed might also contributed to the carbon contents of the soil. The soil inoculated with Pseudomonas aeruginosa with 10t/ha PM and Bacillus subtilis with 10t/ha PM had the highest and high organic carbon content while soil treated with 10t/ha poultry manure without microbes inoculation had the least organic carbon content.

This may probably be due to the fact that hydrocarbon is a source of carbon and hydrogen only, while organic matter is the source and sink of nitrogen, phosphorus and sulphur for energy needed by microbes for the proper functioning and ability to degrade pollutants . This result corroborated earlier reports by Mbah et al., (2009, 2006). They stated that organic carbon and organic matter from wastes can influence the ability of microorganisms to degrade pollutants. There was a general increase in total nitrogen content in all the inoculated and amended soils compared to the uninoculated and unlamented soils. It ranged from 0.14% to 0.28% with the mean value of 0.21%. Nitrogen content in the inoculated soils is moderately high compared to the control. The increase in total nitrogen and available phosphorus might have been as a result of input of organic manure which is source of mineral nutrients capable of increasing the limiting nutrients as reported by (Mbah et al., 2009; Tanee and Kinako, 2008). Samples inoculated with bacterial species had the highest available phosphorus content followed by those seeded with fungi species. The sample amended with poultry manure had least available phosphorus. The available phosphonus ranged from 19.45mg/kg to 44.77mg/kg with the mean value of 31.39mg/kg. Exchangeable bases (Ca, Mg, K and Na) were found to be in range of 1.7 to 5.1 Cmol/kg 1.3 to 2.3 Cmol/kg, 0.06 to 0.11 Cmol/kg, and 0.03 to 0.08 Cmol/kg with the mean of 2.73 Cmol/kg, 1.73 Cmol/kg, 0.058 Cmol/kg and 0.08 Cmol/kg, respectively. Coefficient of Variability for Ca was 30.10%, Mg 11.43%, K14.69% and Na 20.77%. Calcium Potassium and Magnesium were all influenced by the inoculation of microbes and poultry manure amendment. Sodium (Na) was not however affected by microbial inoculation. The increment in exchangeable bases may be attributed to organic manure addition. Similar results was observed by (Mbah 2006, 2009) who stressed that addition of organic matter to the soil will lead to increase in nutrients content and subsequently lead to improve soil fertility. Percentage base saturation greater than 60 percent are considered high.

Table 1: Physico-Chemical Properties of the Soils

	pH	%OC	Total N(%)	Avial p (mg/kg)	Exchangeable cation and acidity(cmol/kg)						Base sat %	ECEC cmol/kg	Sand (%)	Silt (%)	Clay (%)	Texture
					Ca	Ma	K	Na	Al	H						
L. soil	5.10	1.75	0.12	68.80	4.60	2.00	0.10	0.07	0.34	0.44	84.00	7.55	85.30	7.70	7.00	L. soil
M_0P_0	5.20	2.11	0.15	19.50	2.00	1.40	0.07	0.05	0.12	0.56	85.00	5.32	84.30	9.00	6.70	L. soil
M_0P_1	5.30	2.37	0.17	24.75	3.00	1.70	0.08	0.06	0.94	0.52	87.00	6.18	85.30	9.00	5.70	L. soil
M_0P_2	5.70	2.55	0.20	32.12	3.00	1.80	0.08	0.06	0.62	0.49	89.00	6.05	83.53	9.70	6.70	L. soil
M_0P_3	5.80	2.97	0.22	37.62	3.20	1.60	0.09	0.07	0.44	0.40	92.00	5.80	84.60	10.70	4.70	L. soil
M_1P_0	5.20	2.37	0.18	24.33	2.00	1.60	0.07	0.06	1.10	0.48	89.00	5.31	84.30	10.70	5.70	L. soil
M_1P_1	5.40	2.55	0.20	30.50	2.20	1.70	0.08	0.06	0.76	0.40	91.00	6.90	83.60	9.00	6.70	L. soil
M_1P_2	5.60	2.99	0.24	36.17	2.40	1.80	0.09	0.07	0.58	0.28	93.00	5.22	84.30	9.00	6.70	L. soil
M_1P_3	5.80	3.09	0.26	41.12	4.20	2.00	0.09	0.07	0.30	0.24	95.00	6.90	84.30	9.97	5.70	L. soil
M_2P_0	5.40	2.37	0.20	25.43	2.10	1.60	0.07	0.04	0.94	0.46	90.00	5.24	85.60	8.70	5.70	L. soil
M_2P_1	5.80	2.95	0.21	32.17	2.80	1.80	0.08	0.05	0.74	0.36	93.00	5.83	83.60	8.70	7.70	L. soil
M_2P_2	5.80	3.13	0.25	37.22	3.60	1.90	0.09	0.06	0.56	0.24	94.00	6.45	84.30	9.00	6.70	L. soil
M_2P_3	5.90	3.15	0.27	44.75	5.00	2.20	0.09	0.06	0.25	0.20	96.00	7.81	84.60	10.70	4.70	L. soil
M_3P_0	5.20	2.33	0.17	21.37	2.00	1.50	0.07	0.05	1.12	0.50	88.00	5.07	82.60	10.70	6.70	L. soil
M_3P_1	5.30	2.55	0.19	25.34	2.20	1.60	0.08	0.06	0.08	0.42	90.00	5.16	83.30	9.00	7.70	L. soil
M_3P_2	5.30	2.97	0.22	34.17	2.30	1.80	0.08	0.06	0.60	0.32	92.00	5.16	84.30	8.00	7.70	L. soil
M_3P_3	5.60	3.01	0.24	39.00	3.20	1.80	0.09	0.07	0.34	0.28	94.00	5.78	84.60	10.70	4.70	L. soil
M_4P_0	5.20	2.23	0.17	22.12	1.80	1.50	0.07	0.04	1.14	0.52	87.00	0.07	83.60	10.70	5.70	L. soil
M_4P_1	5.30	2.48	0.19	26.11	2.10	1.70	0.08	0.05	0.82	0.46	89.00	5.21	84.60	10.70	4.60	L. soil
M_4P_2	5.40	2.77	0.23	34.17	2.30	1.83	0.09	0.06	0.60	0.36	91.00	6.24	85.30	9.00	5.70	L. soil
M_4P_3	5.70	3.01	0.24	39.87	3.10	1.77	0.09	0.06	0.34	0.32	94.00	5.68	83.60	10.70	5.70	L. soil
Range	5.20-5.90	2.09-3.17	0.14-0.28	19.48-44.77	1.70-5.10	1.3-2.3	0.06-0.11	0.03-0.08	0.23-1.26	0.06-0.11	84-97	4.75-7.97	82.50-85.70	7.90-10.80	4.50-7.80	
Mean	5.51	2.70	0.21	31.39	2.73	1.73	0.08	0.05	0.71	0.08	90.97	5.68	84.21	9.68	6.10	
SD	0.25	0.33	0.03	7.26	0.82	0.199	0.01	0.01	0.30	0.01	3.09	0.72	0.732	0.92	0.99	
CV(%)	4.56	12.35	16.12	23.14	30.10	11.48	14.69	20.77	41.55	14.69	3.39	12.59	0.87	9.47	16.22	

I. soil=Initial soil M_0 = Microbes M_1= *Bacillus* spp. M_2= *Pseudomonas* spp. M_3 =*Aspergillu* spp. M_4 =*Penicillium* spp.
P_0=No poultry manure , P_1=5ta/h poultry manure P_2 = 7.5ta/h poultry manure P_3 =10t/ha poultry manure L. soil = Loamy soil.

Effect of microbial inoculation and poultry manure on concentration cadmium, Lead and Mercury

The results in table 2 showed the influence of microbial inoculation and poultry manure amendment on cadmium, Lead and Mercury contents in soil polluted with crude oil. Cadmium has no known beneficial effects on any living organism. In man it can cause kidney damage, and its affect aquatic organisms even in low concentration (Horsfall and Spiff, 1998). Cadmium content in the control was 11.580mg/kg, but when poultry manure was applied at 5,7.5 and 10t/ha without microbes, there were various percentages of reduction in cadmium content. The reduction in cadmium concentration when 5t/ha poultry manure was applied was 16.58%, but when 7.5 and 10t/ha poultry manure was applied the decrease in cadmium concentration was 63.73 and 65.48% respectively, statistically, application of 7.5t/ha and 10t/ha poultry manure did not give significant reduction in cadmium content in the soil at ($p > 0.05$). Therefore, to save cost of transportation and purchase of poultry manure the use of 7.5t/ha against 10t/ha may be recommended or to achieved maximum result and upward increment from 10t/ha to 15t/ha can reduce cadmium to zero level. In the control before the inoculation of *Bacillus subtilis* cadmium concentration was 11.210mg/kg, but when *Bacillus subtilis* was inoculated with 5t/ha poultry manure 40.59% reduction was archived. At the rate of 7.5and 10t/ha application rates of poultry manure with Bacillus subtilis, the reduction in cadmium concentration was 64.85 and 66.10% respectively although the difference between 7.5and 10t/ha poultry manure was not significantly different at ($P<0.05$) with each other. Before inoculation with Pseudomonas aeruginosa cadmium concentration was 11.130mg/kg soil but on inoculation of Pseudomonas aerugnose and poultry manure amendment at 5,7.5 and 10t/ha, there was reduction in cadmium to 6.330, 3.870 and 3.130 mg/kg respectively or 43.13, 65.23 and 71.87% reduction. Similarly, before the *inoculation* with *Aspergillusochraceus*or amended with poultry manure, cadmium concentration in the soil was 11.300 mg/kg, but on application of 5,7.5 and 10t/ha poultry manure and inoculated with *Aspergillusochraceus*, Cadmium concentration reduced to 8.310, 4.100 and 3.860mg/kg or 43.98, 63.71 and 65.84% respectively. *Penicilliumoxalicum and Aspergillusochraceus* did not differ in their ability to degrade Cadmium concentration in crude oil contaminated soil. *The slight reduction of cadmium concentration in this study suggests a necessity for optimization of the biochemical process of cellular sequestration and growth of Bacillus sp, Pseudomonas and fungi. In an earlier study, Bagat et al (2005) selected Bacterial (*Bacillus*) fungi and *Actinomycetes* for bioremediation of cadmium contaminated agricultural soils. Their results indicated that Bacillus and actinomycetes successfully reduced cadmium. Our study also corroborates the reports by Ike et al. (2007). They successfully had remediation of cadmium contaminated soils using symbiosis between leguminous plants and recombinant rhizobia.

In the control with no microbial inoculation or poultry manure application, lead concentration was 12.60mg/kg soil. On application of poultry manure at the rates of 5, 757 and 10t/ha with no microbial inoculation the concentration of lead was reduced to 8.52, 2.84 and 2.22mg/kg soil respectively or 32.38, 77.46 and 82.38% reduction. When *Bacillus subtilis* was inoculated with 5,7.5 and 10t/ha poultry manure addition, lead content was reduced to 7.52, 2.49 and 1.00mg/kg or 18.082, 72.87 and 89.10% reduction respectively. Inoculation of *Pseudomonas aeruginosa* with 5,7.5 and 10t/ha poultry manure reduced lead to 7.49, 2.46 and 0.81 mg/kg soil or 9.50; 70.39 and 90.25% reduction respectively, At the rate of 5,7.5 and 10t/ha poultry manure amendment, *Aspergillusochraceus* reduced lead to 8.24, 4.00 and 1.64mg/kg or 11.68, 57.12 and 82.42%. Similarly, *Pencilliumoxalium* reduced lead to 8.31, 2.7 and 1.68mg/kg at 5,7.5 and 10t/ha poultry manure application rates respectively or 22.19, 74.43 and 84.26% reduction. The reduction of Lead in the soil might be due to various resistance mechanisms employed by lead resistant bacteria such as extracellular sequestration, bio-sorption, precipitation, alteration in cell morphology. A further reduction of Lead in this study may be due to the present of Bacillus sp, and Psendomonassp inoculated in the sampled soil and subsequent growth and proliferation aided heavy metal concentration reduction by adherence to the extracellular protein structure and intracellular protein.

The United State Environmental Protection Agency (2001) also reported that acute effects of exposure to high levels of cadmium in humans may result in adverse effects on the lungs such as bronchial and pulmonary irritation. The metal is considered to have a high acute toxicity based on short term animal tests on rats. The Agency also details chronic effects to long term exposure to cadmium to include kidney diseases, including protencuica effects on the lungs including bronchioloitis and emphysema adverse effects are also reported on the liver, bone, immune system, blood and nervous systems. Human exposure to cadmium can be from consumption of contaminated water or consumption of plants grown on cadmium polluted sites. Lead is a very toxic heavy metal that does not break down in the environment, thus, elevated levels are found in soil, water and air. The United States Environmental Protection Agency, (USEPA, 2001) reports that Lead can be harmful to humans when ingested or inhaled, particularly to children, under the age of six. The most prominent adverse health effect of lead is the impairment of neurological development. The Agency further stresses that explosive routes can be through contaminated water, soil, paint, chips or dust caused muscle coordination, never damage to the sense organs and never controlling the body increased blood pressure, hearing and vision impairment reproduction problems (e.g. decreased sperm count) retarded faetal

development event at relatively low exposure levels. In children Lead poison can cause damage to the brain and nervous system, behavioural problems, anemia, liver and kidney damage, hearing loss, hyperactively, development, and delays and in extreme cases death. Lead as the potential to be bio-accumulated rising from low concentrations to potentially high concentrations that have detrimental effects on human health.

Table: Effects of microbial inoculation and poultry manure on the cadmium (mg/kg), lead (mg/kg) and mercury (mg/kg) contents of crude oil polluted soil 90 DAP.

Microbes	Poultry manure application rates (tones/ha)				
	0	5	7.5	10	mean
			Cadmium		
No microbes	11.580	9.660	4.200	4.00	7.360
Bacillus subtilis	11.210	6.660	3.940	3.800	6.403
Pseudomonas aeruginosa	11.130	6.330	3.870	3.130	6.115
Aspergillusochraceus	11.310	8.310	4.1000	3.860	6.895
Penicilliumoxalicum	11.310	8.310	4.120	3.890	6.908
Mean	11.308	7.854	4.046	3.736	
			Lead		
No microbes	12.60	8.52	2.84	2.22	6.55
Bacillus subtilis	9.18	7.52	2.49	1.00	5.05
Pseudomonas aeruginosa	8.31	7.49	2.46	0.81	4.77
Aspergillusochraceus	9.33	8.24	4.00	1.64	5.80
PenicilliumOxalicum	10.68	8.31	2.73	1.68	5.85
Mean	10.02	8.02	2.91	1.47	
			Mercury		
No microbes	0.2880	0.2040	0.1410	0.1110	0.1860
Bacillus subtilis	0.2040	0.1890	0.1050	0.1020	0.1515
Pseudomonas aeruginosa	0.1980	0.1410	0.1050	0.1020	0.1365
Aspergillusochraceus	0.2460	0.1950	0.1230	0.1050	0.1673
Penicilliumoxalicum	0.2610	0.1950	0.1260	0.1050	0.1725
Mean	0.2394	0.1854	0.1212	0.2394	

	Cadmium	Lead	Mercury
F- LSD (0.05) for microbes (M) Mean =	0.010	0.43	0.0010
F-LSD (0.05) for poultry manure (p) Means =	0.009	0.38	0.0010
F-LSD (0.05) for (M xP) interaction Means =	0.020	0.86	0.00

Mercury released into the environment is converted into methyl Mercury, which is extremely toxic to living organisms. The main route of human intake of Mercury from the external environment is food. The crucial factor is how much fish with a high Mercury content do we eat, Mercury may damage the central nervous system, as been shown by catastrophes such as the Miamian disease in Japan in 1959 (Horsfall and Spiff 1989). In the control with no microbial inoculation or poultry manure amendment, Mercury content was 0.2880mg/kg but

on addition of poultry manure at the rates of 5,7.5 and 10t/ha without microbial inoculation, Mercury concentration in the soil reduced to 0.2040, 0.1410 and 0.1110mg/kg or 29.17, 38.15 and 61.49% reduction respectively. At the rates of 5,7.5 and 10t/ha poultry manure application and *Bacillus subtilis,* Inoculationreduced Mercury concentration in the soil to 0.1890, 0.1050 and 0.1020mg/kg or 7.35, 48.52 and 50% reduction respectively. *Pseudomonas aeruginosa* similarly reduced Mercury concentrations to 0.1410, 0.1050 and 0.1020 mg/kg at poultry manure application rates of 5,7.5 and 10t/ha or 20.73, 50 and 57.31% reduction. Also the fungus *Penicilliumoxalicum* reduced Mercury concentration to 0.1950, 0.1260 and 0.1050mg/kg soil or 25.52, 51.72 and 59.77% reduction. The microbial inoculation significantly ($p<.05$) reduced the concentrations of the three heavy metals (Cd Pb and Hg), but differed in their ability to reduce the heavy metals. Among the four microbes, *Pseudomonas aeruginosa* was more efficient followed by bacillus, subtilis and the two fungi also showed substantial amount of efficiency in reduction. The concentrations of the three heavy metals in the control and polluted soils were however not above permissible limits given by FEPA (1991) and USEPA standard. The acceptable limit for Cadmium is 12mg/kg FEPA (1991) Lead, 300mg/kg, USEPA (1997), Mercury 1mg/kg USEPA (1997). Although the concentration of these heavy metals in the treated soil were not at a worrysome level, but a build up with time may result in health hazard. The decrease in these heavy metals may be due to the activity of the microbes in the polluted soil. The microbe might have reduced the metals from available or soluble form to less available forms. This finding was in consistent with Odokuma and Akponah (2010) findings. They highlighted that crude oil degrading microbes are capable of assimilating the heavy metals under favourable conditions.

CONCLUSION

Effect of microbial inoculation and poultry manure amendment on heavy metals in crude oil contaminated soil was investigated. It was observed that among the four microbes Pseudomonas aeruginosa reduced the three heavy metals better than Bacillus subtilis, *Aspergillusochraceus* and *Penicilliumoxalicum*. In the second factor, poultry manure amendment at the rate of 10t/ha reduced the three heavy metals better than 5 and 7.5 t/ha, but was not statistically different from 7.5 t/ha application rate at ($P>.05$). Among the treatment combinations, *Pseudomonas aeruginosa* and 10t/ha performed better than 5 and 7.5t/ha application rates, but was not statistically different from 7.5t/ha application rate. Therefore for the purpose of saving cost transportation and purchase of poultry manure, the use of *Pseudomonas aeruginosa* combined with 7.5t/ha poultry manure will equally reduce heavy metals in crude oil polluted soils as if *Pseudomonas aeruginosa* with 10t/ha poultry manure were used.

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