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Assessment of Heavy Metals Bioremediation Potential of Microbial Consortia from Poultry Litter and Spent Oil Contaminated Site

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Abstract Heavy metals are high density metallic chemicals that are potentially toxic at low concentrations and present a danger to human and environmental health. This study was conducted to ascertain the efficiency of microorganisms present in animal wastes in bioremediation of heavy metals present in spent engine oil contaminated soil. Spent engine oil impacted soil was excavated from a mechanic workshop in Ugbowo, Edo State, Nigeria and transported to the laboratory in a plastic container. Air dried spent engine soil samples were homogenized and measured into four plastic buckets used as test cells (1 kg each) and mixed thoroughly with poultry manure in a soil to manure ratio of 80% weight of soil to 20% weight of manure, 70% weight of soil to 30% weight of manure and 60% weight of soil to 40% weight of manure and labeled as PL 20%+SEOCS (Poultry Litter + Spent Engine Oil Contaminated Soil), PL 30%+SEOCS and PL 40%+SEOCS and CONTROL respectively. The study lasted ten (10) weeks and analytes were obtained on a weekly basis for soil pH, microbial counts, and heavy metals analysis. Results obtained indicate that pH was 6.9 in control soil initially while it ranged from 8.1 (Week 1 PL 20% +SEOCS) to 8.4 (Week 7 PL 20% SEOCS) for the treatment categories. The differences in pH between treatment categories and control were not statistically significant ($P<0.05$). Initial soil moisture content was 2% which was improved after watering to 17.8% (lowest) in PL 30%+SEOCS and 18.4% (highest) in PL 40% +SEOCS. Moisture content in control sample was 18.9%. The difference moisture content in all treatment categories were not statistically significant ($p<0.05$). Microorganisms identified in poultry litter and spent engine oil contaminated soil were; Pseudomonas Spp, Enterococcus Spp, Micrococcus Spp, Corynebacterium Spp, Arthobacter Spp, Klebsiella Spp, Acinetobacter Spp, Bacillus Spp, Penicillum Spp, Sachoromyces Spp, Mould and Trichoderma Spp. Heavy metal analysis indicate that Arsenic (mg/kg) in the control sample had 2.73% reduction, compared to 26.6%, 32.5% and 41.17% for PL 20%+SEOCS, PL 30%+SEOCS and PL 40%+SEOCS, respectively. Barium in the control sample had 6.28% reduction compared to 35.9%, 11.1% and 64.21% for PL20%+SEOCS, PL 30% +SEOCS, and PL 40%+SEOCS respectively. There was no significant difference between PL 30%+SEOCS and control while reduction in PL 20%+SEOCS and PL 40% +SEOCS was significantly different from control ($P<0.05$). Cadmium in the control sample had a drop of 25% while PL 20%+SEOCS, PL 30%+SEOCS and PL 40% +SEOCS had <0%, 38% and 33.3% reduction respectively. Cadmium reduction in the treatment categories was not significantly different from the control. Chromium in the control sample had 20% reduction while there was 26%, 58.06% and 46.57% reduction in the PL 20%+SEOCS, PL 30% +SEOCS and PL 40%+SEOCS respectively, there was a significant reduction in the concentration of Chromium. Cobalt in the control sample reduced by 5.86%; it had reduction of 53.3%, 56.0% and 61.4% in PL 20%+SEOCS, PL 30% +SEOCS and PL 40%+SEOCS respectively. Reductions in all treatment categories were significantly different from the control (<0.05). Lead (mg/kg) in the control reduced by 2.70% while in the treatment categories, PL 20%+SEOCS, PL 30%+SEOCS and PL 40%+SEOCS, had 15.3%, 24.06% and 34.5% reduction respectively. There was a significant reduction in the concentration of Lead ($P<0.05$) when compared with the control. The research findings indicate that bioremediation using growing microorganisms present in contaminated soil and animal wastes can reduce the concentration of heavy metals in soil. The research can further be implemented in a pilot scale study and subsequently on spent oil contaminated sites.

Keywords: *heavy metals bioremediation, poultry litter, heavy metals, spent engine oil contaminated soils*

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1. Introduction

Environmental degradation is assuming an alarming rate worldwide. This problem might not be unconnected with the rapid industrialization, population growth and technological advancement being experienced in most parts of the world today. A long list of potential hazardous substances is released on a daily basis which contributes to the global pollution burden. As part of this 'list', heavy metals result from industrial wastes; electronic wastes etc, and pose serious threat to both man and animals if not properly abated.

Indiscriminate disposal of engine oil into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics (Okonokhua *et al*, 2007). Heavy metals such as Vanadium, Lead, Aluminium, Nickel and Iron usually below detectable limits in unused lubricating oil have been reported by Whisman *et al*, (1971) to give high values (ppm) in used oil. These metals may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations, and/or bound to organic matter in soil (Yong *et al.*, 1992). There is a growing global concern because of the numerous health risks to animals and humans following exposure.

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals that are harmful to humans include Mercury, Lead, and Arsenic. Chronic exposure to these metals can have serious health consequences. Humans are exposed to heavy metals through inhalation of air pollutants, consumption of contaminated drinking water, exposure to contaminated soils or industrial waste, or consumption of contaminated food grown on contaminated land. Food sources such as vegetables, grains, fruits, fish and shellfish can become contaminated by accumulating metals from surrounding soil and water. Heavy metal exposure causes serious health effects, including reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as Mercury and Lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system. Metals are particularly toxic to the sensitive, rapidly developing systems of foetuses, infants, and young children. Some metals, such as lead and mercury, easily cross the placenta and damage the foetal brain. Childhood exposure to some metals can result in learning difficulties, memory impairment, damage to the nervous system, and behavioural problems such as aggressiveness and hyperactivity. At higher doses, heavy metals can cause irreversible brain damage. Children may receive higher doses of metals from food than adults, since they consume more food for their body weight than adults (Roane, 1999).

Environmental contamination by heavy metals is consequently the most critical environmental problem as it poses significant impacts to the health of humans as well as the numerous ecosystems that support it. The contaminants infiltrate deep into underground, pollute ground water and surface water. There is a probability of entering the human food web through plants and aquatic animals that bioaccumulate them, consequently transferring them from one food chain to another in a process known as biomagnification.

The effectiveness of commonly employed methods of treating heavy metals polluted wastes, including among others, precipitation and ion exchange remains limited, (Kratochril *et al.,* 1997), particularly for dilute metal wastes (Corder and Reeves, 1994).

Over the last decade, biosorption developed as an alternative treatment method (Volesky, 1990; Williams *et al.,* 1998). This technology involves the accumulation of heavy metals by biological material either by metabolically mediated methods or by purely physico-chemical means (Sakaguchi and Nakijama, 1991) unlike physical and chemical treatments, biosorption is relatively cheap.

However, this technology relies on active microbial presence and metabolism which in some environmental matrices is inhibited by nutrient availability and bioavailability as there exist a direct relationship between nutrient supply and microbial growth.

This study seeks to assess and evaluate the efficacy of providing a low cost, environmentally friendly and socially acceptable means of remediating some selected heavy metals from spent oil contaminated soil using microbial consortia from poultry litter. This study is justified because of the common problem associated with conventional treatment namely; the difficulties encountered in treating the solid waste subsequently generated might not exist since biological treatment concentrates the wastes into smaller volumes which are subsequently easier to dispose of appropriately (Bhude *et al.*, 1996).

2. Microorganisms and Heavy Metals

Microorganisms have evolved various mechanisms of metal resistance and scientists have tried to exploit genetic/metabolic basis of all such mechanisms for the production of superior strains. Silver *et al* (2001) reported that although chromosomal genes may be involved, bacterial resistance to heavy metals is often conferred by products of genes simulated on plasmids rendering genetic manipulation for strain improvement easy and feasible.

A possible way to confer resistance to heavy metals on microorganisms is to progressively increase the concentration of the toxicants microbes are exposed to and observe microbial metabolism and proliferation. Scientists have explored this attribute to ascertain if tolerance of higher concentrations can subsequently aid in biodegradation of such toxicants. Donmez and Aksu (2001) tried to adapt strains of *Candida* species (isolated from sewage) to Copper and Nickel by serial subcultures in Copper and Nickel supplemented growth medium. Adapted cells could grow well in presence of higher metal concentrations while non adapted ones perished. Moreover the specific metal uptake capacity and metal removal (%) by adapted cells were higher than the non-adapted cells at all the concentrations tested. Although the authors could not elucidate the mechanism of adaptation, they implied constitutive synthesis of methalothionein (other copper binding proteins or changes in the genetic

makeup) as one of the factors in adaptation. Baillet *et al* (1997) adapted *Thiobacillus ferroxidans* strain via successive exposure to higher concentrations of Cadmium. The biomass (living but not growing) of adapted strain had cadmium uptake capacity of 0.31 g/g dry weight as compared to 0.21 g/g dry weight in case of non adapted cells.

Other non conventional techniques reported by Soares *et al* (2002) are the comparison of flocculent strain and non flocculent strain of *S. cerevisiae* for metal bioremediation. They inferred that flocculent strain S646-1b accumulated more copper than non flocculent S646-8D strain in the first 10 minutes of contact with the metal. The authors attributed this to the presence of additional metal binding on cell surface of flocculent strains.

3. Statement of Problem

There are relatively large amounts of hydrocarbons in spent engine oil including highly toxic Polycyclic Aromatic Hydrocarbons (Wang *et al.,* 2000). Ekundayo *et al.,* (1989) have shown that a marked change in the properties occur in soils polluted with petroleum hydrocarbons affecting the physical, chemical and microbial properties of the soil. Oil pollution leads to a buildup of essential organic nutrients such as carbon, phosphorus, calcium and magnesium and non essential Lead, Zinc, Iron, Copper and Cobalt. These elements if in excess will lead to translocation of plant tissues. Although some heavy metals at low concentrations are essential micro nutrients for plants, at high concentrations they could cause high metabolic disorder and growth inhibition for most plant species. This coupled with the potential of some plants to bio accumulate heavy metals make the presence of spent oil in soil worrisome. Soil contamination with engine oil that is rampant in the Nigerian environment has adverse effects for seasonal crops such as tomato (*Lycopersicum esculentum*) and maize (*Zea mays*). (Okonokhua *et al.,* 2007).

4. Significance of the Study

Since contamination of soil and groundwater by the indiscriminate discharge of petroleum products has become a significant problem today, a number of technologies have been investigated to remedy the situation. Treatment processes have incorporated chemical, physical and biological methods or a combination of them. Treatment options include excavation and landfill disposal, incineration, surfactant application and a host of others that are expensive, environmentally unfriendly and only transfer the contaminants from one place to another. Bioremediation is inexpensive, naturally and socially acceptable and environmentally friendly. The technology under study will help minimize the prohibitive cost associated with soil remediation, prevent significant soil texture alteration, prevent the transfer of pollutants from one medium to another and ensure a healthier technique for remediating heavy metals.

5. Materials and Methods

The experiment was carried out in a private laboratory in Benin City, Edo State, Nigeria. Top soil (0.7cm-15cm), was obtained from a mechanic site along Federal Government Girls College Road Benin City and transported to the laboratory in a plastic container. Poultry Litter was obtained from the University of Benin poultry farm within the Ugbowo Campus in Benin City, Edo State, Nigeria and air dried in the laboratory. The dried poultry droppings were carefully sorted, sampled for the presence of microorganisms, ground to semi-powder and used as organic fertilizer to support the growth of microorganisms and remediation of heavy metals. Air dried spent engine soil samples were measured into four plastic buckets used as test cells (1 kg each) and mixed thoroughly with poultry manure in a soil to manure ratio of 80% weight of soil to 20% weight of manure, 70% weight of soil to 30% weight of manure and 60% weight of soil to 40% weight of manure and labelled as PL 20%+SEOCS (Poultry Litter + Spent Engine Oil Contaminated Soil), PL 30%+SEOCS and PL 40%+SEOCS and CONTROL respectively. Each bucket was watered as necessary to achieve optimum moisture requirement for microbial growth. Leachate was re-introduced into the test cells before sampling every week.

6. Methods

6.1. Process Description

Soil samples were carefully sorted out to remove impurities and weighed (20 kg). Soil samples were characterized for their physico-chemical and microbial parameters using standard methods.

6.2. Soil pH

Soil pH was determined using the method of Bates (1954).

6.3. Soil Moisture Content

Soil moisture content was determined by evaporation on Whatman filter paper No. 1 at 103°C in an electrical oven.

6.4. Total Heterotrophic Bacterial and Fungal Counts

Samples were enumerated by making ten-fold dilutions of the soil samples from 10^1 to 10^3. Aliquot 0.1ml of the 10^{-3} dilution was transfer plated in nutrient agar amended with nystatin (0.5-1 µg/ml) for isolation of bacteria while potato dextrose agar amended with streptomycin (0.02-1 µg/ml) was used for the isolation of fungi. The plates were prepared and inoculated in duplicates. The inoculated nutrient agar plates were incubated at 37°C for 24 hours while the potato dextrose agar plates were incubated at 28°C for 72 hours. After incubation, the colonies of the isolates were counted and expressed in CFU/g.; isolated colonies were further purified by sub-culturing and identified using bio-chemical tests and microscopy.

6.5. Isolation of Degrading Microorganisms

The culture medium used for the isolation of hydrocarbon degrading bacteria was mineral salt agar which is the enrichment medium for the isolation of

hydrocarbon degrading bacteria. Aliquot of 0.1 ml of the 10^{-3} dilution was plated in the medium and the plates were incubated at 30°C for 5 days. Discrete colonies that developed were counted and expressed in CFU/g.

6.6. Identification of Isolates

Each isolate was examined for its size, shape, margin, consistency, elevation, pigmentation, Gram reaction and cell morphology. The isolates were characterized as described by Holt *et al.,* (1999). Biochemical tests which were carried out included production of catalase, indole and oxidase enzymes. Spore production and oxidation/ fermentation of sugars were also carried out.

6.7. Heavy Metals Analysis

Heavy metals analysis was carried out using the wet oxidation method for soils (APHA, 2000); soil samples dried at room temperature was sieved using 2mm mesh sieve. About 2.5 g of the soil was weighed into a $300m^3$ conical flask. 1 ml of concentrated HF under a fume hood was added. Heating was continued until dense white fumes appeared. Finally, it was heated strongly (medium to high heat) for half a minute. It was allowed to cool, and the 40-50 ml of distilled water was added. The solution was cooled and filtered completely with a wash bottle into a 100 ml pyrex volumetric flask. It was made up to the mark with distilled and filtered with Whatman No. 42 filter paper. The soil extracts were aspirated into the air acetylene flame of Varian 220 (fast sequential) Atomic Absorption Spectrophotomer (AAS)

6.8. Experimental design

Three different quantities of poultry litter were measured into separate test cells and thoroughly mixed. The quantities were in ratio of 20: 80, 30: 70 and 40: 60 respectively. Microbial growth/density as well as heavy metal concentration was considered as experimental responses. Heavy metal concentration was measured on a weekly basis for 10 weeks. Untreated soil (spent engine oil contaminated soil without poultry litter) was analysed as control.

6.9. Statistical analysis

All statistical parameters (ANOVA at 95% probability level of significance) were carried out using Statistical Package for Social Sciences and Microsoft Excel 2007.

7. Results and Discussion

7.1. pH

The results of the hydrogen ion analysis are presented in Figure 1.

pH for untreated soil at baseline week (week 0) was 6.9 while it was 7.20 for poultry litter used as nutrient additive. The results obtained at the end of the experiment indicate a range of 8.1 (Week 1 PL 20% + SEOCS) and 8.4 (week 7 PL 20% + SEOCS). These results show that pH in treatment categories was slightly higher than the control throughout the experiment although this difference was insignificant.

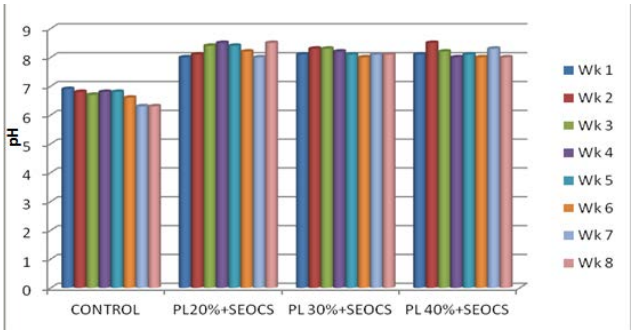

Figure 1. pH of experimental groups

Biological activities are regulated by enzymes that operate within a fairly stringent range of pH. When the extremes of this range are exceeded then microbial growth and physiology is significantly distorted. Boonchan, (2000) and Joanne *et al.*, (2008) reported that optimum pH for bioremediation is between 6.0 and 8.9. They opined that changes from initial levels of pH could be as a result of the release of acidic and alkaline intermediates and final products during biodegradation of hydrocarbons which has an effect on the pH. The findings of this research indicate that pH level fell within a suitable range to support microbial growth in all the treatment categories and control.

7.2. Soil Moisture Content

Table 1 shows the percentage water content by mass in the treatment categories.

In this study, percentage initial moisture content was 2.0%. Watering during the research phase improved moisture content and analysis revealed that the lowest average percentage moisture content by mass was 17.8% in PL 30% + SEOCS, while the highest average percentage moisture content by mass was 18.4% in PL 40% + SEOCS. In control samples, moisture content was 18.9%. The values in the treatment categories had no significant differences with the control ($P<0.05$).

Table 1. Percentage moisture content of experimental groups

Experimental Group	Soil moisture content by mass (%)	Lowest – Highest (%)
PL 20% + SEOCS	18.2	16.5-19.2
PL 30%+SEOCS	17.8	16.6-18.4
PL 40%+SEOCS	18.4	18.2-19.4
CONTROL	18.9	18.0-19.4

Microorganisms require water for microbial growth and for diffusion of nutrients and by-products during the degradation process (Malik, 2004). If the soil is too dry, many rnicroorganisrns will die. If water content of the soil is too high, oxygen transfer to microorganisms will be resisted by the flooded soil and the rate of the hydrocarbon degradation will be reduced. The optimum soil water content for bioremediation is dependent on the soil type. Generally, the optimum activity occurs when the soil moisture is 15-40% of the field capacity, also termed the water holding capacity which is defined as "the amount of the water remaining within the soil after gravitational water has drained away" or the percentage of water in a soil when it was (Baker, 1994). When moisture content is lower than 10% of the holding capacity, the bioactivity becomes marginal (Testa and Winegardner, 1991). Water was added to the entire experimental group so as to achieve optimum moisture content of at least 17%. Based

on the results observed, moisture content was optimum in all the treatment categories as well as control during the period of experiment.

7.3. Biotic count

Table 2 presents results of Total Microbial Count.

In this study, identification of microorganisms in poultry litter and contaminated soil revealed the following heterotrophic microbial species; *Pseudomonas, Enterococcus, Micrococcus, Corynebacterium, Arthobacter, Klebsiella, Acinetobacter, Bacillus, Penicillum, Sachoromyces, Mould and Trichoderma*. The microbial density and diversity prevalent in a contaminated medium play important roles in degradation of pollutants.

Table 2. Microbial isolates from poultry litter and soil samples

Bacteria	Fungi
Pseudomonass Sp.	*Penicillum Sp.*
Enterococcus Sp.	*Sachoromyces Sp.*
Micrococcus Sp.	*Mould Sp.*
Corynebacterium Sp.	*Trichoderma Sp.*
Arthobacter Sp.	
Klebsiella Sp.	
Acinetobacter Sp.	
Bacillus Sp.	
Thiobacillus Sp.	
Leptospitillum Sp.	
Enterobacteriaceae Sp.	
Clostridium Sp.	
Desulfosporosinus Sp.	

The extent of bioremediation depends on microbial diversity present which can survive in highly concentrated areas. However, reports abound (Yong *et al*, 1992; Volesky, 1990; Bhude *et al.*, 1996 and Baillet et al., 1997) suggesting that microbial performance in bioremediation is hindered by such factors as nutrient availability, temperature, pH, pollutant concentration, moisture content, oxygen content as well as soil properties of impacted soils. These finding agrees with some studies (Malik, 2003, Bade *et al*, 2012) which reported that *Bacillus, Acinetobacter, Pseudomonas and Arthobacter* have great potentials for accumulating and removing some heavy metals. This finding also corroborates the study by Akpokona (2011) who observed an increase in uptake of Chromium and TPH degradation in brackish water impacted with crude oil when NPK and poultry litter were used as nutrient additives to stimulate microbial growth.

8. Heavy Metals Degradation

8.1. Arsenic (mg/kg)

The results of arsenic analysis are presented in Figure 2. In this study, Arsenic (mg/kg) in the control sample reduced from 2.19 mg/kg at the start of the experiment to 1.91 mg/kg and 2.13 mg/kg (2.78%) on the final sampling week. In PL 20%+SEOCS, the concentration reduced from 1.8 mg/kg to 1.08 mg/kg on the third week and 1.32 mg/kg (26.6%) on the final week of the experiment. In PL30%+SEOCS the initial concentration was 1.78 mg.kg and it dropped to 1.11 mg/kg and eventually to 1.2 mg/kg (32.5%) on the final sampling week. In PL 40%+SEOCS the initial concentration of 1.87 mg/kg dropped to 1.1 mg/kg (41.17%) on the final week of the experiment. The peaks observed in the plots might be as a result of experimental errors. ANOVA shows that there was a significant difference between the treatment categories and the control ($P<0.05$). There was also a significant difference between week 0 and week 10 ($P<0.05$).

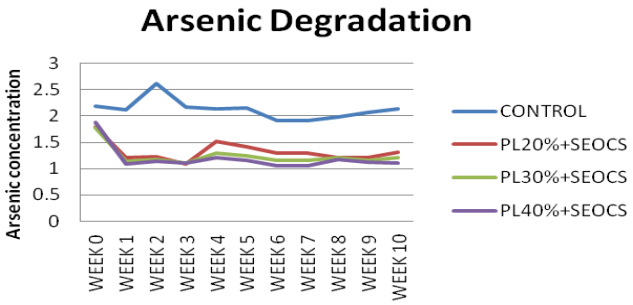

Figure 2. Arsenic degradation (mg/kg)

Arsenic is naturally present at high levels in the soil in a number of countries and can be further increased by anthropogenic activities. This heavy metal is a known carcinogen and causes numerous other serious health problems (Santhosh, 2008). It is highly toxic in its inorganic form and exposure to arsenic is usually through drinking contaminated water, using contaminated water in food preparation and irrigation of food crops, industrial processes, eating contaminated food and tobacco smoking. Katerina *et al* (2008) reported the removal of arsenic from contaminated soil using bioremediation. This research corroborates the fact that arsenic can be reduced using a biological treatment scheme. The decrease of arsenic might be as a result of the presence of *Bacillus species* in the soil samples that have high adsorptive capacity due to high peptidoglycan and techoic acid content in their cell walls which enables an ion exchange reaction.

8.2. Barium (mg/kg)

The results of barium analysis are presented in Figure 3. In this study, Barium (mg/kg) in the control sample reduced from 1,290 mg/kg in the first week to 1202 mg/kg (6.28%) in the final week of the experiment. In PL 20%+SEOCS barium (mg/kg) reduced from 1,130 mg/kg to 724 mg/kg (35.19%) in the final sampling week. In PL 30%+SEOCS, initial concentration was 790 mg/kg and this value reduced to 702 mg/kg (11.1%) while in PL 40%+SEOCS the initial concentration of 503 mg/kg reduced to 180 mg/kg (64.21%). The peaks observed in the plot might be due to experimental errors. ANOVA shows there was no significant difference between PL 30%+SEOCS and control ($P>0.05$) while there was a significant difference between PL 20%+SEOCS, PL 40%+SEOCS and control. There was also a significant difference ($P<0.05$) between week 0 and week 10.

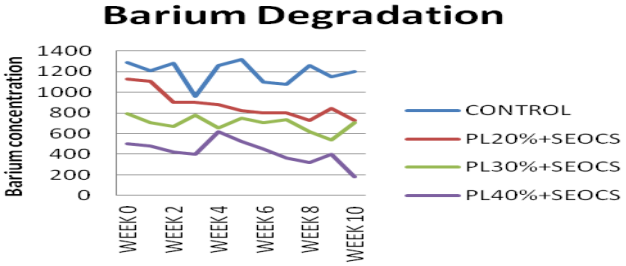

Figure 3. Barium degradation (mg/kg)

Barium causes respiratory effects such as benign pneumonoconiosis, silicosis (Seaton *et al*, 1986) and pulmonary lesions (Tarasenko *et al*, 1977). This metal has also been incriminated in cardiovascular and gastrointestinal effects with abdominal cramps, nausea, vomiting as acute symptoms of exposure to barium carbonate powder (Shankle and Keane 1988). Other significant health effects include renal failure, body weight loss; metabolic effects such as decrease in plasma potassium concentration as well as reproductive impairment especially disturbances in spermatogenesis. The reduction in the concentration of barium over time during this experiment might not be unconnected with the presence *Pseudomonas Sp.* which produces rhamnolipids, a surfactant that shows specificity for certain metals such as barium and cadmium. This report is in agreement with the findings of Sand *et al.,* (1992) who studied the leaching potential of *Leptospirillum feroxidans* and reported the significance of surfactants in the remediation of barium and other heavy metals.

8.3. Cadmium (mg/kg)

Figure 4 shows the results for cadmium (mg/kg) during the research period. In the study, cadmium (mg/kg) dropped from 0.6 mg/kg to 0.45 mg/kg (24%) in control, in the treatment categories; cadmium mg/kg had an initial concentration of 0.32 mg/kg in PL 20%+SEOCS and fluctuated throughout the research duration and eventually was 0.75 mg/kg on the final week of sampling. In PL 30%+SEOCS the initial concentration of 0.13 mg/kg dropped to 0.08 mg/kg (38%), while in PL 40%+SEOCS the initial concentration of 0.15 mg/kg dropped to 0.1 mg/kg (33.3%). The peaks observed were probably due to experimental errors. Analysis of variance shows that cadmium reduction in the treatment categories was not significantly different from the control and although there were reductions observed during the research duration, they were however not significant (P>0.05).

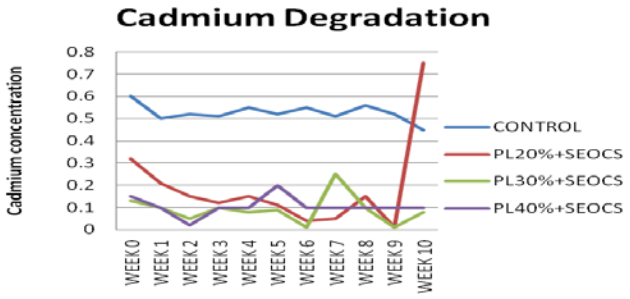

Figure 4. Cadmium degradation (mg/kg)

The United States Environmental Protection Agency (2001) states 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 protenuria, effects on the lungs including bronchioloitis and emphysema, adverse effects are also reported on the liver, bone, immune systems, blood and nervous systems. Human exposure to cadmium can be from consumption of contaminated water or consumption of plants grown on cadmium polluted sites. Studies on the remediation of cadmium conducted by Ike *et al*, (2007) report a technique of successfully remediating cadmium contaminated soil using symbiosis between leguminous plants and recombinant rhizobia, Bagot *et al* (2005) selected bacteria, (*Bacillus),* fungus and actinomycetes for bioremediation of cadmium contaminated agricultural soils. Their results indicate that *Bacillus* and actinomycetes successfully reduced cadmium. 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.

8.4. Chromium (mg/kg)

Figure 5 shows the results for chromium.

In this study, there was a gradual reduction of chromium in the treatment categories. Chromium (mg/kg) in the control sample was 19.5mg/kg intitially, this concentration was 13.4mg/kg in the seventh week and ended at 15.6mg/kg (20%) on the final week of the experiment. In the PL20%+SEOCS the initial concentration of 18.9mg/kg reduced to 13.9mg/kg (26%) on the final sampling week. In PL30%+SEOCS the initial concentration of 15.5mg/kg reduced to 6.5mg/kg (58.06%) while in PL40%+SEOCS, the initial concentration of 14.6mg/kg reduced to 7.8mg/kg (46.57%). Analysis of Variance indicates that there was no significant difference (P>0.05) between PL20%+SEOCS and control while PL30%+SEOCS and PL40%+SEOCS were significantly different from the control. There was a significant reduction in the concentration of chromium over time (P<0.05).

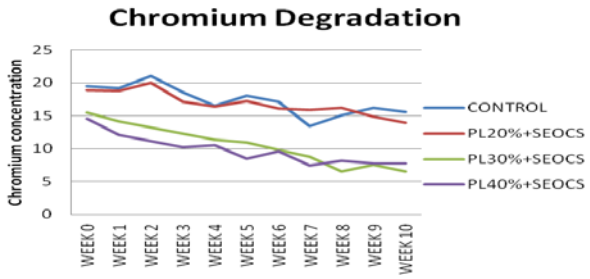

Figure 5. Chromium degradation (mg/kg)

The Occupational Safety and Health Administration's fact sheet (2006) reports that adverse health effects associated with chromium exposure include occupational asthma, eye irritation and damage, perforated ear drums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer, skin irritation and erosion, allergic skin reaction and contact dermatitis. Human exposure to environmental chromium is usually from handling chromium contaminated medium and contact with contaminated sites either through dermal exposure or through ingestion of contaminated drinking water.

For Chromium, bioremediation relies on the reduction of soluble and mobile hexavalent chromium, Cr (VI), to its reduced form, Cr (III). Chromium reduction may occur if the growth of microbes is stimulated via the addition of a reduced carbon source, such as lactate, acetate, or molasses. The product, Cr (III), is often assumed to precipitate as relatively insoluble hydroxide solid.

Studies on specific bioremediation of chromium by Jeyasingh (2005) using optimization of operating parameters under laboratory conditions revealed a reduction aerobically and anaerobically by indigenous microorganisms that were isolated from a previously contaminated site. After studying the mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential, Cheung *et al* (2007), reported that microbial reduction of chromium is commonly catalyzed by soluble enzymes with *Pseudomonas spp* and *E. coli* capable of secreting chromium reductases. They also reported that through the utilization of membrane associated reductases, *Pseudomonas maltophilia* and *Bacillus megaterium* could detoxify chromium. The presence of *Pseudomonas Sp.*, *Bacillus Sp.* and *E. coli* and subsequent reduction in the concentration of chromium during the experiment is in agreement with the findings of these researchers.

8.5. Cobalt (mg/kg)

Results of cobalt degradation are presented in Figure 6. In this study, cobalt (mg/kg) in the control sample was 2.9 mg/kg on the first week of sampling; it reduced to 2.73 mg/kg (5.86%) on the final sampling week. In the treatment categories, PL 20%+SEOCS had initial cobalt concentration of 2.4 mg/kg and this concentration reduced to 1.12 mg/kg (53.3%). Cobalt concentration in PL 30%+SEOCS was 2.3 mg/kg initially and it reduced to 1.01 mg/kg (5.60%) on the final sampling week. In PL 40%+SEOCS the initial concentration of 2.1 mg/kg reduced to 0.81 mg/kg (61.42%) on the final sampling week. Analysis of Variance indicates that reductions in all the treatment categories were significantly different from the control. Overall reduction throughout the duration of the research was significant ($P<0.05$).

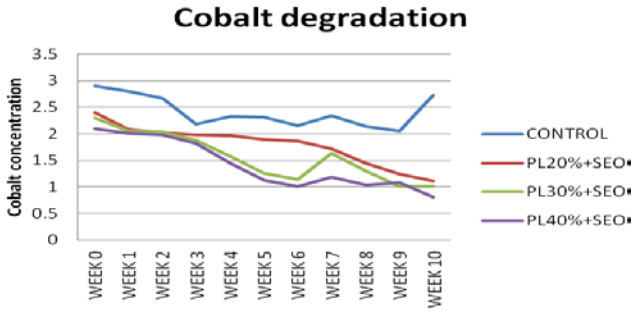

Figure 6. Cobalt degradation (mg/kg)

Swennel *et al*, (1993) reported adverse haematological effects of exposure to cobalt, Jordan *et al*, (1990) reported neurological effects including memory loss, Bucher *et al.*, (1990) reported reproductive effects in animals while Mur *et al.*, (1987) studied carcinogenecity of cobalt and reported that an increase in deaths due to lung cancer was found in workers exposed to cobalt in a plant refining cobalt and sodium. Studies on microbial remediation of trace cobalt conducted by Gogada (2008) using *E. coli*, expressing NiCoT genes revealed the viability of *E. coli* strains in the remediation of trace cobalt. Santhosh studied the biosorption of heavy metals by *Paenibacillus polymyxa* and reported that the heavy metal cobalt was reduced to up to 90% primarily due to the binding of the metal to the cell walls through the extra cellular polymeric substances and precipitation of sulphides. This study idenfied the presence of *E.coli* and the possibility of biosorption to the cell surfaces and subsequent reduction in the concentraion of cobalt. These results agree with the findings of Gogada (2008) and Santhosh (2008).

8.6. Lead (mg/kg)

Results of Lead degradation are presented in Figure 7. In this study, lead (mg/kg) in the control sample was 11.1 mg/kg on the first week, it dropped slightly to 9.98 mg/kg on the 8th week and eventually ended at 10.8 mg/kg (2.70%) on the 10th week. In the treatment categories, PL 20%+SEOCS had initial concentration of 6.14 mg/kg and reduced to 5.2 mg/kg (15.3%). In PL 30%+SEOCS initial concentration of lead was 7.23 mg/kg, it reduced to 5.49 mg/kg (24.06%) on the final sampling week. In PL 40%+SEOCS, initial Lead (mg/kg) concentration was 8.1 mg/kg, it reduced to 5.3 mg/kg (34.5%) on the final sampling week. There was a significant reduction in the concentration of Lead ($P<0.05$) when compared with the control.

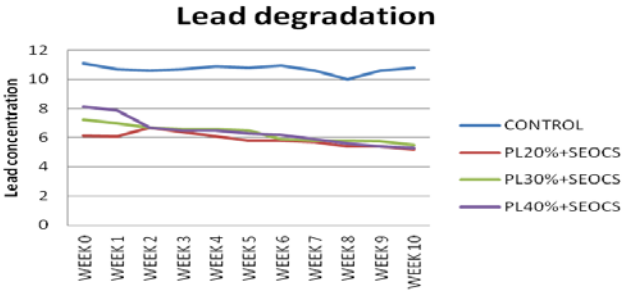

Figure 7. Lead degradation (mg/kg)

The United States Environmental Protection Agency, (USEPA, 2001) reports that Lead can be harmful to humans when ingested or inhaled, partciularly to children under the age of six. The most prominent adverse health effect of Lead is the impairment of neurological development. The agency further states that exposure routes can be through contaminated water soil, paint chips or dust causing poor muscle coordination, nerve damage to the sense organs and nerves controlling the body, increased blood pressure, hearing and vision impairment, reproductive problems (e.g. decreased sperm count), retarded fetal development even 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, hyperactivity, developmental delays and in etxreme cases death.

Lead also has the potential to be bioaccumulated rising from low concentrations to potentially high concentrations that have detrimental effects on human health.

Chatterjee *et al*, (2012) reports that various lead resistance mechanisms employed by lead resistant bacteria includes efflux mechanism, extracellular sequestration, biosorption, precipitation, alteration in cell morphology, enhanced siderophore production and intracellular lead bioaccumulation. Adsorption, ion exchange, precipitation, and complexation with organic matter are other factors that contribute to the high adsorption rate of Lead in soil (Evanko and Dzombak, 1997).

Boricha and Fulekar, (2009) reported that potent metal biosorbents under the class of bacteria include the genera

of *Bacillus, Pseudomonas, Streptomyces* and *P. aeroginosa* they found that *P. plecoglossicida* as a novel organism for the bioremediation of cypermethrin while *P. aeruginosa, Bacillus sp., Streptomyces sp., P. fluorescens* were effective against lead.

Macaskie *et al.* (2000) found that sulfate-reducing bacteria successfully treated the metal leachates generated by sulfuric-acid producing *Thiobacillus sp.*

Puranik and Puranik (1997) reported that amongst the various microorganisms, fungal biomasses were very effective due to presence of high percentage of cell wall material, which may have the excellent metal binding capacity. Many fungi and yeast have excellent biosorption potential includes the genera of *Rhizopus*, *Aspergillus*, *Streptoverticullum* and *Sacchromyces*.

The article Metal Bioremediation Through Growing Cells demonstrated that many bacterial and fungal strains can assist with lead removal. Organisms like *Pseudomonas marginalis*, *Plectonema boryanum*, and *Desulfosporiosinus* orients can all assist with lead uptake (Malik, 2003).

Other findings corroborate the fact that many grown bacterial and fungal strains can assist with lead removal, research today is focusing on the precipitation of metal sulfides utilizing sulfate-reducing bacteria. The Mine Waste Technology Program of the United States Environmental Protection Agency and Department of Energy uses sulfate-reducing bacteria for bioremediation purposes. They also use passive treatment systems like compost for remediation. Although this is currently found not to be an effective treatment because the precipitates are not guaranteed to stay in their precipitated forms (Malik, 2003).

However there exist the challenge to find fungal or bacteria biofilms that can work in many different conditions and keep the metals from leaching into the environment. It is important for researchers to discover ways to tackle this challenge; otherwise industries will continue to be skeptical about using bioremediation at metal-contaminated sites. (Beyenal and Lewandowski, 2004).

This study showed a significant reduction in Lead concentration, the presence of *Bacillus sp,* and *Pseudomonas sp* in the sampled soil and subsequent growth and proliferation aided heavy metal concentration reduction by adherence to the extracellular protein structure and intracellular protein sequestration respectively. *Sacchoromyces cerevisiae's* presence might have further aided reduction through the adherence of Lead onto the cell wall and membrane and also internally in the cytoplasm. These mechanisms of microbial accumulation of heavy metals have been documented previously by Roane (1999), Sub *et al.,* (1998) and Torres *et al*, (1998).

9. Conclusion

The research findings indicate that bioremediation using growing microorganisms present in contaminated soil and animal wastes can reduce the concentration of heavy metals in soil. The presence of consortia of microorganisms improved the metals scavenging properties and ensured a continuity in the process irrespective of the external environmental fluctuations. Ability to grow and utilize cells sourced from potential waste streams also promises an economically viable option. The exploitation of locally available support materials and cheap carbon/nutrient sources for the cells can further improve the economic imperative for heavy metals remediation.

However, choosing the right consortia must depend on initial heavy metal concentrations and should be carefully carried out so as to ensure some microorganisms metabolism do not inhibit the growth of others.

If there is an improvement in laboratory analysis to identify the exact mechanisms of uptake, the bioaccumulative 'store houses' of the cells and the genetic materials/properties responsible for biodegradation, then the gap between successful reproduction of the process at commercial scale as well as ability of these strains to continually take in heavy metals would have been bridged.

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