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CADMIUM LEACHABILITY AND MICROBIAL POPULATION DYNAMICS IN A STABILIZED AND BIOAUGMENTED MODEL CONTAMINATED SOIL

U. E. DURU

A. AL-TABBAA

University of Cambridge

ABSTRACT

This article presents a laboratory study on the consequences of the application of combined soil stabilization and bioaugmentation in the remediation of a model contaminated soil. Stabilization and bioaugmentation are two techniques commonly applied independently for the remediation of heavy metal and organic contamination respectively. However, for a cocktail of contaminants combined treatments are currently being considered. The model soil was contaminated with a cocktail of organics and heavy metals based on the soil and contaminant conditions in a real contaminated site. The soil stabilization treatment was applied using either zeolite or green waste compost as additives and a commercially available hydrocarbon degrading microbial consortium was used for the bioaugmentation treatment. The effects of stabilization with or without bioaugmentation on the leachability of cadmium and copper was observed using an EU batch leaching test procedure and a flow-through column leaching test, both using deionized water at a pH of 5.6. In addition, the population of hydrocarbon degrading microorganisms was monitored using a modified plate count procedure in cases where bioaugmentation was applied. It was found that while the stabilization treatment reduced the metal leachability by up to 60%, the bioaugmentation treatment increased it by up to 100% Microbial survival was also higher in the stabilized soil samples.

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INTRODUCTION

Techniques such as soil stabilization and bioremediation are commonly used independently for target contaminants (Chen, Lee, & Liu, 2000). Bioremediation refers to the use of microorganisms to reduce organic contaminant concentrations in contaminated materials. Bioremediation processes applied in soil remediation include bioaugmentation, which involves the enhancement of contaminant biodegradation by the introduction of microbial population with the desired biodegrading capacity. This method, which is dependent on environmental factors, is considered effective in cases where the contaminants are recalcitrant to the degradation in the presence of naturally occurring microbial community and requisite microbial nutrients in the contaminated soil (Iwamoto & Nasu, 2001). In addition, different microbial processes and products are also reported to affect metal leachability and can lead to increased leachability due to the acidification of the surrounding environment. The process of acidification could enhance metal mobility through a number of routes including competition between the protons and the metal ions for sorption and complexation sites which invariably leads to release of free metal cations (Burgstaller & Schinner, 1993; Diels, De Smet, Hooyberghs, & Corbisier, 1999).

Zeolite and compost, two less commonly used additives for soil stabilization, are considered here. Zeolites are aluminosilicates characterized by their high cation exchange capacity (Dyer, 1998) and are known to increase ion exchange sites in soils. Consequently, zeolites are able to retain heavy metals in soil more than other aluminosilicate constituents of soils like clays, and may have the capacity of reducing the detrimental effect of heavy metals on soil microorganisms (Chander & Joergensen, 2002). Organic materias, such as green waste compost, are able to affect the transformation of heavy metals from soluble and labile forms to fractions associated with organic matter or carbonates as well as by specific and non-specific ion exchange mechanisms (Huang, Xu, Gu, Wang, Cao, Du, et al., 2005; Lofts, Tipping, Sanchez, & Dodd, 2002).

Our aim was to investigate the effect of the different remediation procedures described above, when applied singularly or in combination, on metal leachability and sustenance of viable hydrocarbon degrading microbial population which is essential for the degradation of hydrocarbon soil contaminants. Soil stabilization was applied using different quantities of zeolite or compost, both applied at 15% and 30%, while bioaugmentation was applied using a commercial available microbial consortium containing hydrocarbon degrading microbes and microbial nutrients which was obtained from Cleveland Biotech, UK (2004). It was envisaged that the soil stabilization procedure would reduce metal mobility and toxicity and thus allow the sustenance of hydrocarbon degrading microbial population, which is a determinant for the biodegradation of the hydrocarbon, as other factors such as nutrients were supplied with the microbial consortium.

Experiments were carried out on a laboratory prepared model soil spiked with salts of five heavy metals, namely copper, cadmium, lead, nickel, and zinc, as well as liquid paraffin as a representative hydrocarbon. Model soil samples were used in this study to ensure consistency in the concentration of soil contaminants and to gain a preliminary insight on possible observations in natural soil conditions. Results on the leachability of cadmium are presented in this study as representatives of the most mobile metal contaminant from preliminary studies on the model soil (Duru, 2004). Results on microbial population are also presented. The effect of different factors such as type and quantity of the stabilization additive and bioaugmentation treatment on cadmium leachability and microbial population was also analyzed.

MATERIALS AND METHODS

Constituents of the Model Contaminated Soil and Microbial Consortium

A model contaminated soil was prepared in the laboratory to represent the soil and contaminant conditions of a reference site, where both the real site soils as well as the model soils have been the subject of extensive related research (Al-Tabbaa & Evans, 1998). The reference site, a former chemical works site of the Ministry of Defence at West Drayton near Heathrow Airport, is contaminated with a cocktail of toxic chemicals. The top 2 m layer of soil is modeled here using the soil constituents detailed in Table 1 which were all purchased from building material suppliers. The idea behind using a model soils in preference to the actual site soil is that the high level of heterogeneity within the site soils, which is typical of many contaminated soil conditions, would complicate the experimental findings. Homogeneous soil and contaminant conditions are required here to pinpoint the effect of the different additives used in the treatment.

The laboratory prepared soil was spiked with a number of chemical compounds, detailed in Table 2, to represent the soil contaminant loading of cadmium, copper, lead, nickel, and zinc, applied as reagent grade chemical compounds, and with liquid paraffin, to represent the total hydrocarbon content in the soil.

Notably, the fresh spiking of the chemicals will clearly not reflect any age related properties of the contaminants within the soil. It was used to ensure a homogeneously contaminated soil which is not usually the case when using a site soil. The microbial consortium used (Amnite P1300) is commercially available (from Cleveland Biotech, UK, 2004) and is described to contain a mixture of naturally occurring hydrocarbon degrading microbes and nutrients. The mixture consists of at least ten *Pseudomonas* species and is provided in a cereal like formulation.

Table 1. Model Soil Constituents by Percentage Weight

Constituents	Percentage by weight (%)
Gravel (fine)	50
Sand (sharp sand)	29.2
Silt (silica flour)	5.9
Kaolin clay	5.3
Bentonite clay	0.6
Water	9.1

Table 2. Contaminant Profile of the Model Soil

Contaminant	Concentration of contaminant (mg/kg) dry soil	Representative chemical compound
Cadmium	8.7	Cadmium nitrate
Lead	2345	Lead nitrate
Copper	962	Copper sulphate
Nickel	232	Nickel nitrate
Zinc	1800	Zinc chloride
Hydrocarbons	8700	Liquid paraffin

Preparation of the Model Contaminated Soil

The preparation of the model contaminated soil, which was carried out according to procedures described in Al-Tabbaa and Evans (1998), involved the mixing of the soil constituents with heavy metal and hydrocarbon contaminants, as listed in Tables 1 and 2 respectively, in a rotary drum mixer for several minutes until a consistent mix is achieved.

The zeolite used was Beli Plast clinoptilolite from Bulgaria which has the chemical formula Al6.1Si29.9O72.21.3H2O. Its chemical composition by percentage weight is: SiO_2 (65.8%), Al_2O_3 (10.9%), Fe_2O_3 (1.6%), CaO (2.9%), MgO (1.1%), K_2O (3.4%), Na_2O (0.8%), and H_2O (13.2%). The green waste compost used was coir compost and was described as peat free compost produced from the coir elements of coconut husks. Both binders were applied singularly at a soil:additive ratio of 70:30 and 85:15 by weight. The soil/additive mixtures were prepared by manually mixing the appropriate weight of the binder with 1 kg of the model soil. Test samples were thereafter derived from this stock. The untreated soil was also tested and served as a control. The samples were tested at 28 days after preparation.

Procedure for Sample Bioaugmentation

For the microbial inoculation of soil and binder mixes, 23 g of the microbial consortium, in cereal formulation, was mixed in deionized water. This mixture was allowed to stand for 1 hour to allow for the activation of microorganisms as directed by the manufacturers (personal communication with Cleveland Biotech, UK). At the end of the activation period, it was mixed manually with 550 g of the stabilized soil and stored in closed containers at 25°C for 28 days prior to testing. All the tests were then performed from this stock. The total moisture content in the bioaugmented samples was the same as in the non-bioaugmented samples. This total moisture content was up to 60% of the total water holding capacity of the bioaugmented samples.

In order to ensure aerobic conditions, the stock material was aerated by opening the container and agitating the stock material on the bottle roller for 15 minutes on a weekly interval. Preliminary hydrocarbon degrading microbial enumeration tests showed that microbial consortium gave about 4.8×10^8 colony forming units (CFU) per gram of inoculation cereal. Therefore, adding 23 g to 550 g of contaminated sample will give approximately 2×10^7 CFU of added microorganisms per gram of mixture. Similar levels of inoculation have been used in previous studies (Ghazali, Rahman, Salleh, & Basri, 2004).

Leaching Tests

The effect of the treatments on the leachability of cadmium was evaluated using the EU batch leaching test BS EN 12457 (British Standards Institution, 2002) and a flow-through column leaching test, which was carried out to assess conditions more representative of in-situ infiltration (Jackson, Garrett, & Bishop, 1984). The concentrations of cadmium in the leachates were quantified using a UNICAM 929 Atomic Absorption Spectrophotometer (AAS) with a detection limit of 0.032 mg/l for cadmium. A Hanna ATC piccolo pH meter with accuracy of ±0.1 was used to measure the leachate pH.

Batch Leaching Test (BS EN 12457)

The BS EN 12457 is a regulatory standard test procedure for assessing contaminated soils which involves mixing of a 100 g representative sample with a leachant composed of carbonated deionized water of pH approximately 5.6 in a liquid to solid weight ratio of 10 to 1. The mixture was contained in a 1 liter polyethylene bottle and agitated on a bottle roller at 30 rpm for 24 hours to allow for equilibration. The leachate was then separated from the solid particles using a vacuum filtration unit with a membrane filter of 0.45 µm pore size. The filtered leachate was then subjected to chemical analysis. Duplicate measurements were carried out on the different test samples and mean values are presented in the results sections.

Flow-Through Column Tests

The test was carried out in glass columns with an internal diameter of 50 mm and 350 mm length. Washed gravel was placed at the base of the column, followed by 250 g of the test sample, which was added in batches of 25 g without being compacted. The remaining space above the test sample was then filled with washed gravel. A membrane filter paper of 0.45 µm pore size was then placed between the gravel sections and the sample. The gravel sections served as end drains and to fill up the column as appropriate since the samples had different densities and for the same weight they were of different heights in the columns. A peristaltic flow pump was used to pump the leachate through the base of the column. The setup was left to stand for 24 hours to allow for equilibration. The leachate was then pumped through the column at a flow rate of 0.6 ml/min, giving a flow velocity of leachate in the column of 0.4 m/day which enabled the collection of 2500 mL of the leachate, corresponding to liquid:solid weight ratio of 10:1, in three days. Both the batch leaching tests and flow-through column tests were conducted in duplicates and the average results presented together with error bars.

RESULTS AND DISCUSSION

The results shown in the different graphs represent mean values from duplicate measurements together with the error bars. The results are presented for the two leaching tests separately. Each graph presented both the leachate concentrations as well as the leachate pH. The results from both the stabilization and bioaugmentation treatments are also presented. The results are then discussed separately and compared with the EU waste acceptance criteria.

Batch Test Leachate pH and Cadmium Leachability

The batch leaching test results for cadmium are presented in Figure 1.

Leachate pH

In the sample without the bioaugmentation treatment the leachate pH ranged between 7.50 in the unstabilized soil and 7.45 in the stabilized soils. This shows little change (0.6%) in the leachate pH due to the stabilization treatment. In the

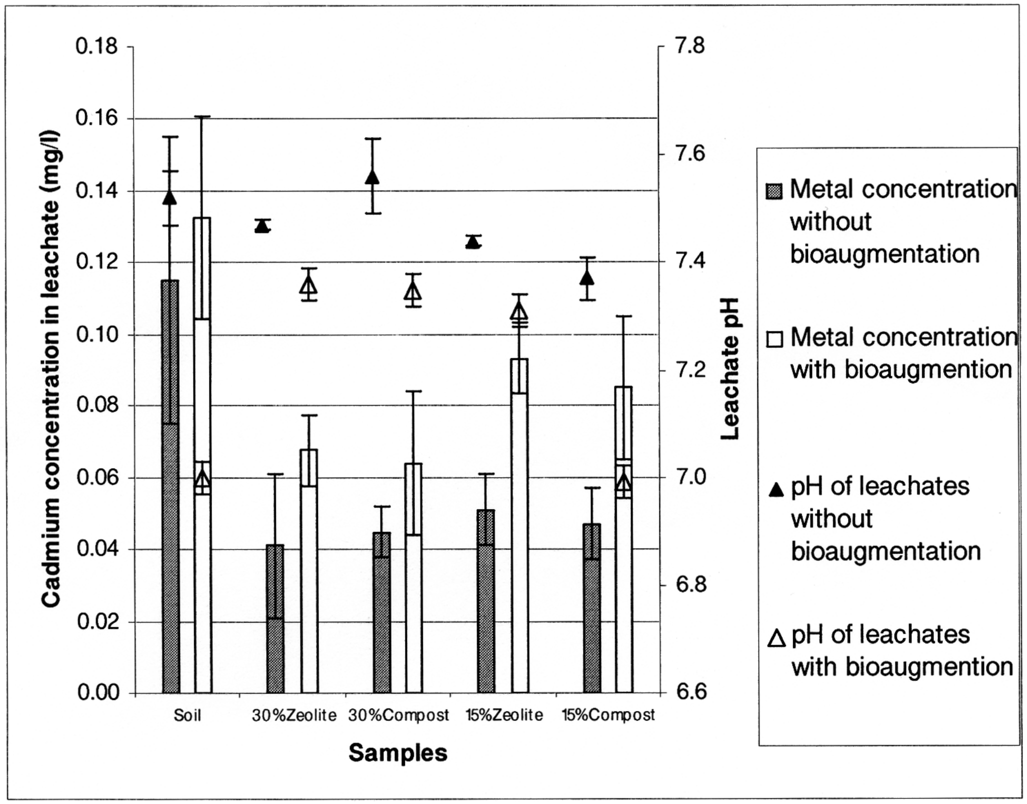

Figure 1. Leachate pH and cadmium concentration in batch leaching test.

bioaugmented mixes, the leachate pH decreased by up to 6% in the bioaugmented soil which was not stabilized. In the stabilized soil samples, which were also bioaugmented, the pH decreased by up to 6.8%. pH values ranged from 7.36 in the sample treated with 30% zeolite to 6.99 in the sample treated with 15% compost.

The pH of non-bioaugmented treatments was higher than that of corresponding bioaugmented treatments by 0.2 units to 0.5 units. Also, the leachate pH of the samples treated with 30% binder being higher than that of the corresponding samples treated with 15% of the same binder by 0.1 units to 0.3 units. On the other hand, there was no consistent difference between leachate pH of samples treated with zeolites and those treated with composts.

Leachability of Cadmium

The concentrations of cadmium in the leachates in Figure 1 show the following: in samples with no bioaugmentation treatment, the untreated soil had a leachate concentration of about 0.115 mg/l, while in the case of treated with stabilization soils cadmium concentration in leachates ranged between 0.04 mg/l to 0.05 mg/l. In the samples treated with bioaugmentation, the leachate concentration was 0.13 mg/l in the bioaugmented soil and between 0.06 and 0.09 mg/l in the soils treated with both stabilization and bioaugmentation. Both sets of results show that the presence of the stabilizing additive decreased the cadmium leachate concentration. The soil itself also reduced the leached concentrations compared to the maximum quantity of cadmium present, which in the worst case scenario would have been 0.87 mg/l, thus the soil reduced this concentration approximately 7.5 times. These results show that cadmium concentration in test leachates were consistently higher in the bioaugmented samples than in the non-bioaugmented samples by at least 0.02 mg/l. Comparing the effects due to difference in the quantity of stabilizing additive added, the samples treated with 15% of the different binders had higher concentrations of cadmium in the test leachates compared to those treated with 30% binder by at least 0.02 mg/l to 0.03 mg/l. However, there was no consistent difference in cadmium concentration in the test leachates due to differences in type of binder.

Flow-Through Test Leachate pH and Cadmium Leachability

The cadmium leachability and leachate pH results obtained from the flow-through column leachate test are shown in Figure 2.

Leachate pH

The leachate pH of the soil with no treatment was 7.60 and in soils treated only with stabilization the leachate pH values varied between 7.74 and 7.90. On the other hand, in the bioaugmented mixes, the leachate pH was 7.50 for the

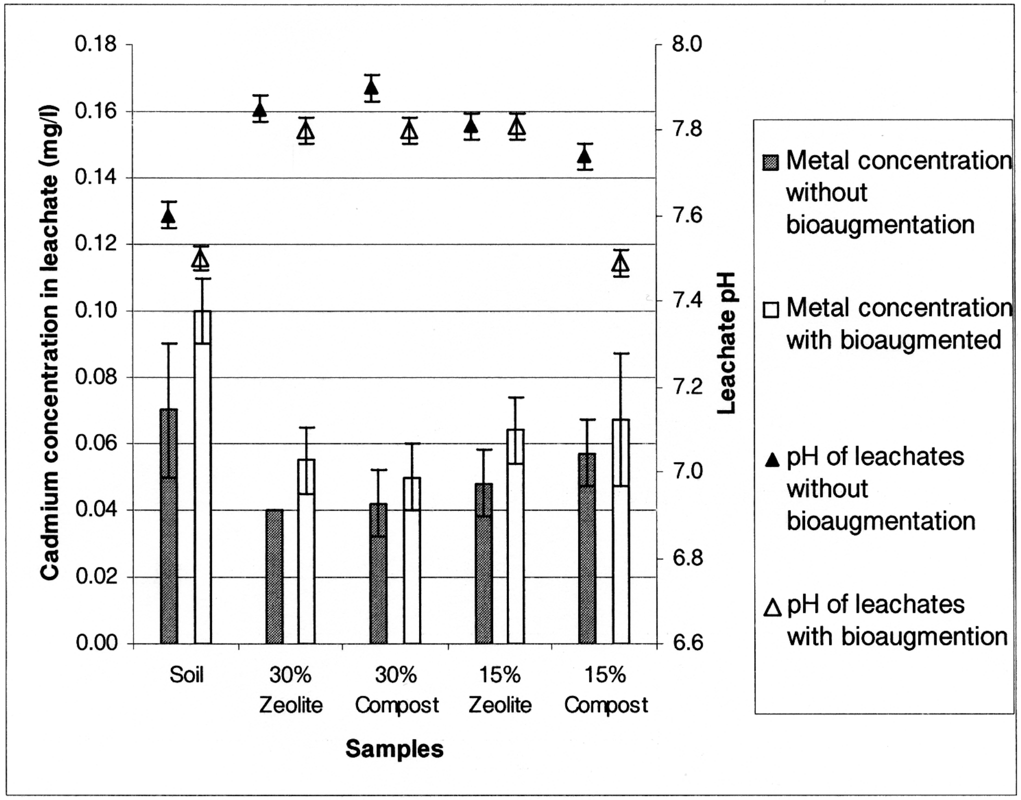

Figure 2. Leachate pH and cadmium concentration in in the flow through column leaching test.

bioaugmented soil and ranged between 7.49 and 7.81 in the bioaugmented and stabilized soil mixes. In both the non-bioaugmented and bioaugmented cases the presence of the binders increased the leachate pH. These results show that the leachate pH value was consistently higher in the non-bioaugmented samples than in corresponding bioaugmented samples by up to 0.2 units. However, there was no consistent difference between the leachate pH of samples treated with 30% of binders when compared to their counterparts treated with 15% binder. Also, comparison between leachate pH of samples treated with the zeolite and compost showed consistent difference due to differences in type of binder.

Leachability of Cadmium

The concentration of cadmium in leachates from the soil with no bioaugmentation or stabilization treatment was 0.07 mg/l. In the case of the soil samples that were treated only with soil stabilization, the leachate concentration ranged between 0.04 and 0.06 mg/L. In the bioaugmented samples, the leached cadmium concentration was 0.10 mg/l in the soil sample treated with only bioaugmentation while in the samples that had been treated with stabilization the leachate concentration was between 0.05 and 0.07 mg/l. The results show that the cadmium leachate concentrations decreased in the presence of the stabilizing additive while the bioaugmentation treatment increased the concentrations. The cadmium concentration in the leachates from the bioaugmented samples was higher than those from the non-bioaugmented samples by up to 0.01 mg/l to 0.03 mg/l. Comparing groups based on quantity of binder used, there was a consistent difference in cadmium concentration in leachates collected from samples treated with 15% and those treated with 30% of the respective binders. Cadmium concentration was higher in leachates from samples treated with 15% of the binder. On the other hand, there was consistent difference in the concentration of cadmium in test leachates due to differences in the type of binder used.

Comparison between the Results of the Batch Leaching and Flow-Through Leaching Test Procedures

The results from the batch leaching test in Figure 1, and flow-through leaching test in Figure 2, show that the trends in the leachate pH and cadmium leachate concentrations were similar in the two different test procedures. However, the leachate pH values were higher and the leachate concentrations lower in the flow-through leaching test compared to values obtained in the batch leaching test. The cadmium concentrations reduced to 75% of their corresponding batch leaching test results. This shows that the batch leaching test conditions are more severe and hence more conservative than the flow-through leaching test conditions.

Comparison with the EU Waste Acceptance Criteria

The cadmium leachate concentrations resulting from the batch leaching test in Figure 1 can be compared to the waste acceptance criteria in the EU Landfill Directives (European Union, 1999). This is relevant where stabilization is applied alone or combined with bioaugmentation as a pretreatment prior to landfilling. The EU Landfill Directive now requires hazardous waste sent to landfill to be pre-treated in order to reduce its hazardous nature, facilitate its handling or enhance its recovery.

The EU waste acceptance criteria for cadmium are 1-5 mg/kg for hazardous waste going to a hazardous waste landfill, 0.1-1.0 mg/kg for hazardous waste going to a non-hazardous waste landfill, and 0.04 mg/kg for inert waste going to an inert waste landfill. These values are based on the results of the same batch leaching test reported in this article. The cadmium leachate concentrations in the untreated soil of 0.115 mg/l are equivalent to 1.15 mg/kg (by multiplying by 10) which classify the contaminated soil as a hazardous waste to be disposed of in a hazardous waste landfill. However, both treatments of stabilization and combined stabilization and bioaugmentation reduced the hazardous nature of the soil so that it can be accepted in a non-hazardous waste landfill.

If a comparison is to be carried out based on the quantity of the original soil present and not applying for the dilution caused by the additives, then the conversion factor for the treated soils with 15% and 30% additive will be a multiplication factor of 11.77 and 14.29 respectively. The results after converting the results in Figure 1 are shown in Figure 3.

The results in Figure 3 show that cadmium leachability in samples treated with soil stabilization was within the range 0.1 to 1 mg/kg while some of the samples treated with bioaugmentation had cadmium leachability exceeding the 1 mg/kg limit. These results allow a direct comparison of metal leachability from the treated and untreated soil based on a unit mass of soil. From the results shown in Figure 3, it is clear that the additive treatments do have a reductive effect on the leachability of the metal, the reduction was not just due to a diluting effect by the addition of the additives.

Microbial Population Dynamics

Experiments were carried out to measure the recoverable population of hydrocarbon bacteria in the bioaugmented mixes within a 28-day test interval. This gives an indication of the extent of survival of the bacteria used in different soil treatment conditions. In addition to the mixes already prepared, a soil contaminated solely with liquid paraffin was also tested.

Microbial enumeration tests on the microbial consortium used in the bioaugmentation process showed that it contained about 4.8×10^8 CFU of hydrocarbon degrading bacteria per gram of cereal. As mentioned earlier, preliminary hydrocarbon degrading microbial enumeration tests showed that 23 g of the

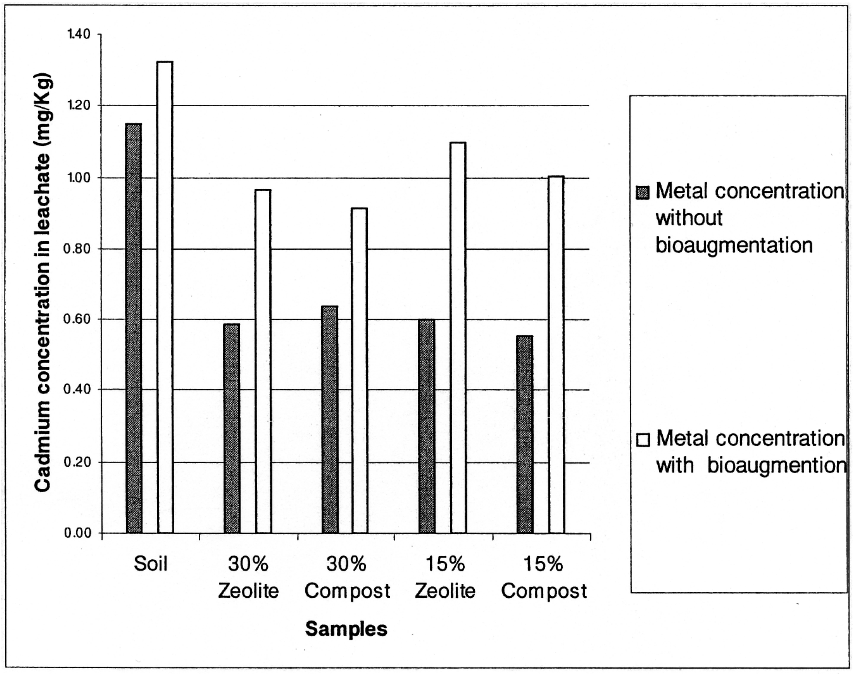

Figure 3. Cadmium concentrations in batch leachate samples in mg/kg not allowing for any effects due to dilution by additives.

microbial consortium would be assumed to give about 200 × 10^5 CFU of added microorganisms per gram of soil. Results from the enumeration test are shown in Figure 4.

The results show that the bioaugmentation treatment employed increased the hydrocarbon degrading population in the contaminated soil samples. Microbial population was higher in the bioaugmented samples than in unbioaugmented soil. In the bioaugmented samples the recoverable bacterial population was lower than the bacterial population used in the bioaugmentation. This could be attributed to a number of factors including the inability of some microbial species in the inoculum to survive under the test conditions or inefficiency of the enumeration technique. It is known that the plate count technique, though widely used in the enumeration of soil hydrocarbon degrading bacteria, significantly underestimates the true viable population density by as much as 90% (Margesin, Labbe, Schinner, & Greer, 2003).

At 28 days, recoverable bacterial population was highest in the soil contaminated only with paraffin in comparison to the soil contaminated with heavy metals and paraffin, reaching a maximum of about 190 × 10^5 CFU/g. In the heavy

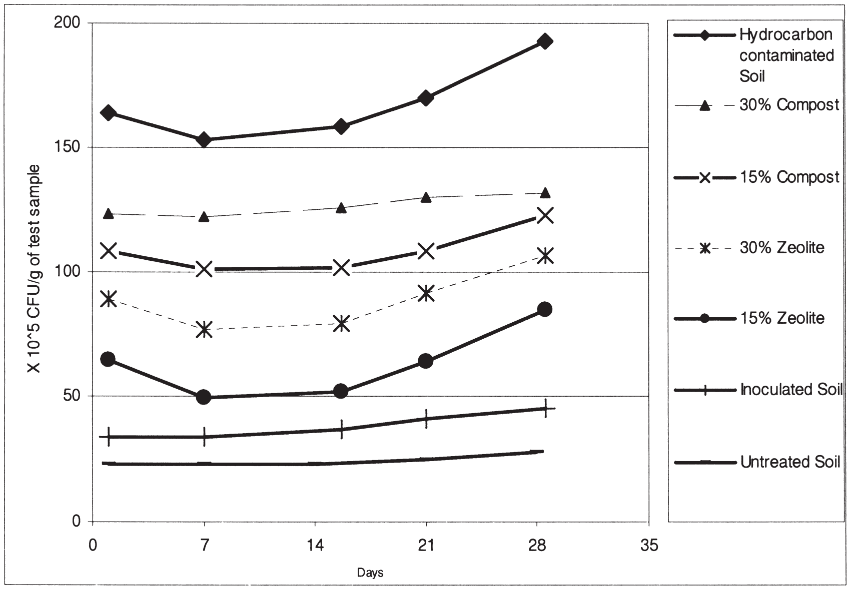

Figure 4. Microbial population changes in test materials treated with bioaugmentation.

metal and paraffin contaminated samples, bacterial population was higher in the compost containing mixes than in the zeolite containing mixes but was generally lower than results observed in the soil contaminated only with paraffin. The difference between the recoverable population of bacteria in the paraffin contaminated soil and the soil contaminated with paraffin and heavy metals clearly points to the detrimental effect of the heavy metals on bacterial inoculum.

The differences between the microbial populations in the different stabilized contaminated soil samples are attributable to the differences in the ability of the two different additives to create conducive conditions for the survival of the microorganisms. These conditions would include reduced bioavailability and toxicity of the heavy metal contaminants.

The reason for the higher bacterial population in the compost containing soil mix compared to the zeolite containing soil mix may indicate that either the compost treatments performed better in supporting the hydrocarbon degrading bacteria population under heavy metal contaminated conditions or that the compost treatment had more residual populations of hydrocarbon degrading microorganisms.

General Discussion

The results presented here show that the effect of both zeolite and green waste compost as stabilization treatments on cadmium leachability was similar and leachability reduced as the binder quantity increased. The cadmium leachability results agree with previous studies on the effect of composts and zeolites as soil amendments on the leachability of heavy metals from soil (Chen et al., 2000; Nissen, Lepp, & Edwards, 2000). Earlier investigation on similar behavior of copper leachability (Duru & Al-Tabbaa, 2005) showed generally that the leachability of copper is reduced more by zeolite compared to the coir compost. Hence it has been shown in that study and other studies that the behavior of different heavy metals differ and that the behavior of a metal in the presence of another could also vary from its behavior when alone. A full investigation of these effects is required in order to develop a broader picture of the behavior of heavy metals in the presence of additives. It has also been shown in other studies (Brown, Chaney, & Angle, 1997) that the application of mixed amendments is likely to lead to different results than the application of the additives in isolation.

From the analysis it is clear that the bioaugmentation treatment invariably increased the leachability of cadmium under the different test conditions. This can be explained as a result of microbial heterotrophic metabolism which could lead to soil acidification as a result of proton efflux to maintain charge balance, the production of respiratory CO_2 as well as other organic acids. This acidified condition would enhance the mobility of heavy metals. The idea of microbial driven acidification is further strengthened by observations which showed that the pH of leachates from bioaugmented samples were consistently lower than that of samples without bioaugmentation. In addition to system acidification, the microbial process could have increased metal mobility through the production of siderophores which would also enhance metal mobility by complexation (Burgstaller & Schinner, 1993; Diels et al., 1999). However, it should be noted that such increases in metal leachability due to an increased level of organic acids or other organic substances may not necessarily lead to an increase in soil toxicity as reported by Kiikkila, Pennanen, Perkiomaki, Derome, and Fritze (2002).

CONCLUSION

The results show that both coir compost and zeolite could be effective soil amendments to reduce heavy cadmium leachability. The results presented here show that the effect of both additives on cadmium leachability was similar and reduced as the binder quantity increased. Under the batch test conditions, treatment with 15% and 30% of the additives reduced cadmium leachate concentrations by 60% and 65% respectively compared to the untreated soil. This effect was also shown as not entirely due to dilution. In the column test condition, the same treatments caused a reduction in leachate concentration of up to 43% and

32% times respectively. Our study has also shown that the application of the different soil amendments encouraged a higher microbial population needed for the biodegradation of hydrocarbon contaminants, while the bioaugmentation treatment increased metal leachability. It is therefore possible through further research to elucidate workable combinations of soil stabilization and bioaugmentation treatments that would be effective, where it is required, to improve reduction in metal leachability, microbial survival, and consequently the degradation of soil hydrocarbon contaminants.

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Direct reprint requests to:

U. E. Duru
Department of Engineering
University of Cambridge
Cambridge CB2 3HU, UK
e-mail: ue.duru@gmail.com