Bioremediation of Polycyclic Aromatic Hydrocarbon contaminated Aqueous-Soil matrix: Effect of co-contamination

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ABSTRACT: This study investigates the effect of lead and chromium on the rate of bioremediation of polycyclic aromatic hydrocarbon (PAH) contaminated clay soil. Naphthalene was used as a target PAH. The soil was sterilized by heating at 120°C for one hour. 100g of the soil was contaminated with lead, chromium, nickel and mercury (40-200mg/l), 200mg/l of naphthalene and finally inoculated with microbes (Bacillus spp and Aspergilus niger). A control experiment containing these microbes, naphthalene but without heavy metals served was also setup. Residual naphthalene concentration was taken and analysed every three (3) days. The result of the study showed that lead at 40mg/l, 80mg/l, 120mg/l, 160mg/l and 200mg/l had the following percentage degradation 84.45%, 75.68%, 70.23%, 60.49%, and 52.95% respectively. Chromium at the same concentrations had 83.89%, 74.95%, 69.99%, 59.95% and 52.89%, nickel had 82.49%, 71.89%, 62.86%, 49.89%, 42.34% while mercury had, 73.99%, 71.89%, 48.98%, 24.22% and 10.17% at the end of the 42 days contact period. The percent degradation for the control sample was found to be 85%. The observed percentages are an indicator that the rate of naphthalene degradation was faster in the control experiment and the results showed that heavy metals inhibited biodegradation of naphthalene by varying degrees. At concentrations of 40mg/l-200mg/l of the different heavy metals, there was a steady reduction in the biodegradation rate, which suggests an increase in the interference with the metabolic activity of the microbes. This can be attributed to the poor colonization of the microbes at these concentrations. @JASEM

Keywords: Relative toxicity, Mineralization, Availability, Co-contamination, Activity series, Complexation.

Biodegradation of multiple contaminants in the environment is a complex process that has been a source of major concern to environmental biologists and scientists. Micro-organisms such as fungi and bacteria are the key agents of bioremediation as they have been very useful in effectively degrading a wide range of contaminants in the ecosystem. However, factors such as the characteristics, content and concentration of PAHs present, the physical, chemical and environmental conditions and the composition of the microbial population dictates the overall microbial degradation process (Rahman et al., 2002; Tam et al., 2002; Owabor and Agarry, 2009a; Bogan et al., 2003; Oleszczuk and Baran, 2003; Owabor and Inheren, 2002; Owabor and Osarumwense, 2008; Obahiagbon and Owabor, 2008; Owabor, et al, 2010). Cleaning areas of oil contamination is of interest because of the resultant threat of such contamination to the natural terrestrial ecosystem and the natural aquatic environment. Concerns have grown even more as researchers have discovered that microbial processes can now be used to effect clean up of radioactive and metallic contaminants.

However, recently, the concern over the persistence, disposition and presence of co-contamination of metals and polycyclic aromatic hydrocarbons in the environment (air, soil and water system) has increased, since these chemicals have been shown to be carcinogenic to humans and livestock. Though some heavy metals (Cu, Zn, Cr, Ni and Fe) are essential for the growth of microorganisms in trace amounts but they have however, been shown to be toxic at high concentrations. Their addition in soil has been known to inhibit soil respiration, nitrogen mineralization and nitrification (Lao et al, 2005; Sobolev and Begonia, 2008; Nwuche and Ugoji, 2008). Heavy metals have also been implicated in the reduction of degradation of vegetable materials and can potentially limit the biodegradation of organic contaminants in the environment (Sokhn et al, 2001; Rii et al, 2002; Atagana, 2010).

Field studies of metal contaminated soils have demonstrated that elevated metal concentrations can result in decreased microbial community size and thus their activity. This occurs either by complete inhibition of various metabolic activities like protein denaturation, inhibition of cell division, cell membrane disruption or the organisms develop resistance or tolerance to the elevated metals (Chaalal et al, 2005; Zukauskaitie et al, 2008; Alisi et al, 2009). The resultant effect of co-contamination is increased time span of remediation and increase in cost implication associated with carrying out effective remediation of contaminant.

Metal pollutants are mostly produced through industrial processes such as mining, metal smelting, refining and electroplating. A key factor to the remediation of metals is that they are non
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biodegradable, but can be transformed through sorption, methylation and complexation and changes in valence state. These transformation often times affect the mobility and bioavailability of metals.

The aim of this work is to unravel the extent of toxicity of heavy metals on soil microbial activity with a view to assessing the feasibility of remediating soil spiked with PAHs and co contaminated with heavy metals.

MATERIALS AND METHODS

Materials: Clay soil from Ikpoba River Bank, Benin-city, Edo state, was obtained through the surface sampling methods at depths between 0-30cm using a stainless steel trowel and gloved hands. The soil was kept in a sealed black polythene bag and taken to the laboratory for analysis.

The soil from site was analyzed to identify the microbes indigenous to the soil, ascertain the quantity of heavy metals in the soil samples and also to ascertain the existence of PAH in the soil sample. The soil samples were subjected to the pre-treatment outlined below:

1. The crushing of large particles or lumps of the soil into smaller sized particles

2. Screening by means of sieves with different meshes to get fine soil particles of the appropriate size range of (0.1-2mm). Pollutants are more attached to soil particles of small diameter, during spiking (Sun et al, 2003; Owabor and Ogunbor, 2006; Torres et al, 2007).

3. Sterilization was carried out by autoclaving to 120°C for approximately one hour. This was done to eliminate any pre existing microbes in the soil. Naphthalene solution was prepared by dissolving naphthalene crystals in ethanol to obtain a concentration of 200mg/l of naphthalene solution. Simulated solution of the chloride or nitrate salts of lead, chromium, nickel and mercury were added at varying concentrations of 40-200mg/l to effect heavy metal contamination in the different soil samples.

Methods: Deionized water was added to 100g of the pre-treated soil sample at 45% of the full soil water holding capacity in a 250ml measuring cylinder and this level was maintained throughout the duration of the experiment to favour the microbial environment (Guoqing and Yutong, 2006; Owabor and Irheren, 2006). Pseudomonas spp. and aspergillus spp. were introduced into soil sample to act as agents of degradation.

The aqueous-soil mixture was inoculated with 200mg/l of naphthalene and varying initial concentrations (i.e. 40-200mg/l) of the different heavy metal salts of lead, nickel, chromium and mercury and stirred vigorously before the mixtures were transferred to the mechanical shaker for effective agitation aimed at creating a homogenous solution.

A control experiment consisting of 200mg/l of naphthalene and microbes in soil was set up to monitor the biodegradation rate and the response of micro-organisms in the absence of the heavy metals. This was used to compare the results from the experimental samples.

The experiments were conducted at room temperature of about 28°C. Source of oxygen was from air to ensure adequate aeration of the soil sample and for the survival of the aerobic microbes. Sampling for quantitative analysis was carried out every three days i.e. 72 hourly.

Determination of residual naphthalene: The residual amount of naphthalene left after 3days was measured by taking 5ml of the slurry from each reactor using sterilized syringes and mixed with ethanol contained in a 100ml centrifuge tube. This was properly shaken for as long as it was necessary to obtain a proper supernatant and also to ensure accuracy in the readings during the measuring of the residual naphthalene, since the slurry will normally contain the PAH and some soil particles that would interfere with readings to be obtained from the T70 UV/VIS spectrometer.

Analysis for residual PAH for both test and control experiments carried out using T70 UV/VIS spectrophotometer at a wavelength of 226.5nm.

RESULTS AND DISCUSSION

The physicochemical characterization of the clay soil used in this study showed an initial pH of 4.4, and the following heavy metal contamination; 0.115mg/kg lead, 0.85mg/kg chromium, 0.03mg/kg nickel and
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0.05mg/kg mercury. Microbial analysis carried out on the soil showed that it contained micrococcus spp, bacillus spp, enterobacter aerogenes, and aspergillus niger with heterotrophic count (cfu/g) $1.5 \times 10^4$, coliform count (cfu/g) $10^3$ and fungal count (cfu/g) $10^2$. The pre-treatment of the soil provided a blank platform for the experimental assay. The effects of the presence of the heavy metals used in this study are presented in Figures 1-4.

Naphthalene mineralization by a mixed culture of bacillus and aspergillus niger indigenous to the soil are shown in Figures 1-4. The microbes exhibited similar profiles which were characterized by a steady decline of the amount of solute removed as concentration of heavy metals increased. The result from the control experiment showed that 85% of naphthalene degraded, while for the heavy metal amended experiment, 83.89%, 74.95%, 69.99% and 52.89% degradation was observed at 40-200mg/l of chromium respectively. About 85%, 75.68% and 70.23% were removed from treatments amended with same concentrations of lead, nickel had 82.49%, 71.89%, 62.86%, while mercury had 73.99%, 71.89% and 48.98% respectively as shown in Figures 2-4.
This is in agreement with earlier literature reports which showed slightly inhibited enzyme production at lower concentrations of heavy metals and subsequent reduction in hydrocarbon content (D’Annibale et al. 2005, Baldrion, 2008). The phenomenon of surface adsorption/desorption effects of naphthalene on soil organic carbon and interstitial voids within the micro and sub-micro pores of the soil particle may also be implicated in the observed results. This is consistent with reports from previous investigations which revealed that less than the total amount of aromatics adsorbed onto soil particles are actually desorbed and released (Nkedi-Kizza et al, 2006, Owabor and Agarry, 2009b, Owabor et al, 2010, Owabor and Osarumwense, 2011). This therefore, limits their bioavailability for microbial degradation.

Generally, there was no significant difference in the treatments containing 40, 80 and 120mg/l of chromium, lead, and nickel (except for 120mg/l) treatments. However, mercury showed high toxicity beginning from 120mg/l.

The inhibitory effects of chromium, lead and nickel were observed at concentrations of between 160 and 200mg/l of treatments as reflected in their final percentage degradation. This could be attributed to the inhibition of soil respiration, nitrogen mineralization and nitrification as reported by (Baldrin et al, 2000, Sokhn et al. 2001, Riis et al. 2002, Atagana, 2009, Atagana, 2010). The least removal was achieved at 200mg/l which showed that approximately half the initial concentrations of naphthalene, 52.9% (lead), 52.89% (chromium) and 42.34% (nickel) were still left undegraded in the amended samples. Microbial activity was significantly impeded at 200mg/l.

The effects of higher concentrations (160-200mg/l) of the heavy metals used in this study as reflected in the 72hourly measurements of the residual concentrations of naphthalene, showed that removal was relatively slower in these treatments when compared with lower concentrations (40, 80 and 120mg/l).

On the one hand, the slow rate of removal of naphthalene in the soil amended with 160 and 200mg/l of chromium, lead and nickel metal suggest that the metals may have become toxic to the microbes at these concentrations, which may have resulted from the inhibition of the enzymatic activities. It is also not unlikely that enzymes may have been denatured at the onset by high concentration of the metals leading invariably to retardation in the growth of microbes and consequently reducing its colonization of the soil system. On the other hand the microbes could have also been preoccupied with finding ways of adapting to the heavy metals environment thereby leaving the naphthalene in the beginning of the treatment.

In addition, the findings associated with mercury in this study i.e its significantly high toxicity at concentrations from 120mg/l -200mg/l (48.98%-10.17%) may also be linked to the fact that mercury by virtue of its affinity for thiol groups in protein acts majorly as an inducer of oxidative stress. The underlying effect is the inactivation of enzymes and ultimately the death of the microbes. Again, this argument is consistent with literature reports of Baldrion, 2008. Thus at 40-120mg/l, the microbes responsible for the degradation process, showed reasonable tolerance for the metals while the influence of these metals on the extent of degradation as concentrations increased was severe impairment of enzymatic activities.

Critical analysis of the variation observed in the degree of impairment of naphthalene degradation exhibited amongst the test metals can be adduced to their relative electro positivity and the presence of the 3d electrons available for bonding which results from the increased ionization energy. Ionization energy is the energy needed to remove one electron from each atom in a mole of gaseous ions with a positive charge. This predisposes the bonding in the transition elements (Cr, Ni and Hg) to be very stable. The densities of the transition metals are also implicated in this study as a factor which has a direct influence on their relative toxicity. A close examination across the series shows that the relative atomic masses increase progressively while atomic sizes remain fairly constant. Using the observed densities of Cr; 7.1g/cm³, Ni; 8.9g/cm³ and Hg; 13.534g/cm³ (Perry and Green, 2004 ), it can be seen that with increase in the atomic numbers and density of the transition elements, there is increased toxicity of the metals as evident in the results of the percentage degradation of naphthalene shown in Figures 2-4.
In contrast, lead (Pb) which belongs to group 4 elements and period 6 does not possess the 3d electrons, and this makes the attraction of the s-electrons and nucleus of its atom weak. The weakness of the bond between the nucleus and the outer electrons is responsible for the susceptibility of lead to complexation with other interfering chemicals such as ethylenediaminetetraacetic acid (Greenwood, and Earnshaw, 1997). This interaction ultimately results to its reduced toxicity to microbes.

**Conclusions:** From the results obtained, it can be seen that bacteria and fungi were capable of degrading the naphthalene in soil in the presence of the different concentration of heavy metals in soil. There was no significant difference between the effects of lead, chromium and nickel on the degradation of naphthalene. This could be attested to their similarity in functions as macro-nutrients to the microbes at reasonable concentrations that ensure microbial survival. It could also be seen from the study that the extent of inhibition was proportional to the concentration of heavy metals present in soil matrix. The mineralization of naphthalene have been found to be affected rather sharply at concentrations ranging from 160mg/l, as significantly high concentrations of naphthalene was left undegraded in the soil contaminated with the target heavy metals.

**REFERENCES**


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