

Interaction of Selected Minerals with Organic Pollutants: Effects of Phyto-Assisted Bioremediation Using *Vigna Subterranea*

Nwaichi EO* and Onyeike EN

Department of Biochemistry, Faculty of Science, University of Port Harcourt, P. M. B. 5323, Rivers State, Nigeria

Abstract

Soils contaminated with hydrocarbons are a serious problem in Nigeria and 70% of such soils are distributed in the Niger Delta. This pot experiment was conducted to determine the interactions of selected soil minerals with organic contaminants from oil spill site. *V. subterranea*, which potential for stimulating the biodegradation processes of petroleum hydrocarbons was established was employed under the application of two fertilizer types. Iron concentrations were greater than maximum permissible levels, MPL for plants grown in contaminated soil and showed significant variation ($p \geq 0.05$) compared to control. Poor zinc concentrations in oil-stressed regimes decreases ear size, slowed growth and maturity of specie. Both iron and manganese became less available at $pH > 7$ in the absence of organic matter and water (dehydrated due to oil-stress). Concentration of available P was sensitive to soil pH and was more available to Poultry manure-amended soil as pH correction was evident. Plant tissue analyses revealed significantly ($p \leq 0.05$) higher increases in accumulation, in comparison with those grown in unpolluted (Control) regimes. Also, soils depleted mineral nutrients were replenished ($> 30\%$) by test plant and best practice. These results indicated that soil minerals affect the dynamics and transformations of organic materials and metabolic processes even in a stressed soil type with modifications due to clean-up.

Keywords: Biodegradation; Soil mineral; Depletion; Microbes; Oil-stress

Introduction

The widespread contamination of our soils with organic pollutants has become a matter of public concern. Various anthropogenic substances, either organic or inorganic in nature, upon entering the soil, may not only adversely affect its productivity potential, but may also compromise the quality of the food chain and groundwater. Minerals, organic matter and microorganisms are intimately associated in soils and closely interact in environmental processes. These interactions are especially important in the soil rhizosphere and the sediment-water interface, where low-molecular weight biochemicals are abundant and microbial activity is intense [1]. One major function of a soil is its use as a habitat for soil organisms including maintenance of biodiversity, assemblage, and activity for both soil microflora and soil fauna. Soil microorganisms themselves are involved in major soil processes, such as humification, recycling and mineralization of organic residues, leading to the plant availability of nutrients [2]. Zinc helps to make acetic acid in the root to prevent rotting; it is used to control blight and allows dead twigs on trees to shed off. Perceived zinc deficiency is often only symptomatic. Iron is second only to aluminum in the list of abundant metals. It makes up about 5% of the earth's crust, so it is rarely absent from soils, although it may not be present in an available form. Iron is necessary for the maintenance and synthesis of chlorophyll and RNA metabolism in the chloroplasts [3]. Manganese is synergistic with iron; they work together in biology in ways that are not well understood, but we do know that they need each other. Perhaps this is a clue to the biological relationships of Mn and Fe, in that the manganese may slow the oxidation rate of iron in living things [4]. In wet, acid soils below pH 5 or so that naturally contain high amounts of manganese, soluble manganese can reach levels that are toxic to plant roots. The objective of this study is to follow the distribution and degradation of selected organic pollutants in oil-stressed soil and to characterize the selected soil minerals in such soil. This exercise not only increases our understanding of the interactions between soil components, but also assist us in establishing and applying methods for remediation and the

cleanup of contaminated soils. The organic pollutants of this study are represented by potentially biodegradable aromatic compounds.

Materials and Methods

17 cores of surface soil (sandy loam) samples (0-20cm depth) from agricultural grassland located at Choba, Eastwest Rivers State of Nigeria with no history of pollution, were collected in a random sampling design. The air-dried, gently crushed soils were sieved (2mm screen) and characterized. The treatments included crude oil contaminated (10% v/w), nutrient un-amended CON; crude oil contaminated, Urea amended UREA and crude oil contaminated, poultry manure amended PM soil regimes. Control (no spiking, no amendments) and Unplanted replicates were also provided for further comparison. The spiked soil was characterized and labeled (PRE-P). 0.8g of amendments were added according to the method of Akobundu [5] and allowed for two weeks while watering. *V. subterranea* plant seeds were sourced from Enugu state of Nigeria and were subjected to formazan test, scarification and imbibition for viability and germination enhancement. Average of 10 seeds/pot were planted and thinned to three plants/pot after 10 days of germination/seedling emergence. Measurement of growth indices commenced 2 weeks after germination at 2-weeks intervals of growth period. At the end of the study, harvested plants were separated into shoots and roots. The plants were thoroughly washed and roots

*Corresponding author: Nwaichi EO, Department of Biochemistry, Faculty of Science, University of Port Harcourt. P. M. B. 5323, Rivers State, Nigeria, Tel: 234 (0)8056031989; E-mail: nodullm@yahoo.com

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were immersed in a solution of 20mmol L⁻¹ Na₂ – EDTA for 30 min to remove extracellular metals before washing. The washed root and shoot samples were dried at 80°C for 48h, their dry weights recorded, ground and digested with aqua-regia (HCl and HNO₃ in the ratio of 3:1).total organic matter was estimated by weight loss method while metals were determined by atomic absorption spectrophotometry (Varian SpectrAA 220 FS, Varian, Palo Alto, CA, USA). ANOVA was performed on each measured variable, means and standard errors (SE) were calculated. The least significant difference (p < 0.05) was used for multiple comparisons among treatment means. Descriptive statistics were calculated using the SPSS v. 13.0 software package.

Results

The contamination of soil with crude oil resulted in a decrease in pH (from 7.04 to 3.84) (Figure 1).

Usually, the degree of acidity or alkalinity is considered a master variable that affects nearly all soil properties: chemical, physical, and biological. If the soil pH is too high or low, the nutrients are either locked onto the soil particles or washed out. Plant height increased with time in control samples and that of the Poultry manure amended, PM regimes but no significant height correlation was observed for the Urea amended, UREA regime and contaminated and unamended, CON treatments at the 95% level of probability. The inorganic nutrients mobilized the contaminants hence the poorer growth at some concentrations. Poultry manure amendments, PM ameliorated the expressed phytotoxicity in preference to Urea inorganic fertilizers employed and also improved the root biomass. Also, the results in Table 1, suggest that increasing root length resulted in increased contaminant degradation.

Decreased leaf formation with increased concentration of crude oil was also observed for all treatments. Zinc uptake by *V. subterranea* declines as pH increases (Figure 1 and Figure 3). Both iron and manganese become less available at moderate pH and in the presence of low organic matter and water (Figure 2 and Figure 4).

In a calcareous soil, most of the potentially available iron is tightly bound to organic matter. Contamination delayed tasseling and maturation time. The presence of the microbial community, which

TMT	Control	CON	Urea	Urea	PM
12WAG	48	24	5	5	15
6WAG	36	38	7	7	22

12WAG = Twelve weeks after germination, 6WAG = Six weeks after germination

Table 1: Observed root length, RL (cm) 12 WAG.

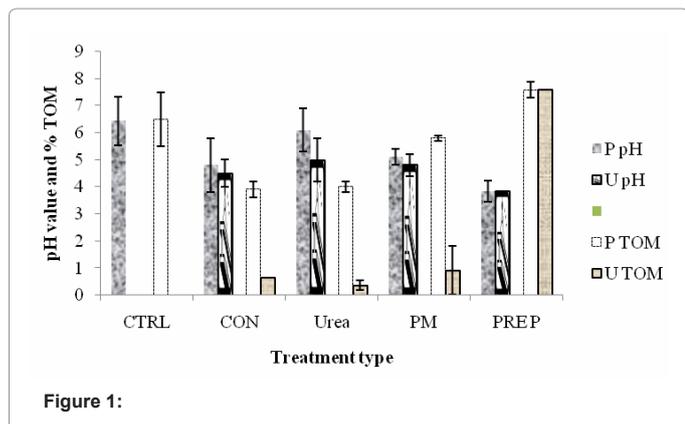


Figure 1:

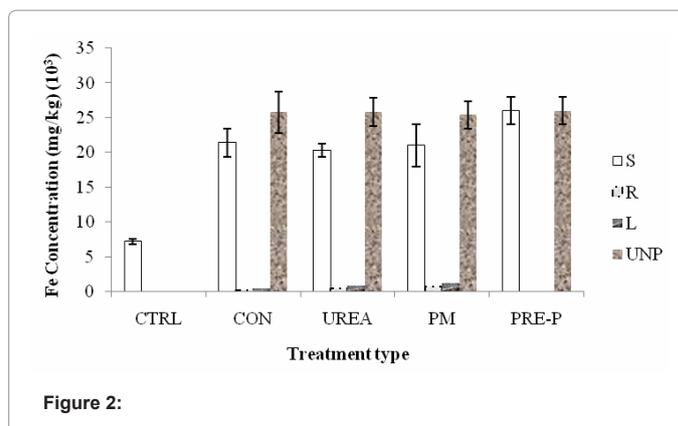


Figure 2:

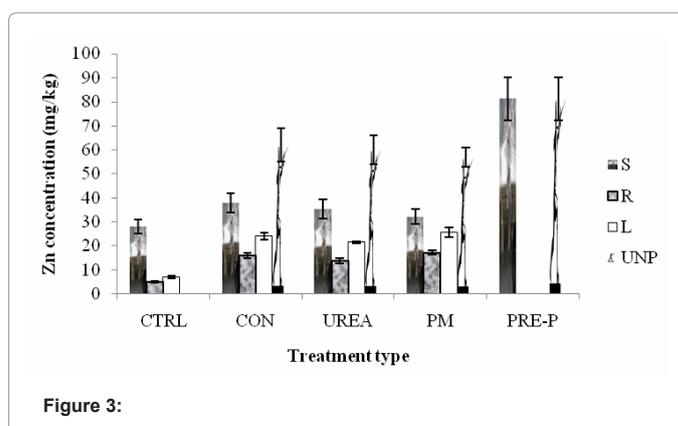


Figure 3:

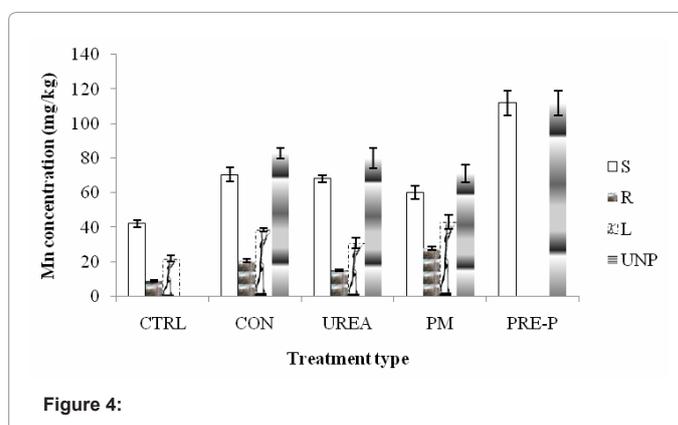


Figure 4:

are the primary factors that influence the fate of organic xenobiotics in soil, enhanced by the participating plant was visible in the increased degradation efficiencies in planted regimes (Figure 5). Some anthropogenic compounds may be used by microorganisms as a source of energy and nutrients for growth [6].

There was a significant decrease in the levels of extractable zinc from the high [7] observed levels of 80mg/kg (Figure 3) when the soils were amended with urea fertilizer.

The observed high concentration of iron in the soil is attributable to the geology of the Niger Delta area and Iron is in great abundance in tropical and subtropical aquifers and is also associated with crude oil deposits. In Bambara beans-assisted clean-up, tissue levels of Fe

showed that photosynthetic processes were effective. Poultry manure amendments gave higher phytoextraction of Fe (Figure 2). Reasonable levels of Fe were also seen in fruits samples (Data not shown).

Each of these important components of the soil system not only participates in the transformation of xenobiotics but also modifies the activity of the other components. Total organic matter, TOM depletion (Table 2) was evident in contaminated soil and was remedied with poultry manure amendment.

Values denote mean ± SEM (n = 3). Values in the same row with same superscript letters are not significantly different at the 5 % level.

There was varied mineral depletion with decreased TOM levels. BTEX and PAHs removal efficiencies were very significant at 95% confidence level for planted regimes (Figure 5) in comparison to the unplanted counterpart.

Over 50% degradation achievement was established for these priority [8] contaminants even without nutrient amendments. The leguminous property of *V. Subterranea* was no doubt very helpful.

Discussion

Most plants grow when the pH is between 5.5 and 6.5 and again, pH greatly affects the solubility of minerals; strongly acidic soils (say with mean pH of 3.0), usually have high concentrations of soluble aluminium and manganese, which are toxic to plants. Nitrogen fixation and decomposition are also hindered in such strongly acidic soils [9].

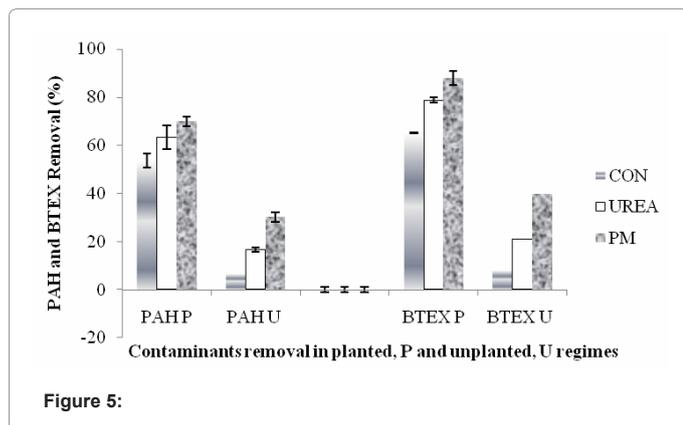
The application of poultry manure to contaminated soils significantly increased the soil pH, and promoted better growth and yields in *V. subterranea*. This is in line with the findings of Chen and Lee [10]. However, *V. subterranea* showed little response to applications of Urea fertilizer. As predicted, the spill led to loss of vegetation compared to control. With anaerobiosis imposed, the rate of glycolysis might have increased sharply to maintain energy supplies near to the aerobic levels. Some bacteria use the same compound as an energy

source and as a source of building blocks for biosynthesis. To do this, they must balance the rate of ATP production with the rate that they 'remove' precursors for biosynthesis. Under aerobic conditions, the molecules pass through glycolysis (glucose + O₂ → CO₂ + H₂O), then enter the citric acid cycle, where it is completely oxidized. The electrons removed during glycolysis and the citric acid cycle (as NADH) is passed through an electron transport chain to create a protonmotive force which generates ATP. However, under anaerobic conditions (Glucose → Lactate), the citric acid cycle cannot be utilized (because it generates too much NADH), and thus cell resorts to a fermentation. Much less ATP is produced from glucose during glycolysis, and the cell tries to compensate by 'eating' glucose faster. Note that the rate that precursors are removed for biosynthesis does not increase. This shift from slow aerobic to rapid anaerobic consumption of glucose was first noted by Pasteur. This shift also happens anytime man is unable to provide oxygen to its own mitochondria – they consume glucose faster in an attempt to produce ATP via the less efficient fermentation to lactate, and lactic acid accumulates in his muscles. This 'Pasteur effect' leads to rapid and inefficient exhaustion of available carbohydrates, a rapid build-up of toxic metabolites, and eventual death of both roots and shoots. Lack of adequate oxygen supply to the roots of the affected species may have initiated conversion of methionine to S-adenosylmethionine, which leads to high concentrations of ethylene (precursor is 1-aminocyclopropane-1-carboxylic acid (ACC) in the plant system. High ethylene concentrations in petioles are known to cause rapid cell expansion and epinasty (the condition in which an organ, as a leaf/other plant parts, turns/bends downward because of the more rapid/excessive growth of the upper layers/sides of the cells), followed by the eventual shedding of leaves and subsequent death of the plant [11]. Iron usually present in deltaic environments, was detected and observed to have a mean value of 18690 mg/kg on site and laboratory monitoring revealed several alterations of chemical and biophysical characteristics of the affected plants.

The availability of metals in waste depends on process type, and is affected by physical and chemical interactions. These interactions are affected by factors like pH, temperature, CO₂ concentration, the type and concentration of available ligands and chelating agents, as well as type and concentration of the metal ions. Concerns over metals relate to their toxicity, bio-accumulation and hazards to human and animal health [12]. The observed zinc-phosphorus antagonism occurs on calcareous soils and may be related to iron availability (Figure 2).

The observed high concentration of iron in the soil is attributable to the geology of the Niger Delta area and Iron is in great abundance in tropical and subtropical aquifers and is also associated with crude oil deposits. In Bambara beans-assisted clean-up, tissue levels of Fe showed that photosynthetic processes were effective. The transformation of xenobiotics in a multicomponent system, such as soil, is a result of the combined activity of microorganisms, extracellular enzymes, mineral colloids and humic materials [1]. Microorganisms are usually responsible for mineralizing most organic matter to carbon dioxide, water and inorganic components [1].

The amount of organic matter in soil depends on the input of organic material [13], its rate of decomposition, the rate at which existing soil organic matter is mineralized, soil texture, and climate. The amount of humus in soil cannot be measured directly but is estimated by measuring the % carbon (C) and multiplying this by 1.72 to give % soil organic matter or humus. Humus is important in maintaining soil structure, slightly increasing the soil's water holding capacity and holding a small store of N, P, S and trace elements in



TMT	CON	Urea	PM	PRE P
Planted	3.9 ^a ±0.01	4 ^{cd} ±0.04	5.8 ^{de} ±0.07	7.58 [±] 0.03
Unplanted	0.64 ^c ±0.2	0.36 ^e ±0.01	0.91 ^{de} ±0.2	7.58 [±] 0.03

Control = 6.5^a±0.06
 CON = contaminated and unamended regime
 Urea = contaminated and Urea - amended regime
 PM = contaminated and poultry - amended regime
 PREP = contaminated regime prior to planting

Table 2: % levels of TOM for soil samples on which *V. subterranea* was grown.

organic forms. Variability in the effects of dissolved organic matter (Table 2) components on mineral dissolution observed suggests that metal mobility in soils will depend on the source and composition of soil organic matter. Also, this is in agreement with the findings of Bremner [14] that soil organic matter is intimately associated with metallic cations. Clean – up [8] of such soil therefore will depend on the understanding of these interactions.

Conclusion

This study demonstrated, adsorption and other binding interactions that occur on both mineral and humic surfaces reduce the bioavailability of xenobiotics. Also, overall, phyto-assisted remediation using choice plants enhanced xenobiotic biodegradation in soil environments more effectively than did indigenous microorganisms, and that biostimulation especially with poultry manure reduced oil phytotoxicity in soil.

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