

The Potentials of Chicken Drops in the Remediation of Petroleum Contaminated Agricultural Soil

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Abstract

A study on the potentials of chicken drops in the remediation of petroleum contaminated agricultural soil was carried out. A total of 18 m² farmland was divided into three sites of 4 m² each with 2 m spaces in between them. Two out of the three sites were polluted with 40 dm³ of bonny light crude oil and remediated for 16 weeks. The results showed a total percentage degradation of 72.18 ± 0.56 and 59.21 ± 0.54 of aliphatic hydrocarbon, 37.92 ± 0.38 and 29.58 ± 0.36 of polycyclic aromatic hydrocarbon and 5.26 ± 0.22 and 0.50 ± 0.08 of heavy metals for bioaugmented and natural attenuated sites respectively. However, the highest percentage degradation for aliphatic hydrocarbon was 97.59 ± 0.95 for C₂₄ of the bioaugmented site and 92.46 ± 0.72 for C₂₆ of the natural attenuated site, while the highest degradation rate for polycyclic aromatic hydrocarbon was 100.00 ± 0.00 for Pyrene, Benzo(k)fluoranthene and Benzo(g,h,i)perylene of both the bioaugmented site and natural attenuated site. However, Chromium had the highest percentage degradation rate for the heavy metals with values of 11.95 ± 0.33 and 1.12 ± 0.25 for the bioaugmented and natural attenuated sites respectively.

Keywords: Hydrocarbon; Pollution; Toxicity; Degradation; Remediation

Introduction

Since the rise of agriculture and forestry in the 8th millennium B.C., there has also arisen by necessity a practical awareness of soils and their management. In the 18th and 19th centuries, the industrial revolution brought increased pressure on soil for the production of raw materials demanded by commerce, while the development of quantitative science offered new opportunities for improved soil management [1]. These initial inquiries expanded into the understanding of soil as complex, dynamic, biogeochemical system that is vital to the life cycle of terrestrial vegetation and soil-inhabiting organisms and by extension to the human race as well. The presence of toxic compounds in soil has increased dramatically due to the accelerated rate of extraction of minerals and fossil fuels and by highly technological industrial processes [1]. Petroleum like all fossil fuels primarily consists of complex mixture of hydrocarbons. Petroleum hydrocarbons are composed of various portions of alkanes (e.g. methane, ethane, etc), aromatics (e.g. benzene, toluene, ethylene and xylene, collectively known as BTEX) and polycyclic aromatics hydrocarbons (PAHs) (e.g. naphthalene, phenanthrene, anthracene and benzo(α)pyrene, etc). Also present in petroleum mixture are organometallo constituents such as Chromium, Cadmium and Nickel [2]. In high concentrations, these hydrocarbons molecules that make up petroleum are highly toxic to many organisms, including humans [3]. The dominance of petroleum products in the world economy creates the conditions for distributing large amounts of these toxicants into populated areas and ecosystem around the globe [4]. Industrial activities release substantial amount of crude oil and refined products into the environment, as a result of accidents such as storage tank leakage, oil spills during routine transportation and shipping operations or sabotage [5]. The contaminant load of soil and water are growing steadily each year in parallel to increasing industrialization and energy demand and therefore necessitate the need for remediation. Hydrocarbonoclastic microorganisms such as bacteria, fungi, yeasts and some algae play paramount roles in bioremediation [6]. These organisms have been isolated from heavily oil polluted deposits or in a variety of soils and water continuously exposed to hydrocarbons for several years [7]. The ability of certain

microorganisms to degrade petroleum seems to be an adaptive process that is controlled by environmental conditions [6]. The presence of petroleum may also affect the microbial community through selection of species. Despite decades of research, successful bioremediation of petroleum hydrocarbon contaminated soil remains a challenge [8]. However, Ijah and Antai [9], reported the ability of chicken drop microorganisms in petroleum hydrocarbon remediation and they identified species of *Micrococcus*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Aspergillus*, *Rhizopus* and *Penicillium*. *Pseudomonas aeruginosa* CDB-06 and *Penicillium* CDF-10 as potential crude oil degraders [9]. Ohiri et al. [10] also reported that the application of chicken drops in bioaugmentation of crude oil polluted site raised the pH of the top soil to a range 6.89 to 7.80 which favours the growth of soil microorganisms thereby enhancing bioremediation. The aim of this research is to assess the potentials of Chicken drops (poultry manure) in the remediation of petroleum contaminated agricultural soil.

Materials

Eighty litres of bonny light crude oil was obtained from Shell Petroleum Development Company (SPDC) flow station at Egbema, Imo State, Nigeria, while Chicken drops (40 kg) was purchased from Godvine Poultry Farm at Elioazu, Obio Akpor, Rivers State, Nigeria.

Study area

The study area was located along Eneka-Oyigbo new link road (longitude 70 10" E and latitude 40 40" N) in Obio Akpor Rivers State, Nigeria. The soil of this area belongs to the ultisols. Approximately the

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entire area consisted of deep uniform sand and clay sand, with slightly humus topsoil and a top soil pH of approximately 4.86 ± 0.12 . There was no record of oil spillage or pipeline vandalization in the study area.

Pollution and bioremediation of research site

Approximately 18 m² farmland was cleared and divided into three sites of 4 m² each with 2 m spaces in between them. These sites were polluted with 40 dm³ of bonny light crude oil and bioremediated for 16 weeks as follows: Site A (Control site) was an unpolluted 4 m² farmland, while site B (Bioaugmented site) was a 4 m² farmland polluted with 40 dm³ of bonny light crude oil and bioaugmented with 40 kg of chicken drops. However, site C (Natural attenuated site) was polluted with 40 dm³ of bonny light crude oil.

Collection of soil sample

Soil samples were collected by the method Bashour and Sayegh [11]. Where a sterilized soil auger was used to make a depth of 20 cm using a grid. The samples for biochemical analyses were collected with unused and sterilized plastic bags sealed with rubber bands. All samples were labelled with a permanent water resistant marker and were taken to the laboratory within 1 hour of collection for analysis. The samples were collected from both the control and the polluted sites as follows:

Topsoil (0-10 cm depth): Samples were collected from the three sites at week 0 and week 16 from date of pollution.

Subsoil (15-20 cm depth): Samples were collected from the three sites at week 0 and week 16 from date of pollution.

Determination of aliphatic and polycyclic aromatic hydrocarbons (PAH) in leaf samples

Extraction of soil samples for aliphatic and polycyclic aromatic hydrocarbons was done by modified method of the Association of Official Analytical Chemist [12]. Composite soil samples from the control and bioremediated sites were air dried and 1.0 g of sample was placed in a separation funnel. Ten millilitres of dichloromethane was added. The mixture was homogenized by stirring with a glass rod and was subsequently poured into a chromatography column packed with glass wool, silica-gel and 0.5 cm³ of anhydrous sodium sulphate. One millilitre of the eluent was transferred into a 10 mm (internal diameter) × 250 mm long chromatographic column packed with glass wool. Slurry of 2 g activated silica in 10 ml methylene chloride and 0.5 cm³ of sodium sulphate were made at the top of the column. The column was pre-eluted with 20 ml of concentrated dichloromethane for 2.0 min and 1.0 ml of the initial eluent was then added. A volume of 1ml dichloromethane was introduced at the receiving end of the column and the stop-cork was opened. The final eluent was collected into a 10 ml graduated cylinder. The extract was concentrated in a water-bath at a temperature of 40°C and 1.0 ml of dichloromethane was added to the concentrated extract (to dissolve it), then 1 µl portion of the dissolved extract was collected with a hypodermic string and injected through a rubber septum at the injection point of the gas chromatography manufactured by Hewlett and Packard (hp), model: HP 5890 Series II. After injection, separation occurred as the vapour constituent partition between the gaseous and liquid phase. Signals were obtained in the form of chromatogram as the vapour phase of the injected extract was being ionized for the fractionating components of aliphatic hydrocarbon or polycyclic aromatic hydrocarbon (PAH). Gas chromatography conditions for determination of aliphatic hydrocarbon were initial and final oven temperatures of 50°C and 310°C. Injection temperature was 275°C while detector temperature was 320°C. The gas chromatography

was calibrated with Accustandard aliphatic hydrocarbons at 100 ppm. Gas chromatography condition for the determination of polycyclic aromatic hydrocarbon was initial and final oven temperatures of 65°C and 310°C. Injection temperature was 275°C while detector temperature was 375°C. The gas chromatography was also calibrated with Accustandard polycyclic aromatic hydrocarbons at 2000 ppm.

Determination of Heavy metal contents of vegetable leaves

Heavy metal contents of soil samples were determined by the emission spectroscopy method [8].

Principle: Sample digests were burned in a carbon arc, causing each element to emit a unique wavelength of light. The density of light emitted by each element is directly proportional to the concentration of the element in the sample.

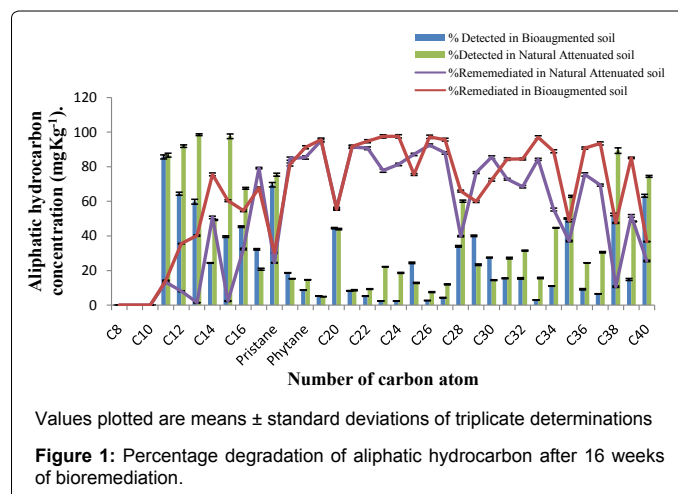
Apparatus: Arc-spark emission spectrography, Analytical weighing balance, Furnace, 10 ml Porcelain crucibles, 50 ml Burette, Drying oven, Wiley mill, volumetric flasks and Pipettes.

Reagents: Lithium carbonate (LiCO₃) use for the analysis was obtained from Sigma Aldrich, MO USA.

Procedure: A quantity of 1.0 g of dried soil samples were ground and placed in 10 ml porcelain crucibles. The crucibles were placed in a cool muffle furnace and the samples were ashed at a temperature of 500°C for 4 hours. The crucibles were removed and allowed to cool. Then 5.0 ml of lithium carbonate buffer was added and swirled gently to dissolve the ash. The digest was transferred to a Teflon boat and analysed on a direct reading arc-spark emission spectrography.

Results and Discussions

The results of this study showed great variation in the ability of chicken drops in the remediation of crude oil polluted soil. The increase in percentage remediation of both aliphatic and aromatic hydrocarbons observed in the bioaugmented site (Figure 1 and 2), may be attributed to the presence of aerobicheterotrophs, total fungi and species of crude oil degrading bacteria such as *Micrococcus*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Aspergillus*, *Rhizopus* and *Penicillium* in the chicken drop [9]. Microbial degradation of contaminants has been reported to be a factor of contaminant concentration, bioavailability and nutrient availability [13]. Moreover, chicken drops contains about 2.3% total nitrogen, 0.5% available phosphorus and 18.5% moisture content in addition to other nutrients [9]. This nutrient availability



Values plotted are means ± standard deviations of triplicate determinations
Figure 1: Percentage degradation of aliphatic hydrocarbon after 16 weeks of bioremediation.

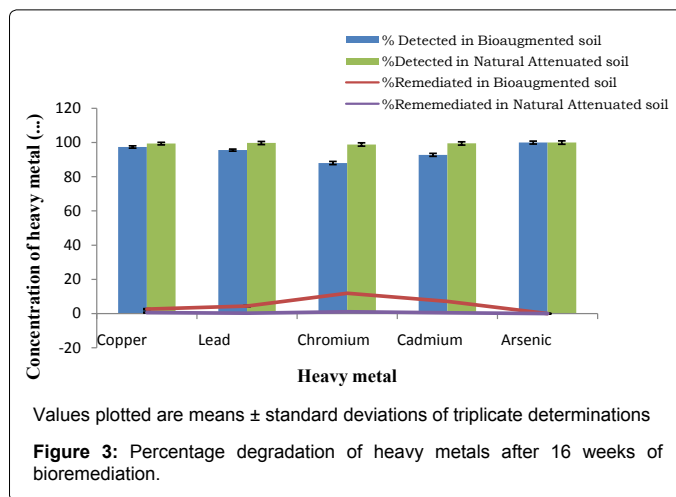
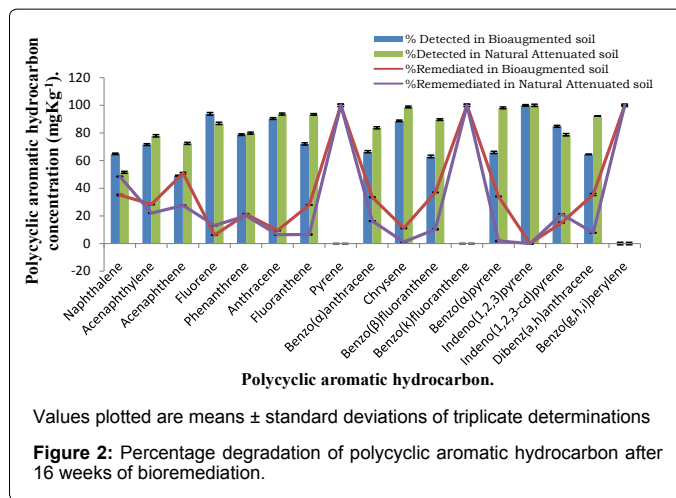
may have also resulted to increase in percentage remediation of both aliphatic and polycyclic aromatic hydrocarbons observed in the bioaugmented site (Figure 1 and 2).

Aliphatic hydrocarbons are preferably degraded under aerobic conditions than polycyclic aromatic hydrocarbons [13]. However, the reduced degradation rate of polycyclic aromatic hydrocarbons as compared to the aliphatic hydrocarbons (Figure 1 and 2) may be attributed to poor aerobic degradation of polycyclic aromatic hydrocarbons which is analogously effected by the degradation of mononuclear aromatics with the individual rings being degraded one after the other [13]. Immobilization of polycyclic aromatic hydrocarbons in the presence of other contaminants has been reported [13]. These hydrocarbons tend to form aggregates which are poorly supplied with oxygen, thereby reducing aerobic degradation [13]. This may also be responsible for the reduced degradation rate of polycyclic aromatic hydrocarbons as compared to the aliphatic hydrocarbons (Figure 1 and 2).

The reduced remediation rate of Indeno(1,2,3)pyrene, Chrysene, Fluorene and Anthracene (Figure 2) may be attributed to the low solubility of these polycyclic aromatic hydrocarbons which may result to a decline in bioavailability, thus the threshold concentration for degradation may not be reached. Sequel to this, higher condensed polycyclic aromatic hydrocarbons may scarcely serve as carbon and energy sources [13]. Moreover, the 100% degradation rate recorded for Pyrene, Benzo(k)fluoranthene and Benzo(g,h,i)perylene can be attributed to the presence of high catabolic microbial superbugs that enhances either their degradation or enzymatic cleavage of some rings, thereby generating other polycyclic aromatic hydrocarbons such as Naphthalene. This may be responsible for the increased detection rate of Naphthalene when compared to Acenaphthylene and Acenaphthene (Figure 2). However, the degradation of some low-molecular polycyclic aromatic hydrocarbons such as Naphthalene, Acenaphthylene and Acenaphthene can take place under anaerobic conditions, dominated by co-metabolic degradation reactions [13]. Thus the increase in percentage remediation of acenaphthylene and acenaphthene may be attributed to the addition of chicken drops (poultry manure) which may generate a co-metabolic degradation process, thereby increasing the rates of degradation of these low-molecular polycyclic aromatic hydrocarbons (Figure 2).

However, bacterial biosurfactant has been proven effective in enhancing the solubility and biodegradation of petroleum hydrocarbons including Polycyclic aromatic hydrocarbons [14,15]. Microbial production of biosurfactant has been linked to the utilization of available hydrophobic substrates by the producing microbes from their natural habitat, presumably by increasing the surface area of substrates and increasing their apparent solubility [16,17]. Therefore the presence of high microbial population in the chicken drops and their subsequent production of biosurfactant may be responsible for the percentage increase in polycyclic aromatic degradation of the bioaugmented site as compared to the natural attenuated site (Figure 2).

The low percentage degradation rate of heavy metals observed in this research (Figure 3) may be attributed to the adverse effect of toxic metals on potentially important biodegradation processes occurring in the environment [18]. Such metallic compounds appeared to be considerably more inhibitory to the biodegradation of an organic chemical than high concentrations of microbial toxic organics [19]. However, the poisonous nature of some heavy metals such as arsenic has long been reported but its mode of toxicity depends very



much on the chemical form of the metalloid [20]. Arsenate has been reported to mimic phosphate and can therefore enter the microbial cell via transporters meant for the uptake of this essential nutrient and subsequently interferes with phosphate-based energy-generating processes, and as a result inhibits oxidative phosphorylation [21]. Arsenite, on the other hand, enters via aqua-glycerolporins and targets a broader range of cellular processes, binding to the thiol groups in important cellular proteins such as pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase [21]. However, these transport and toxicity mechanisms culminate to a reduced degradation of heavy metals as observed in this research (Figure 3). In conclusion, the results of this study encourage the use of chicken drops in bioremediation, especially in the remediation of aliphatic hydrocarbons. However, a combined remediation approach involving a sequential application of nutrient augmentation, microbial seeding and phytoremediation methods should be encouraged as a single step remediation process in crude oil abatement programme.

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