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Full Length Research Paper

Safety Evaluation of Oil Polluted Soil by Phytoremediation

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Abstract
Oil polluted soil was remediated using lemon grass (Cymbopogon citrate) and the plot was assessed by planting maize to cross check the effectiveness of the phytoremediation. The study focused on oil contaminated soil that was remediated with lemon grass (C. citrate) and another oil contaminated soil that was allowed to remediate naturally, and it was replicated 9 times. Remediation was carried out for the duration of 80 days. The results show that the level of concentration of crude oil contamination caused significant difference in the plants development. It is therefore, concluded that plant has potential for phytoremediation as it grew successfully relative to unremediated soil.

Key words*: Engine oil, Lemon grass, Phytoremediation, Viable maize.*

introduction

Phytoremediation is the name given to a set of technologies that use plants to clean contaminated sites. The term *phytoremediation* (phyto = plant and remediation = correct evil), comes from a variety of research areas including constructed wetlands, oil spills. Phytoremediation is used to mean the overall idea of using plant-based environmental technologies. Today, environmental managers can choose from a variety of approaches to remediate petroleum contaminated soil and groundwater. Various plants have been identified for their potential to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons. In the majority of studies, grasses and *legumes* have been singled out for their potential in this regard (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996). Examples are; Prairie grasses (*Buchloe dacotyloides*), western wheatgrass (*Agropyron smithi*), Lemon grass (*Cymbopogon citratus*), carrot (*Dnucus carota*) Bermuda grass (*Cynodon dactylon* L.) are thought to make superior vehicles for phytoremediation because they have extensive fibrous root systems. Grass root systems have the maximum root surface area (per m3 of soil) of any plant type and may penetrate the soil to a depth of up to 3 m (Aprill and Sims, 1990). They also exhibit an inherent genetic diversity, which may give them a competitive advantage in becoming established under unfavorable soil conditions (Aprill and Sims, 1990).

Legumes are thought to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen; i.e., legumes do not have to compete with microorganisms and other plants for limited supplies of available soil nitrogen at oil-contaminated sites (Gudin and Syratt, 1975).

Crude oil spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. It has been recorded that oil contamination causes slow rate of germination in plants. Adam and Duncan (2002) reported that this effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen.

Phytoremediation mechanisms depend on the type of contaminant, bioavailability and soil properties (Cunningham and O.W, 1996). There are several approaches to selecting candidate plants for phytoremediation of soils contaminated with organic pollutants, these approaches have been based on the occurrence of plants under specific climatic conditions (Gudin and Syratt, 1975; Banks et al., 2003) their resistance to pollutant phytotoxicity (Kirk et al., 2002). Most studies on the phytoremediation of petroleum hydrocarbon contaminated soils have employed grasses (Poacae) and legumes (Leguminosae) (Aprill and Simms, 1990; Gunther *et al.,* 1996; Merkl *et al.,* 2005; Kirkpatrick *et al.,* 2006; Qui *et al.,* 1997; Schwab *et al.,* 2006; Kaimi *et al.,* 2006). According to Adam and Duncan (1999), Merkl *et al.* (2004, 2005) it has been concluded that grasses and legumes are the best candidates for the process of phytoremediation because of their root systems. This study is to evaluate the suitability of phytoremediated soil for safety of Agricultural products.

Materials and methods

Materials

Soil Sample Collection

Top soil sample (approximately 250kg) was collected from depth 0-20cm from a fallow land, 4m from the nearby roadside at a site located at the National Centre for Agricultural Mechanization, Ilorin, Kwara state. The area was chosen because it was assumed to be minimally contaminated by hydrocarbon.The soil sample was air-dried and sieved with a 2mm mesh.

Collection of Lemon Grass

The plant utilized in the phytoremediation, namely lemon grass *(Cymbopogon citratus)* was also collected from a garden that was 2m away from the roadside. The garden was cited in Adewole area in Ilorin city. The plant samples collected were cut to 30cm in length prior to planting; this gave the initial length at planting.

Collection of spent engine oil

About 20 liters of spent engine oil were collected from a mechanic village at Ipata-Oloje, Ilorin in a sterile container.

Methodology

The experimental design consisted of three sets namely; Control which focused on non-oil contaminated soil, oil contaminated soil but remediated with lemon grass *(Cymbopogon citratus)* for 80 days called phytoremediation denoted with code PHY and the last set is also oil contaminated soil which was remediated naturally called natural attenuation denoted with code NAT. Each set was replicated 9 times. Plastic pots were used and each was labeled as described in the Tables 1 and 2. Each pot was filled with the soil at 6kg per pot. The pots were then arranged in randomized design; the soils in the pots were spiked with the spent engine oil at 100ml per pot, and stabilized via watering and stirred together to have homogenous polluted soil samples before planting 30cm lemon grass.

Table 1: Phytoremediation

S/N	Description of set up	Sample code
1.	Soil + oil + C.citraus	**PHY-O-01**
2.	Soil + oil + C.citraus	**PHY-O-02**
3.	Soil + oil + C.citraus	**PHY-O-03**
4.	Soil + oil + C.citraus	**PHY-O-04**
5.	Soil + oil + C.citraus	**PHY-O-05**
6.	Soil + oil + C.citraus	**PHY-O-06**
7.	Soil + oil + C.citraus	**PHY-O-07**
8.	Soil + oil + C.citraus	**PHY-O-08**
9.	**Soil + oil + C.citraus**	**PHY-O-09**

Table 2: Natural attenuation

S/N	Description of set up	Sample code
1.	Soil + oil	**NAT-O-01**
2.	Soil + oil	**NAT-O-02**
3.	Soil + oil	**NAT-O-03**
4.	Soil + oil	**NAT-O-04**
5.	Soil + oil	**NAT-O-05**
6.	Soil + oil	**NAT-O-06**
7.	Soil + oil	**NAT-O-07**
8.	Soil + oil	**NAT-O-08**
9.	**Soil + oil**	**NAT-O-09**

Viable maize (2per pot) seeds were planted in all the treatments. Plant toxicity to germination, plant height and leaves number were monitored for 8 weeks. The plants were watered as required every other day with (UNILORIN) pure water. The plant height and leaves number were monitored on a weekly basis. The growth assessment of the crop was terminated when the plants growth started depreciating.

Determination of pH

The measure of acidity and alkalinity of the soil is known as the pH of the soil. Exactly 10g of soil samples was weighed into a plastic sampling bottle. 20ml of distilled water was added and this was shook in the shaker for 30 minutes. The pH values were read by dipping the tip of the electrode of pH meter into the resulting mixture of the soil and water.

Assessment of soil microbial, diversity and population

The soil sample was mixed and a suspension of 1g (dry weight equivalent) in 10ml of sterile water was prepared. 1ml of the soil suspension was then diluted serially (ten-fold). The sterile plates were labeled (in duplicate) with the dilutions 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. The pre-poured agar plates were allowed to remain for a few days at room temperature to allow the surface to dry. The dry surface absorbed more rapidly the suspension that was introduced. Aseptically 1ml of the 10^{-4} dilution was introduced into the dry agar surface of 2 plates, previously labeled 10^{-4}. This was repeated for the other dilutions 10^{-5}, 10^{-6} and 10^{-7}.

In each case, a sterilized glass spreader was used to spread the suspension on the surface of the agar. The plates were inverted and incubated at 35^{o}C for 48 hours. After the incubation period, the number of total bacteria and fungi colonies on plates that contained 30-300 colonies were counted. The number of colonies on a plate multiplied by the dilution factor gave the plate count per ml of the soil sample and this was expressed as CFU/g soil.

Microbial Identification

Nutrient agar containing 0.015% (w/v) (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35^{o}C for five days. Potato dextrose agar to which 0.05% (w/v) choramphenicol has been added (to inhibit bacteria growth) was used for fungi isolation and incubation was at ambient temperature for seven days. Pure isolates of representative communities was maintained on agar slant at 4^{o}C. Identification of isolates was based on cultural, microscopic and biochemical characteristic.

Results

Table 1: The average means value of plants height

Weeks	Phytoremediation	Natural	Control
1	13.40	6.73	18.93
2	34.53	13.07	43.73
3	49.40	18.47	61.67
4	63.73	25.67	79.73
5	72.73	34.20	89.20
6	76.13	35.87	96.80
7	71.93	34.13	100.00
8	68.87	32.00	99.47

Table 2: The average mean value of leaves number

Weeks	Phytoremediation	Natural	Control
1	2.86	2.43	2.86
2	4.43	2.86	3.93
3	5.07	3.43	4.43
4	5.36	3.57	5.36
5	5.79	3.93	5.93
6	5.71	4.36	6.64
7	5.79	4.93	7.07
8	6.14	5.07	7.86

Table 3: The average means value of pH observation for the soil

Treatments	pH before	pH after
Phytoremediation	6.69	6.80
Nat. Attenuation	6.46	6.65
Control	6.50	6.37

Table 4: The mean value of plate counts (CFU/ml) of soil and isolated organisms

	Bacteria	Fungi	Selected organisms
Phytoremediation	352.91	19.45	49.3
Natural attenuation	51.73	13.89	16.58
Control	625.92	28.75	79.73

CFU/g soil means the number of colonies on a plate multiplied by the dilution factor gave the plate count per ml of the soil sample.

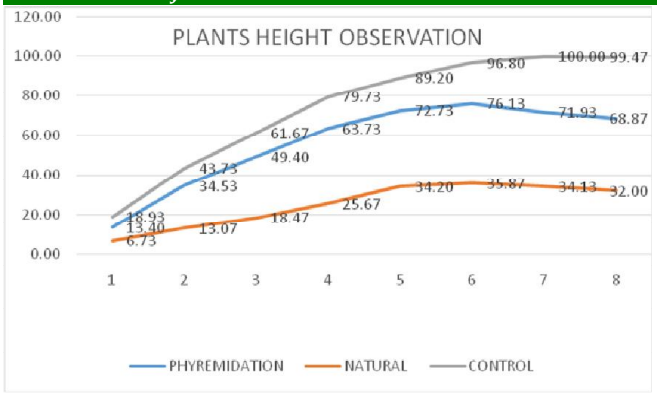

Fig 1: Mean values of the plant height

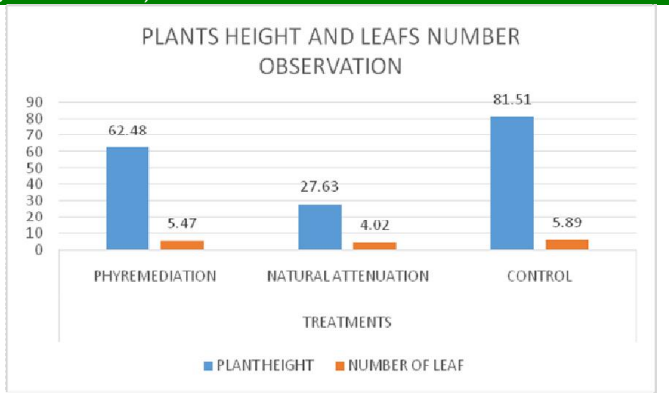

Fig 2: Mean values of plant height and leafs number

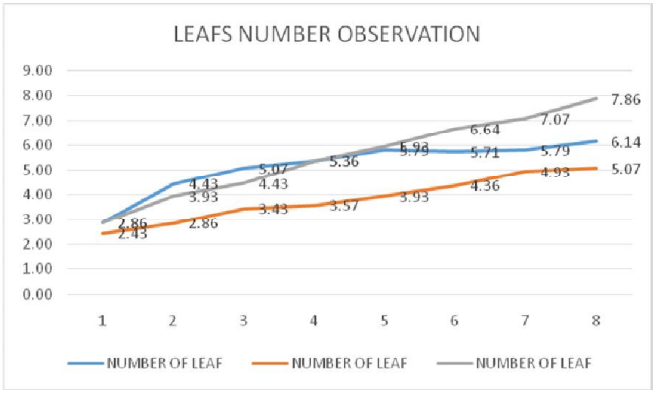

Fig 3: Leafs development observation represented by mean values

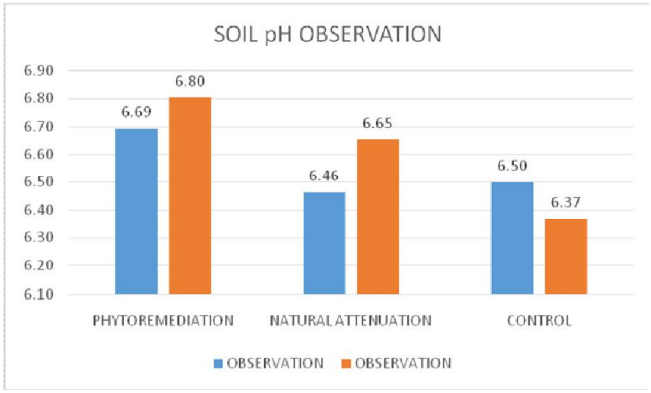

Fig 4: Soil acidity level

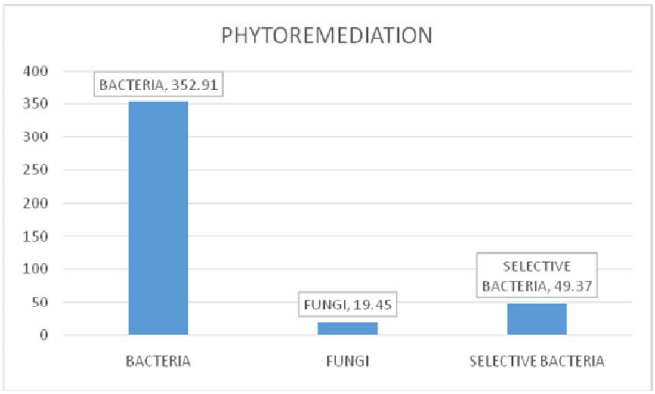

Figure 5a: Phytoremediation

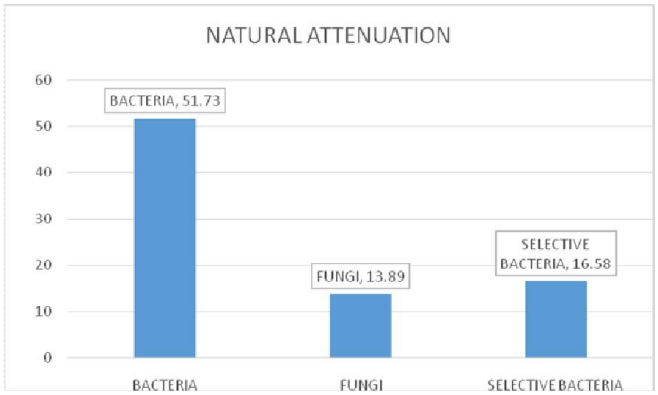

Figure (5b): Natural Attenuation

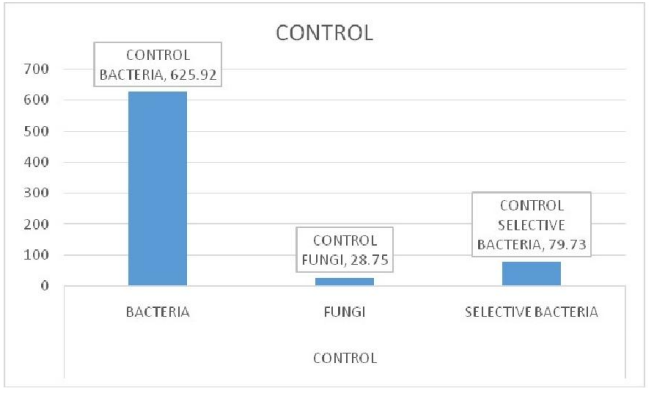

Figure (5c): Control

Figure 5: The mean value of plate counts (CFU/ml) of soil and isolated organisms

Discussion

Figure 1 is the mean values of the plant height treatments which show remarkable development for plants height treatments in phytoremediation compare to plants height in natural attenuation in which soil was allowed to remediate naturally.

Figure 2 shows the bar charts representing combination of Plants height and leaf number for the assessment of plants development for the three set of experiments.

Figure 3 represents Leafs development observation represented by mean values

Figure 4 represent soil acidity level mean values before planting and after the experiment in all treatments as shown above. Therefore, in natural attenuation the soil is more acidic due to the presence of petroleum hydrocarbons which make soil unfavorable for plant survival than in phytoremediation, purposely because the treatments were not subjected to remediation procedure with *(Cymbopogon citratus).*

Figures 5a, 5b & 5c shows the level at which microorganisms can survive in oil contaminated soil. In phytoremediation ***(Figure 5a)*** despite that the soil was remediated it also shows limitation at which microorganisms can survive due to its acidity compare to control samples ***(Figure 5c)*** which referred to as uncontaminated while in natural attenuation ***(Figure 5b*** ,the soil is more acidic makes microorganisms difficult to survive as shown in the figures above, observation shows that the presence of Bacteria, Fungi & selective Bacteria is much more higher in phytoremediation than in natural attenuation, because the presence of oil creates adverse conditions which make essential nutrients like oxygen and nitrogen needed for organisms to survive and multiply insufficient in natural attenuation in comparison with phytoremediation.

Conclusion

The results led to the conclusion that soil degradation due to oil pollution could not be completely safe for plant survival; it could only be minimized environmental degradation. Graphs and bar charts show depreciation in plant height sat a certain stage during the experiment and insufficient leaf number in phytoremediation treatments while very poor development in plant heights and leaf number was observed in natural attenuation.

Recommendation

It is therefore necessary to remediate oil polluted soil that is intended for agricultural purpose. Although, phytoremediation may not necessarily result in 100% reclamation of total petroleum Hydrocarbon (TPH) but support plant growth compare to unremediated soil.

Ethics

All the authors read and approved the manuscript and no ethical issues involved.

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