

## Biorecovery of a Model Oil-Polluted Soil after Exposure to Solutions of Typical Salts Found in Irrigation Water

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**SUMMARY.** The enhanced biorecovery of a model oil-polluted soil by soil wetting with solutions of typical salts found in irrigation waters was investigated. Garden soil was sampled from a selected location of predetermined weed composition for the purposes of determining soil seed bank composition. The air-dried soil was immediately polluted with spent lubricating oil (SLO) to obtain a constant 5% w/w concentration of oil in soil and emptied into wide bowls of 65 cm diameter, and 32 cm in height and set up in a screen house. Aliquots of 2.5 g of each  $\text{Ca}_2\text{SO}_4$ , (SCA)  $\text{MgSO}_4$ , (SMG)  $\text{Na}_2\text{SO}_4$  (SNA) and  $\text{K}_2\text{SO}_4$  (SKA) were weighed into distilled water to obtain constant 0.025 g/l salt solution. Distilled water served as the control (CTR). The oil-polluted soils were wetted with 1500 ml of control or salt solution. The experiment lasted for three months, after which study showed that there was reduction in total poly Aromatic volatile Hydrocarbon (24111.44 ppm) at the start of the experiment to 5.54 ppm. Compared to the control experiment, reduction in the total petroleum hydrocarbon (TPH), reduction in TPH was highest in SNA, being 97.02% remediation efficiency, compared to 72.44% in the SNS treatment. Bacterial species identified during the study included *Corynebacterium kutscheri*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Bacillus licheniformis* and *Staphylococcus* spp., whereas fungi species included *Penicillium* spp., *Aspergillus niger*, and *Fusarium* spp. The abundance of the weed *Mariscus alterenifolios* in SCA (24), SMG (13), and CTR (20) may indicate a favoured environment for growth. Regeneration efficiency (RE) of weeds in the treated and control soils 62.5% by *Anelima aequinotiale* in CTR, 50% in SCA, and 12.5% in SNA.

**Keywords:** biorecovery, bioremediation, hydrocarbon, irrigation, salinity

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## Introduction

As a primary recipient of waste products as well as diverse chemicals used to advance our technological development, the soil is constantly under threat in modern society. The fact that technology is basically powered by the petroleum industry, whether directly or indirectly leaves more to ponder. In most oil-producing economies like Nigeria, pollution caused by petroleum and its derivatives is the most prevalent problem, where it has led to the damage of the soil, water and both plants and animals (Essien and John, 2010). Soils are also rendered unproductive for years after spillage, reducing the growth performance of plants (Dale *et al.*, 2006). Odugwu and Onianwa (1987) demonstrated the effect of pollution on germination, growth and nutrient uptake of pawpaw. The chronic effects of oil on soil properties and microflora in a rainforest system was also investigated by (Amadi *et al.*, 1996). However, such several methods as physical (vapor extraction, stabilization, solidification), chemical (photo-oxidation, dissolution, detergent use), and biological methods (bioremediation), have been employed to remove oil wastes, its constituents as well as derivatives from soil and water. All these methods are useful depending on the priorities and circumstances of each oil pollution incident. Bioremediation, a biological method that uses microorganisms, plants and/or associated microorganisms to remove or render harmful material harmless is one of the promising cost and environmental effective approach (Merkl, 2005; Eman, 2008). The success of bioremediation of any oil-polluted soil depends upon a number of factors, including moisture as well as soil-nutrient status.

Biostimulation may have been less effective in accelerating the disappearance of oil on certain oil-contaminated ecosystems due to either the presence of high background nutrient concentrations or oxygen limitation. However, a few field studies did show enhanced oil biodegradation through nutrient addition (Lee and Levy, 1991). The implication, therefore, is that nutrient amendment may still be viable options the remediation hydrocarbons from ecosystems, especially when nutrients are limiting. Commonly used water-soluble nutrient products include mineral nutrient salts (e.g.  $\text{KNO}_3$ ,  $\text{NaNO}_3$ ,  $\text{NH}_3\text{NO}_3$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgNH}_4\text{PO}_4$ ), and many commercial inorganic fertilizers (e.g. the 23:2 N:P garden fertilizer used in Exxon Valdez case). Some soils get nutrients indirectly from irrigation waters; examples being salts of nitrates and sulphates in irrigation waters. The present study hopes to investigate the effects some of the salts found in irrigation waters in the recovery of an oil-polluted soil.

Irrigation waters contain a significant amount of chemical substances in solution, varies according to the source and properties of the constituent chemical compounds. These chemicals including  $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{NaHCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{KCl}$ , and  $\text{K}_2\text{SO}_4$  affects the growth of resident plants and soil microorganisms. Provin and Pitt in an undated report suggested that these compounds formed a list of characteristic salts found in irrigation waters.

These salts derive their source from the earth's crust from weathering. When they dissolve in surface of groundwater, they become available to plants particularly when such waters are used in irrigation purposes.

In remediation activities, the role of regular wetting of the soil to improve soil moisture has been reported in previous study to enhance remediation of oil-polluted soil (Ikhajiagbe *et al.*, 2013). The dilemma therefore is with the eventuality of having to inadvertently expose the oil-polluted soil to saline irrigation waters. It is therefore the object of the present study to investigate the possibility for enhanced or retarded recovery a model oil-polluted soil upon exposure to solutions of typical salts found in irrigation waters.

## **Materials and methods**

### ***Preparation of soil***

Topmost garden soil was sampled from a selected location marked 15 m x 15 m, and then air-dried to constant weight. The air-dried soil was immediately amended with spent lubricating oil (SLO) to obtain a constant 5 % w/w concentration of oil in soil. Prior to collection of soil, a survey of all weed species growing within the designated 15 m x 15 m partition was done. This would serve, in the study, as possible plants to make up the soil's seed bank. The polluted soils were distributed into wide bowls of 65 cm diameter, and 32 cm in height. The bowls were not saturated; this was to ensure that contents within the bowl remain within the bowl for the entire period of 3 months that the soils would be exposed to experimental conditions. Measured 25 kg of soil was originally measured into each bowl prior to amendment with SLO. The set up was laid out in a well-ventilated screen house with temperature ranges of  $32.2 \pm 3.7$  °C. The polluted soils were eventually irrigated first with water (1000ml) and was left for 3 days for natural attenuation in the screen house.

### ***Preparation of salt solution***

Ca<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub>, Na<sub>2</sub>SO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub> were required for preparation of each salt solution. Aliquots of 2.5 g of each salt were weighed into distilled water to obtain constant 0.025mg/l salt solution. Distilled water served as the control (CTR).

### ***Wetting of oil-polluted soil bowls with salt solutions***

Prior to pollution of garden soil with SLO, the water-holding capacity (WHC) of the garden soil was previously determined to be 218.92 ml/kg soil. Each 25 kg soil per bowl was wetted daily with 1500 ml of control or salt solution. The experiment lasted for three months.

### ***Experimental parameters***

The development and appearance of weeds in each bowl from soil seed bank was monitored. After 3 months soil was taken to the laboratory for microbial and PAH determinations. This was done by collecting soil from 10 random spots in each bowl, and an arbitrary uniform depth of 7.5 cm from soil surface.

Standard methods described by Dean and Xiong (2000) were used to determine aliphatic hydrocarbon fractions of the soil; whereas the methods of Cowan and Steele (1974) and Cheesebrough (1998) were used to isolate and characterize bacterial and fungal isolates.

Regeneration efficiency of each weed in remediated oil-polluted soil was determined at 3 months after application of treatments, using the formula developed below:

$$\frac{\text{No of weed at 3 months} \times 100}{\text{No of weed at 1}^{\text{st}} \text{ day}}$$

Tolerance index of weeds in the oil-polluted soil at 3 months was also determined as follows;

$$\frac{\text{Table 8 (3 MAP)} \times 100}{\text{Table 8 (STAT)}}$$

Where MAP – months after pollution and exposure

STAT - Concentration of contaminants in the oil-polluted soil just before soil was wetted with salts.

### **Results and discussion**

This study therefore, was carried out to determine the recovery of oil-polluted soil exposed to saline waters for irrigation. Parameters used in the study to evaluate recovery were total hydrocarbons, weed regeneration, as well as soil microbial composition. The record of weeds that most likely constituted the soil seed bank of the soil used have been presented below (Table 1); the most predominant weed being *Mariscus alterenifolios* (Cyperaceae) with a composition of 13 individuals per square meter, compared to *Andropogon virginatus* (Poaceae) with 1 m<sup>-2</sup>.

Results showed significant reduction in total petroleum hydrocarbons (TPH) of wetted soils, compared to the control. Fundamentally, soil wetted with Na<sub>2</sub>SO<sub>4</sub> solution showed improved TPH remediation efficiency of 97.02%, compared to a range of 72.44 – 89.76% reductions in other wetted soils and 75.41% in the unwetted oil-polluted soil (Table 2).

**Table 1.**

Record of soil seed bank of the soil used in the present study.

| Weeds                           | Family        | *Total individual species m <sup>2</sup> | Frequency of occurrence (%) |
|---------------------------------|---------------|--|-----------------------------|
| <i>Andropogon virginatus</i>    | Poaceae       | 1  | 0.826                       |
| <i>Anelima aequinotiale</i>     | Commelinaceae | 8  | 6.612                       |
| <i>Asystasia gangetica</i>      | Poaceae       | 7  | 5.785                       |
| <i>Croton hirtus</i>            | Euphorbiaceae | 5  | 4.132                       |
| <i>Centrosema pubscers</i>      | Fabaceae      | 6  | 4.959                       |
| <i>Cyperus haspan</i>           | Cyperaceae    | 3  | 2.479                       |
| <i>Chromolina benghanlensis</i> | Commelinaceae | 5  | 4.132                       |
| <i>Eleusin indica</i>           | Poaceae       | 6  | 4.959                       |
| <i>Fimbisstylis ferruginea</i>  | Cyperaceae    | 8  | 6.612                       |
| <i>Gomphrina celosoides</i>     | Amaranthaceae | 4  | 3.306                       |
| <i>Kyllinga erecta</i>          | Cyperaceae    | 7  | 5.785                       |
| <i>Mariscus alterenifolios</i>  | Cyperaceae    | 13                                       | 10.734                      |
| <i>Pennisetum purpureum</i>     | Poaceae       | 6  | 4.959                       |
| <i>Synedrella nodiflora</i>     | Asteraceae    | 4  | 3.306                       |
| <i>Sporobolus pyramidalis</i>   | Poaceae       | 7  | 5.785                       |
| <i>Tridax proambens</i>         | Asteraceae    | 4  | 3.306                       |
| Unidentified (< 5 cm tall)      | -             | 27                                       | 22.314                      |
| Total                           |               | 121                                      | 100.00                      |

\*The space within which plants were surveyed was 15 m x 15 m.

**Table 2.**

Total petroleum hydrocarbons after 3 months of exposure to various treatments.

|                                | STAT     | After 3 months |         |         |        |         |
|--------------------------------|----------|----------------|---------|---------|--------|---------|
|                                |          | CTR            | SMG     | SCA     | SNA    | SKS     |
| Nonane (C9)                    | 3143.78  | 850.71         | 354.46  | 644.47  | 103.12 | 952.79  |
| Decane (C10)                   | 4326.36  | 1170.71        | 487.80  | 886.90  | 141.90 | 1311.20 |
| Dodecane (C12)                 | 5526.11  | 1495.37        | 623.07  | 1132.85 | 181.26 | 1674.81 |
| Tetradecane (C14)              | 3276.43  | 886.60         | 369.42  | 671.67  | 107.47 | 992.99  |
| Hexadecane (C16)               | 39.67    | 4.19           | 1.75    | 3.17    | <0.005 | 4.69    |
| Octadecane (C18)               | 2543.55  | 688.28         | 286.79  | 521.43  | 83.43  | 770.88  |
| Nonadecane (C19)               | 835.84   | 226.18         | 94.24   | 171.35  | 27.42  | 253.32  |
| Eicosane (C20)                 | 2041.56  | 552.45         | 230.19  | 418.52  | 66.96  | 618.74  |
| Docasane (C22)                 | 1053.21  | 285.00         | 118.75  | 215.91  | 34.55  | 319.20  |
| Tetracosane (C24)              | 783.49   | 212.01         | 88.34   | 160.62  | 25.70  | 237.45  |
| Hexacosane (C26)               | 237.83   | 64.36          | 26.82   | 48.76   | 7.80   | 72.08   |
| Tricosane (C30)                | 303.61   | 82.16          | 34.23   | 62.24   | 9.96   | 92.02   |
| TAH (mg/kg)                    | 24111.44 | 6518.01        | 2715.84 | 4937.89 | 789.55 | 7300.17 |
| PAVH (mg/kg)                   | 2412.32  | 5.54           | 1.43    | 3.98    | 0.95   | 8.48    |
| TPH (mg/kg)                    | 26523.76 | 6523.55        | 2717.27 | 4941.87 | 790.50 | 7308.65 |
| TPH remediation efficiency (%) | -        | 75.405         | 89.755  | 81.368  | 97.019 | 72.444  |
| Contamination factor           | 1562.05  | 6506.57        | 160.027 | 291.041 | 46.554 | 430.426 |

STAT - Concentration of contaminants in the oil-polluted soil just before soil was wetted with sulphate salts, TAH Total Aliphatic Hydrocarbon, PAVH Poly Aromatic volatile Hydrocarbon, TPH Total Petroleum Hydrocarbon, CTR soil wetted with water, SMG soil wetted with MgSO<sub>4</sub>, SCA soil wetted with CaSO<sub>4</sub> solution, SNA soil wetted with Na<sub>2</sub>SO<sub>4</sub> solution, SKS soil wetted with K<sub>2</sub>SO<sub>4</sub> solution

Total heterotrophic bacterial count after three months of soil exposure to various sulphate salt solutions was  $7.36 \times 10^4$  cfu/g in SNA and in  $1.21 \times 10^4$  cfu/g SKS, compared to  $2.39 \times 10^4$  cfu/g in CTR. Total Heterotrophic Fungi Count in the control was  $0.29 \times 10^4$  cfu/g and  $1.54 \times 10^4$  cfu/g in SNA. Hydrocarbon utilizing bacteria was highest in SNA ( $4.36 \times 10^4$  cfu/g), compared to  $0.15 \times 10^4$  in SKS.

Comparatively, a look at soil microbial population (see Table 4) also showed that all microbial isolates present in soil prior to exposure to experimental conditions were recurrent three months after exposure. Bacterial species identified during the study included *Corynebacterium utsceri*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Bacillus licheniformis* and *Staphylococcus* spp., whereas fungi species included *Penicillium* spp., *Aspergillus niger*, and *Fusarium* spp (Table 4). *Streptococcus* spp. and *Staphylococcus* spp. were both identified only in the soil wetted with  $\text{Na}_2\text{SO}_4$  solution (SNA). The control soil, although oil-polluted, did not show presence of the bacteria. Perhaps,  $\text{Na}_2\text{SO}_4$  affected soil conditions that enhanced performance of both soil microbes in the oil-polluted soil and also enhanced contaminant degradation.

One good reason for which salinity effects soil microbial population is because of the differences in tolerance of low osmotic potential by different soil microbial genotypes (Mandel, 2006; Gennari *et al.*, 2007; Llamas *et al.*, 2008; Chowdhury *et al.*, 2011). In the present study, total fungal colony forming units was lower than total bacterial composition. Pankhurst *et al.* (2001); Sardinha *et al.* (2003); Wichern *et al.* (2006) earlier reported that fungi are more sensitive to osmotic stress than bacteria. Accordingly, while sensitive microbial cells may be impaired by the low osmotic potential necessitated by the saline condition of the irrigation water, Oren (2001) and Hagemann (2011) reported that some microorganisms, including fungi, can adapt by taking up osmolytes that enable them retain water (Beales, 2004). In this study, fungi species including *Penicillium* spp., *Aspergillus niger*, and *Fusarium* spp, which were initially isolated from the clean soil before exposure to saline water and oil, were recurrent three months after exposure (Table 4). It is perhaps suggested that these organisms have developed a strategy for survival in salt-treated oil-polluted soils. This is hereby presented for further study on possible survival mechanisms.

**Table 3.**

|     | Total microbial count after three months of the oil-polluted soil to sulphate solutions |      |      |
|-----|---|------|------|
|     | THBC  | HUB  | THFC |
|     | (x $10^4$ cfu/g)  |      |      |
| CTR | 2.39  | 1.23 | 0.29 |
| SMG | 4.14  | 2.76 | 0.51 |
| SCA | 2.06  | 1.52 | 0.25 |
| SNA | 7.36  | 4.35 | 1.54 |
| SKS | 1.21  | 1.06 | 0.15 |

THBC – Total Heterotrophic Bacteria Count, THFC - Total Heterotrophic Fungi Count, HUB – Hydrocarbon Utilizing Bacterial, cfu/g – Colony forming unit per gram, CTR soil wetted with water, SMG soil wetted with  $\text{MgSO}_4$ , SCA soil wetted with  $\text{CaSO}_4$  solution, SNA soil wetted with  $\text{Na}_2\text{SO}_4$  solution, SKS soil wetted with  $\text{K}_2\text{SO}_4$  solution.

*Andropogon Virginatus*, *Chromolina benganhensis*, *Pennisetum purpureum*, and *Tridax procumbens* were absent from soils 3 months after exposure to experimental conditions (Table 5). The abundance of *Mariscus alterenifolios* in SCA (24), SMG (13), CTR (20), and UCTR (23) may indicate a favoured environment for growth. However, lower presence of the weed was recorded in SKS (2) and SNA (1). The Table also shows that unidentified weed were 5cm below, with SMG having the highest number of unidentified weeds (30), compared to SNA (1). *Andropogon Virginatus*, *Asystasia gangetica*, *Croton hirtus*, *Chromolina benganhensis*, *Fimbisstylis ferruginea*, *Gomphrina celosoides*, *Kyllinga erecta*, *Sporobolus pyramidalis*, *Tridax proambens* were absent in the salt-treated soils. There were a total of 48 plants per pot in SCA, 45 in SMG, 44 in CTR, 24 in SKS and 7 in SNA respectively.

**Table 4.**

| Microorganism distribution of oil-polluted soil at 3 months after application of treatments |   |     |     |     |     |     |
|---|---|-----|-----|-----|-----|-----|
| Isolates  | UCTR  | CTR | SMG | SCA | SNA | SKS |
|   | Oil-polluted soils after 3 months following salt exposure |     |     |     |     |     |
| <b>Bacteria</b>   |   |     |     |     |     |     |
| <i>Corynebacterium kutscheri</i>  | +   | +   | +   | +   | +   | +   |
| <i>Streptococcus</i> spp.   | +   | -   | -   | -   | +   | -   |
| <i>Pseudomonas aeruginosa</i>   | +   | +   | +   | +   | +   | +   |
| <i>Escherichia coli</i>   | +   | -   | +   | -   | +   | -   |
| <i>Klebsiella</i> spp.  | +   | +   | +   | +   | +   | -   |
| <i>Bacillus licheniformis</i>   | +   | +   | +   | +   | +   | +   |
| <i>Staphylococcus</i> spp.  | +   | -   | -   | -   | +   | -   |
| <b>Fungi</b>  |   |     |     |     |     |     |
| <i>Penicillium</i> spp.   | +   | +   | +   | +   | +   | +   |
| <i>Aspergillus niger</i>  | +   | +   | +   | +   | +   | +   |
| <i>Fusarium</i> sp  | +   | +   | +   | +   | +   | +   |

+ present, - absent; UCTR unpolluted control Soil as used for the experiment. CTR soil wetted with water, SMG soil wetted with MgSO<sub>4</sub>, SCA soil wetted with CaSO<sub>4</sub> solution, SNA soil wetted with Na<sub>2</sub>SO<sub>4</sub> solution, SKS soil wetted with K<sub>2</sub>SO<sub>4</sub> solution.

The regeneration efficiency (RE) of weeds in the treated and control soils have been presented on Table 6. RE of *Anelima aequinotiale* 62.5% in CTR, 50% in SCA, and 12.5% in SNA. The highest RE was obtained for *Mariscus alterenifolios* in SCA (218.2%). Generally, this weed had a significant regeneration capability compared to other weeds in the salt-treated oil-polluted soils. Significant regeneration of *Synedrella nodiflora* were also obtained in SNA and SKS, both being 100%. As observed earlier, Na<sub>2</sub>SO<sub>4</sub>-impacted soils showed enhanced performance of both soil microbes in the oil-polluted soil and also enhanced contaminant degradation; this may also be favourable for plant recovery. Plant recovery in SNA was comparatively lowest in the experiment. However, plants' tolerance index for recovered plants in SNA was better compared to other plants in the other wetted soils (Table 7).

Tolerant index of *Mariscus alterenifolios* in oil-polluted soil wetted with  $MgSO_4$  was 28.88%, and 5.0 % in soil wetted with  $CaSO_4$  solution, compared to 45.45% in the control oil-polluted soil (Table 7).

**Table 5.**

Abundance of weeds on remediated oil-polluted soil at 3 months after application of treatments (soil surface area in bowl is 2828.57 cm<sup>2</sup>)

| Weeds                           | UCTR | CTR | SMG | SCA | SNA | SKS |
|---------------------------------|------|-----|-----|-----|-----|-----|
| <i>Andropogon Virginatus</i>    | 0    | 0   | 0   | 0   | 0   | 0   |
| <i>Anelima aequinotiale</i>     | 5    | 5   | 0   | 4   | 1   | 0   |
| <i>Asystasia gangetica</i>      | 1    | 0   | 0   | 0   | 0   | 0   |
| <i>Croton hirtus</i>            | 2    | 0   | 0   | 0   | 0   | 0   |
| <i>Centrosema pubscers</i>      | 5    | 0   | 0   | 0   | 0   | 4   |
| <i>Cyperus haspan</i>           | 0    | 2   | 0   | 0   | 0   | 0   |
| <i>Chromolina benghanlensis</i> | 0    | 0   | 0   | 0   | 0   | 0   |
| <i>Eleusin indica</i>           | 5    | 3   | 0   | 1   | 0   | 0   |
| <i>Fimbisstylis ferruginea</i>  | 2    | 0   | 0   | 0   | 0   | 0   |
| <i>Gomphrina celosoides</i>     | 1    | 0   | 0   | 0   | 0   | 0   |
| <i>Kyllinga erecta</i>          | 2    | 0   | 0   | 0   | 0   | 0   |
| <i>Mariscus alterenifolios</i>  | 23   | 20  | 13  | 24  | 1   | 2   |
| <i>Pennisetum purpureum</i>     | 0    | 0   | 0   | 0   | 0   | 0   |
| <i>Synedrella nodiflora</i>     | 2    | 1   | 2   | 2   | 4   | 4   |
| <i>Sporobolus pyramidalis</i>   | 3    | 0   | 0   | 0   | 0   | 0   |
| <i>Tridax procumbens</i>        | 0    | 0   | 0   | 0   | 0   | 0   |
| Unidentified (< 5 cm tall)      | 18   | 13  | 30  | 17  | 1   | 14  |
| Total                           | 48   | 44  | 45  | 48  | 7   | 24  |

Weeds accounted for on Table 5 comprise the soil seed bank of soil originally used for the experiment. UCTR unpolluted control Soil, CTR soil wetted with water, SMG soil wetted with  $MgSO_4$ , SCA soil wetted with  $CaSO_4$  solution, SNA soil wetted with  $Na_2SO_4$  solution, SKS soil wetted with  $K_2SO_4$  solution.

Although the result shows that *Andropogon Virginatus*, *Asystasia gangetica*, *Croton hirtus*, *Cyperus haspan*, *Chromolina benghanlensis*, *Fimbisstylis ferruginea*, *Gomphrina celosoides*, *Kyllinga erecta*, *Sporobolus pyramidalis*, *Tridax proambens* had a tolerance index of 0%, the unidentified weed had a tolerance index of 29.54% in CTR, 66.66% in SMG 35.41% in SCA, 14.28% in SNA and 58.33% in SKS respectively. Comparatively, in the oil-polluted soils that wetted with salt solutions, average tolerance index was 5.88%, compared 5.88% in the control. However, average tolerance index in SCA was 3.23%.

The presence of salts in the soil negatively affects growth and development of resident plant species particularly owing to osmotic stress, ion toxicity or plants' reduced capability for essential nutrient absorption (Lauchli and Epstein, 1990). Essentially, increased salt concentrations in soils often lead to poor physicochemical condition of the soil which in turn inhibits seedling development and plant growth (Levy *et al.* 2002; Choudhary *et al.* 2004; Sharma and Minhas, 2005).



**Table 6.**

 Regeneration efficiency of weeds in salt-wetted oil-polluted soils at 3 months after exposure to treatments. Soil surface area in bowl is 2828.57 cm<sup>2</sup>.

| Weeds                           | CTR    | SMG                          | SCA    | SNA   | SKS    |
|---------------------------------|--------|------------------------------|--------|-------|--------|
|                                 |        | (Regeneration efficiency, %) |        |       |        |
| <i>Andropogon Virginatus</i>    | 0      | 0                            | 0      | 0     | 0      |
| <i>Anelima aequinotiale</i>     | 62.5   | 0                            | 50     | 12.5  | 0      |
| <i>Asystasia gangetica</i>      | 0      | 0                            | 0      | 0     | 0      |
| <i>Croton hirtus</i>            | 0      | 0                            | 0      | 0     | 0      |
| <i>Centrosema pubscers</i>      | 0      | 0                            | 0      | 0     | 66.67  |
| <i>Cyperus haspan</i>           | 66.67  | 0                            | 0      | 0     | 0      |
| <i>Chromolina benghanlensis</i> | 0      | 0                            | 0      | 0     | 0      |
| <i>Eleusin indica</i>           | 50     | 0                            | 6.67   | 0     | 0      |
| <i>Fimbrisstylis ferruginea</i> | 0      | 0                            | 0      | 0     | 0      |
| <i>Gomphrina celosoides</i>     | 0      | 0                            | 0      | 0     | 0      |
| <i>Kyllinga erecta</i>          | 0      | 0                            | 0      | 0     | 0      |
| <i>Mariscus alterenifolios</i>  | 181.8  | 118.2                        | 218.2  | 9.1   | 18.2   |
| <i>Pennisetum purpureum</i>     | 0      | 0                            | 0      | 0     | 0      |
| <i>Synedrella nodiflora</i>     | 25     | 50                           | 50     | 100   | 100    |
| <i>Sporobolus pyramidalis</i>   | 0      | 0                            | 0      | 0     | 0      |
| <i>Tridax proambens</i>         | 0      | 0                            | 0      | 0     | 0      |
| Unidentified (< 5 cm tall)      | 48.15  | 111.11                       | 62.96  | 3.70  | 51.85  |
| Total                           | 434.12 | 279.31                       | 387.83 | 125.3 | 236.72 |

CTR soil wetted with water, SMG soil wetted with MgSO<sub>4</sub>, SCA soil wetted with CaSO<sub>4</sub> solution, SNA soil wetted with Na<sub>2</sub>SO<sub>4</sub> solution, SKS soil wetted with K<sub>2</sub>SO<sub>4</sub> solution.

Increased salt concentrations in the soil results in osmotic stress, which in turn affects the microbiological properties of soil, reducing soil microbial biomass (Pathak and Rao, 1998; Oren, 1999). Incidentally, the soil microbial biomass and quality are significant soil properties for accessing the potentiality of the soil to remediate contaminants. Aside from osmotic stress, there are a number of other related factors necessitated by increased soil salinity; these include adverse pH changes, ion toxicities, as well a decline in potentially mineralizable N (Zahran, 1997).

Bandyopadhyay and Bandyopadhyay (1983) reported decreased mineralization and immobilization of soil nitrogen. The rates of nitrification and ammonification were also negatively impacted by saline soils (Wollenweber and Zechmeister-Boltenstern, 1989). These factors are *sin-qua-non* to successful microbial proliferation.

However, studies have shown the capability of *Pseudomonas* to significantly enhanced early plant growth in low fertility soil (Defreitas and Germida, 1992). In the present study, *Pseudomonas* was a prominently occurring bacterial species in the salt-treated oil-polluted soils. Invariably, there is improvement of resident plant development resulting from a concomitant compensation for soil nutrient deficiency by the bacteria, which may produce plant growth regulators within the rhizosphere.

Klopper and Beauchamp (1992) and Wu *et al.* (2005) earlier reported improved root development and better water and nutrient absorption as a result of the microbial action. This also justifies the relative plant recovery percentages in the soil-polluted soils, where plants' ability to access water and nutrients were hitherto hindered (Defreitas and Germida, 1992; Lazarovits and Norwak, 1997; Burdman *et al.* 2000). Lindberg *et al.* (1985) and Frankenberger and Arshad (1995) earlier noted that root-colonizing bacteria may help stimulate plants growth and thus inhibit the damaging effects of environmental stressors by producing phytohormones when in association with the plant. Hasnain and Sabri (1996) also showed that *Pseudomonas* spp. initiated increases in plants auxin content as well as reduced accumulation of harmful ions in wheat plant.

**Table 7.**

Tolerance index of weeds on remediated oil-polluted soil at 3 months after application of treatments

| Weeds                           | CTR   | SMG   | SCA   | SNA   | SKS   |
|---------------------------------|-------|-------|-------|-------|-------|
| (Weed tolerance index, %)       |       |       |       |       |       |
| <i>Andropogon Virginatus</i>    | 0     | 0     | 0     | 0     | 0     |
| <i>Anelima aequinotiale</i>     | 11.36 | 0     | 8.33  | 14.28 | 0     |
| <i>Asystasia gangetica</i>      | 0     | 0     | 0     | 0     | 0     |
| <i>Croton hirtus</i>            | 0     | 0     | 0     | 0     | 0     |
| <i>Centrosema pubscers</i>      | 0     | 0     | 0     | 0     | 16.66 |
| <i>Cyperus haspan</i>           | 4.545 | 0     | 0     | 0     | 0     |
| <i>Chromolina benghanlensis</i> | 0     | 0     | 0     | 0     | 0     |
| <i>Eleusin indica</i>           | 6.818 | 0     | 2.08  | 0     | 0     |
| <i>Fimbisstylis ferruginea</i>  | 0     | 0     | 0     | 0     | 0     |
| <i>Gomphrina celosoides</i>     | 0     | 0     | 0     | 0     | 0     |
| <i>Kyllinga erecta</i>          | 0     | 0     | 0     | 0     | 0     |
| <i>Mariscus alterenifolios</i>  | 45.45 | 28.88 | 5.0   | 14.28 | 8.33  |
| <i>Pennisetum purpureum</i>     | 0     | 0     | 0     | 0     | 0     |
| <i>Synedrella nodiflora</i>     | 2.272 | 4.44  | 4.16  | 57.14 | 16.66 |
| <i>Sporobolus pyramidalis</i>   | 0     | 0     | 0     | 0     | 0     |
| <i>Tridax proambens</i>         | 0     | 0     | 0     | 0     | 0     |
| Unidentified (< 5 cm tall)      | 29.54 | 66.66 | 35.41 | 14.28 | 58.33 |
| Average                         | 5.88  | 5.88  | 3.23  | 5.88  | 5.88  |

CTR soil wetted with water, SMG soil wetted with MgSO<sub>4</sub>, SCA soil wetted with CaSO<sub>4</sub> solution, SNA soil wetted with Na<sub>2</sub>SO<sub>4</sub> solution, SKS soil wetted with K<sub>2</sub>SO<sub>4</sub> solution.

The importance of soil microorganisms cannot be overemphasized. Apart from their prominent role in soil decontamination, they are also a central factor in nutrient cycling, soil organic content, as well as in sustaining plant production. Although a number of soil microorganisms exist that are tolerant to a number of environmental stress (Ikhajiagbe, 2010), however, stresses can be detrimental for sensitive microorganisms and decrease the activity of surviving cells, due to the

metabolic load imposed by the need for stress tolerance mechanisms (Schimel et al, 2007; Yuan *et al.*, 2007, Ibekwe *et al.*, 2010; Chowdhury *et al.*, 2011). This particularly informs the reduction in presence of some organisms in the present study, which were hitherto present in the soil prior to amendment with either saline solution or with oil. Most importantly, apart from the deleterious effects of oil on sensitive soil microbes, salinity also inhibits development of these microbial populations. The idea of this research is to investigate whether the imposition of salinization on the already stressed soils (with oil pollution) offers any respite for the remediation purpose of the soil microorganisms, particularly given the fact that these organisms may be inadvertently exposed to saline irrigation waters.

### Conclusion

The impact of saline irrigation waters on the recovery of a model oil-polluted soil has been reported. Significant changes in soil TPH contents of the oil-polluted soils were reported, with enhanced remediation reported in the soil wetted with Na<sub>2</sub>SO<sub>4</sub>. Results also showed that for plants available three months after exposure to the experimental conditions showed below average tolerance indices, apart from which *Synedrella nodiflora* showed 57.14% tolerance index. Further study is therefore required to ascertain the mechanism of effects of these salts in both plants and the soil microorganisms in order to further clarify the results presented herein.

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