International Journal of Plant & Soil Science

32(16): 13-25, 2020; Article no.IJPSS.56683
ISSN: 2320-7035

Stimulating the Rate of Phytoremediation of *Chromolaena odorata* in Crude Oil Contaminated Soil Using Inorganic Urea

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2020/v32i1630376
Editor(s):
(1) L. S. Ayeni, Adeyemi College of Education, Nigeria.
Reviewers:
(1) Adamu Yunusa Ugya, Kaduna State University, Nigeria.
(2) Araceli Amaya Chávez, Autonomous University of the State of Mexico, México.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56683

Original Research Article

Received 12 March 2020
Accepted 18 May 2020
Published 04 December 2020

ABSTRACT

Investigation on the use of urea in stimulating the phytoremediation of *Chromolaena odorata* in a crude oil contaminated soil was carried out at a crude oil spilled site at Botem-Tai, Ogoni, Nigeria. Three phytoremediation treatments labeled A – C in addition to the control (D) were used. The treatments were: A (*Chromolaena odorata* only), B (*Chromolaena odorata* + 20 g/m^2 urea), C (*Chromolaena odorata* + 40 g/m^2 urea), D (polluted soil without phytoremediation) arranged using Latin Square Design (LSD). Total petroleum hydrocarbon (TPH) and Total hydrocarbon content (THC) in soil and plant samples from the different treatment plots in addition to other soil nutrients were analyzed. The percentage reduction in TPH and THC in soil were as follows: Treatment B, {TPH (92.08%) and THC (95.37%)} > treatment A {TPH (88.95%) and THC (93.37%)}> C {TPH (78.78%) and THC (83.29%)} > Control {TPH (14.76%) and THC (32.90%)}. Treatment C had the highest TPH (2.67 mg/kg) and THC (20.57 mg/kg) accumulation in test plant. Combining stimulant (urea) with phytoremediation also improved soil properties such as pH, Nitrogen and Potassium. With the highest reduction of TPH and THC in treatment B

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(phytoremediation with 20 g/m^2 urea) than other treatments is an indication that low concentration of urea has a stimulatory effect on phytoremediation of crude oil by *Chromolaena odorata*.

Keywords: Phytoremediation; stimulant; Chromolaena odorata; pollution; crude oil; soil.

1. INTRODUCTION

Environmental pollution from crude oil is one of the consequences of oil exploration and exploitation in the Nigeria Niger Delta [1]. Crude oil spills which usually occur during production operation, accidental discharges, leakages from corroded pipes, transport vessels and human errors contaminate the surrounding environment and negatively affect the ecosystem [2] thus capable of causing abrupt physical, chemical and biological damages to affected ecosystems [3].

Contaminants have environmental impacts on both biotic and abiotic components of the environment [4,5]. The impacts which include; alteration of soil physicochemical properties [6], toxicity to flora and fauna of polluted environment which in turn affects the quality of human life [7]. People who depend solely on agriculture (farming) as means of livelihood have been forced to abandon their means of livelihood. This is because petroleum hydrocarbons render the soil sterile; preventing crop growth and yield for a long period of time.

The quest for efficient and economical strategies to break down petroleum hydrocarbons at contaminated sites has led to the development of several remediation methods. Remediation of crude oil contaminated soils holds a lot of promise for oil producing nations where crude oil pollution has impacted negatively on ecological media. Remediation has helped in restoring and rehabilitating the negative impact of crude oil pollution on ecosystems [8,9]. Remediation of the petroleum contaminated soil is essential to maintain the sustainable development of local ecosystem. Various methods have been employed in soil remediation. Some of these methods usually involve excavation and transport of contaminated soil from area of occurrence to landfills where it is treated. Most of these methods are time consuming, costly and also poses other environmental issues such as resultant contamination arising from the dispersal of the pollutant during transportation of the impacted or contaminated soil [10]. One of such commonly used remediation technique is the use of chemical. This method has gained attention as it can be used for treatment of organic and inorganic contaminant in soil within a short time but also with lots of side effects on the ecosystem biota. Another method of remediation that has been rated as more aesthetically acceptable and ecofriendly is bioremediation. This involves the use of microorganisms and / or plants for the treatment of polluted soil [11,12].

Bioremediation has been proven to be relatively cheap compared to other soil remediation techniques. Remediation studies has shown that there are several bioremediation approaches which include biostimulation (the growth of indigenous oil degraders are stimulated by the addition of nutrient or other growth limiting substrates), bioaugumentaton (the addition of a known oil degrading bacteria to supplement the existing ones) and phytoremediation (the use of plant to immobilize, degrade, reduce or render contaminant harmless).

Phytoremediation refers to all plant based technologies that uses green plants to remediate contaminated sites [13,14]. It is an emerging technique in which plant are employed to absorb/degrade pollutant from a polluted environment and mobilize them into various biomolecules in their tissues [15]. Phytoremediation makes use of green plants and associated microorganisms alongside soil amendments and agronomic practices to either contain, remove or render toxic contaminants less toxic or harmless [16]. The technology draws its idea from the numerous physical, chemical and biological interactions occurring between plants and the environmental media. Its applications have been tested for clean-up of contaminated soil, water and air and confirmed feasible in several field trials [14,17,18]. Several features make phytoremediation an attractive alternative to many of the currently practiced remediation technologies. These include low capital and maintenance costs, non-invasiveness, easy start-up, high public acceptance and the pleasant landscape that emerges as the final product [19-22].

One of the major limitations of phytoremediation is that it is slow as compare to other remediation techniques such as engineering and chemical method. Hence, there is need to develop strategies that will stimulate and quickening the process. So this study is designed to evaluate the effectiveness of the use of urea to stimulate the rate of phytoremediation by *Chromolaena odorata* in crude oil contaminated soil. The result obtained in this study will contribute to our knowledge of phytoremediation and also proffer alternatives in dealing with crude oil pollution problems in Nigeria especially in the Niger Delta.

2. MATERIALS AND METHODS

2.1 Study Area Description

The study was carried out on a crude oil polluted land at Botem Community in Tai Local Government Area (Ogoni) of Rivers State, Niger Delta Region of Nigeria. The study site is situated between Latitude: 4°43' 29.5608'' N and Longitude: 7°16' 8.382'' E. The farmland has been abandoned due to impact of crude oil spill from broken pipe (owned by Shell BP); a year prior to the study. The area experienced two distinct seasons; the rainy and dry season and is characterized by high temperature, high rainfall (2000 – 2500 mm/yr.), and high relatively humidity.

2.2 Materials Used and Sources

Urea (inorganic fertilizer) used as amendment/stimulant in the study was obtained from a fertilizer vendor at Kpite daily Market in Tai Local Government Area of Rivers State. Urea is a single element fertilizer; the forty six: zero: zero (46-0-0) which supplies the major essential element (nitrogen). Young seedlings of *Chromolaena odorata* used as the phytoremediationplant were obtained from the wild (unpolluted sites) in Botem community.

2.3 Experimental Design

The work was done between April and August, 2018 (rainy season). A Latin Square Design (LSD) consisting of four (4) phytoremediation treatments and 4 replications each was used for the experiment. The crude oil contaminated site was demarcated and an 8 m x 8 m plot was mapped out. The plot was subdivided into 1 m x 1 m (1 m^2) subplot each with an interval of 30 cm between them giving a total of 16 subplots (Fig. 1). The intervals were heap to prevent exchange of materials between the subplots.

The four treatments were as follows:

A. Phytoremediation with *Chromolaena odorata* only.
B. Phytoremediation with *Chromolaena odorata* stimulated with 20 g/m^2 of urea.
C. Phytoremediation with *Chromolaena odorata* stimulated with 40 g/m^2 of urea.
D. A crude oil polluted soil alone (control) with neither phytoremediation nor stimulant.

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

Fig. 1. Experimental design

2.4 Planting

The phytoremediation site was tilled in preparation for planting. Urea (20 g and 40 g) was added to soil as stimulant to treatments B and C respectively and left for one week. These subplots were tilled again with shovel before planting was done on them. Young seedlings of *Chromolaena odorata* of same size and vigor were collected from the wild and were transplanted into their respective plots (A, B and C). Treatment D (the control) was without plant and Stimulant (amendment). A minimum of twenty (20) seedlings of each plant were planted per subplot.

2.5 Soil Collection and Analysis

2.5.1 Pre-treatment collection

Before planting was done, samples of soil were collected from all subplots in the polluted site. The samples were collected from the soil at a depth of 0 – 15 cm using a soil auger. Soil samples collected from different subplots were mixed homogenously to form a composite sample. This was put into a nylon bag and then labeled. This was taken for analysis (Initial).

2.5.2 Post-treatment collection

Soil and plant samples were collected at every two (2) month interval from the different treatments (A-D). Soil samples were collected within the rhizosphere (root zone) of the phytoremediation plants. Plant samples were collected by total harvest method in which the plants were uprooted with the roots intact and then washed in water to remove all soil particles.

2.5.3 Analysis of samples

The samples (soil and plant) were taken to the laboratory for analysis. Soil chemical properties examined were: Potassium (K), Phosphorus (P), Total Organic Carbon (TOC), Total Nitrogen (N), Total Hydrocarbon Content (THC), Total Petroleum Hydrocarbons (TPH), Soil pH and electrical conductivity, Total Petroleum Hydrocarbon (TPH) and Total Hydrocarbon Content (THC) in Plant were also analyzed.

2.6 Determination of Measured Parameters

The electrical conductivity and pH of the soil were determined electronically using a glass electrode pH metre (PHS. 25 Model) and conductivity metre (Labtech Model), respectively. TNRCC Tx Method 1005 [23] was used to determine the total petroleum hydrocarbon in soil and plant. The API-RP45 Colorimetric method used by Aigberua et al. [24] was used to determine the Total Hydrocarbon Content (THC) of soil and plant samples. Black [25] Method was used to determine both Total Organic Carbon (TOC) and potassium. Total Organic matter content of soil was determined as a derivation using the formula outlined by Combs and Nathan [26]. Total Nitrogen was determined by Kjeldahl Method as outlined by Stewarte et al. [27]. Bray and Kurtz [28] No. 1 Method was used to determine available phosphorus in soil.

2.7 Data Analyses

Statistical evaluations were done using means, standard error means (SEM) and two- way ANOVA. Means were separated using Duncan Multiple Range Test (DMRT) (2018 version). Results were presented as mean ± SD using bar graphs.

3. RESULTS

It was observed that *Chromolaena odorata* is an effective phytoremediation plant for the reduction of petroleum hydrocarbon content in a contaminated soil. However, better performance was obtained in the phytoremediation treatments with addition of stimulant (urea).

Result showed reductions in total petroleum hydrocarbon (TPH) of soil in all the treatments. Observation showed a progressive decrease in TPH with time. That is, month 4 had a greater reduction than month 2 in all the treatment options. Treatment B (*Chromolaena odorata* + urea- 20 g/m^2) recorded the highest (p=0.05) percentage TPH reduction (92.08%) in soil while the least reduction (14.76%) was obtained in the control (no remediation) at both 2 and 4 month post-phytoremediation, respectively (Fig. 2).

Similar trend in TPH was also observed in the Total Hydrocarbon content (THC) of the soil (Fig. 3). Total hydrocarbon content of soil reduced progressively in all the treatments with time. Significant difference in the percentage reduction was observed between treatments (p=0.05). Highest percentage reductions (73.62% and 95.37%) were obtained in soil phytoremediated with *Chromolaena odorata* and stimulated with 20 g/m^2 of urea (treatment B) while the least reduction (24.75% and 32.90%) was in the control at 2 and 4 month, respectively.

These observations showed that lower concentration of urea (20 g/m^2) had higher stimulatory effect on biodegradation of both soil TPH and THC than higher concentration (40 g/m^2).

Figs. 4 and 5 show the stimulatory effects of the different urea concentrations in phytoremediation treatments on soil pH and electrical conductivity. Increase in pH was recorded in all the treatments at both 2 and 4 month post-phytoremediation trial with *Chromolaena odorata*. Phytoremediated soil stimulated with 40 g/m^2 urea (Treatment C) recorded highest pH at both months (Fig. 4).

Electrical conductivity increased in treatment C (phytoremediation with 40 g/m^2 urea) at 2 month and significantly dropped at 4 month post-phytoremediation. Other treatment options showed significant (p=0.05) increase in electrical conductivity with time (Fig. 5).

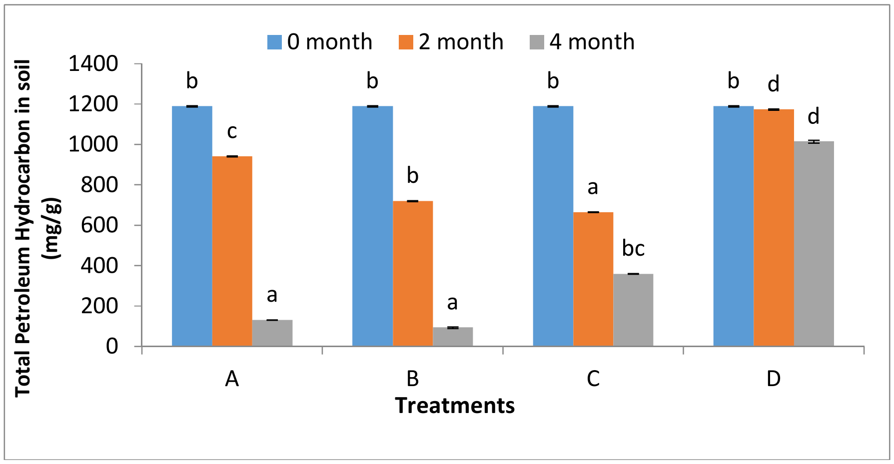

Fig. 2. Effects of treatments on total petroleum hydrocarbon in soil

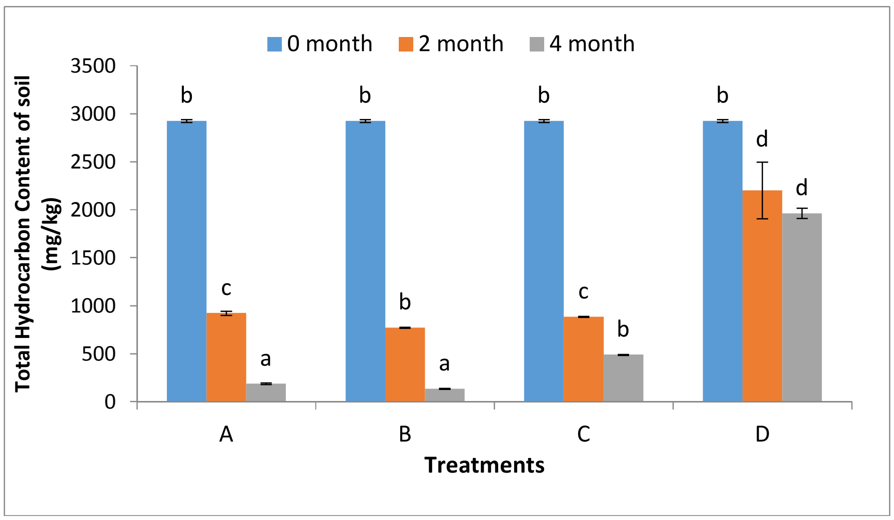

Fig. 3. Effects of treatments on total hydrocarbon content of soil

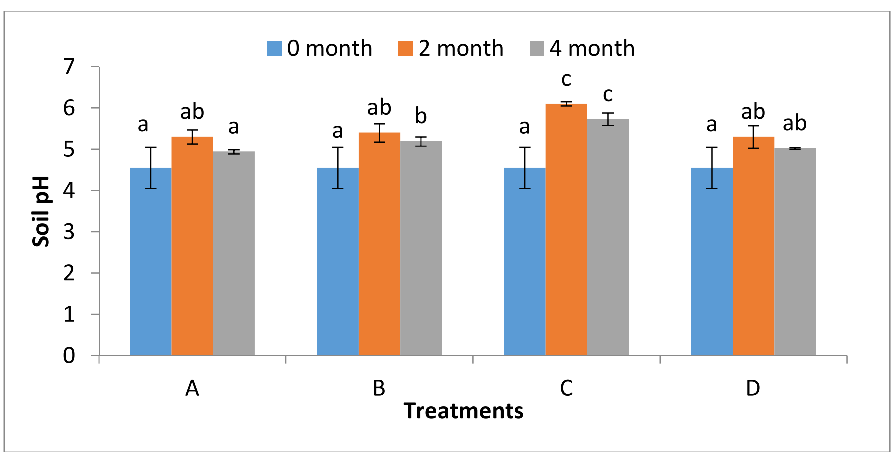

Fig. 4. Effects of treatments on soil pH

There was reduction in Total nitrogen in all the phytoremediation treatments (with or without addition of urea). This decrease was proportional with time with the exception of phytoremediation with 40 g/m^2urea treatment (especially at 4 month post -phytoremediation) (Fig. 6).

Total Organic Matter (TOM) (Fig. 7) and Total Organic Carbon (TOC) (Fig. 8) were observed to decrease in all the treatments with time. At 4 month post-phytoremediation period, there was significant difference (p=0.05) in the TOC and TOM between the different treatment options with the highest decrease obtained in treatment A (phytoremediation without stimulant) while the least was recorded in treatment C (phytoremediation with 40 g/m^2 urea).

Results showed fluctuation in Potassium content of soil in all the treatments (Fig. 9). Increase in Potassium was observed in all the treatments at 2 month. Highest increase was recorded in treatment A while the least was obtained in treatment B. At 4 month, Potassium in soil decreased in treatment A, C and D while increase was observed in treatment B. Increases in phosphorus were observed in all the treatments from 0 to 2 month but at 4 month a decline in phosphorus was observed across treatment (except treatment) (Fig. 10). Increases

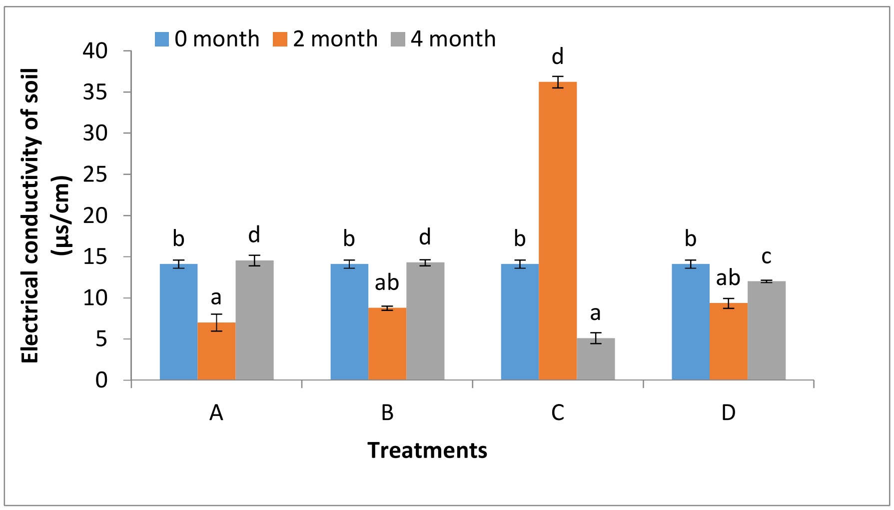

Fig. 5. Effects of treatments on electrical conductivity of soil

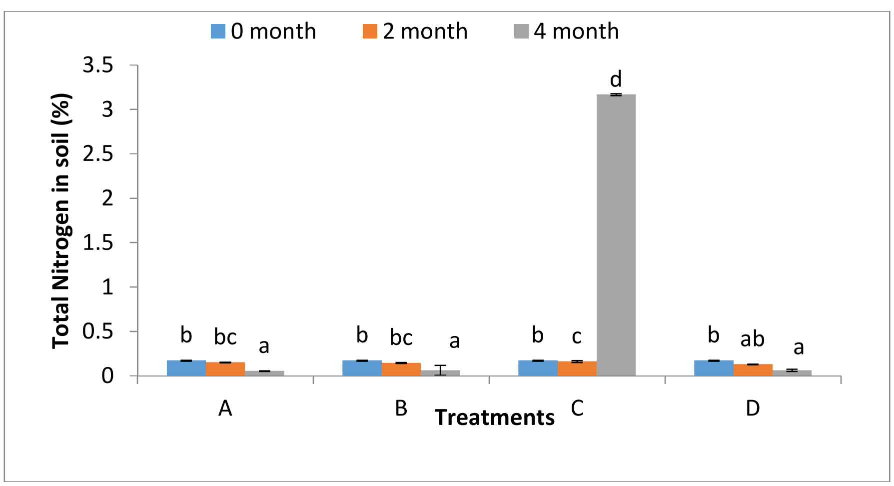

Fig. 6. Effects of treatments on soil nitrogen

in Phosphorus were observed in all the treatments from 0 to 2 month (except treatment C) but at 4 month a decline in phosphorus was observed across treatments (Fig. 10).

The result presented in Figs. 11 and 12 showed that total petroleum hydrocarbon (TPH) and total hydrocarbon content (THC) were accumulated in the plant in all the treatments. Highest TPH accumulation was obtained in treatments A at 2 month after planting and in treatment C at 4 month and the least in treatment C and A at 2 month and 4 month, respectively. But total hydrocarbon was accumulated in the plant with in Treatment C recording the highest accumulation at the end of the study.

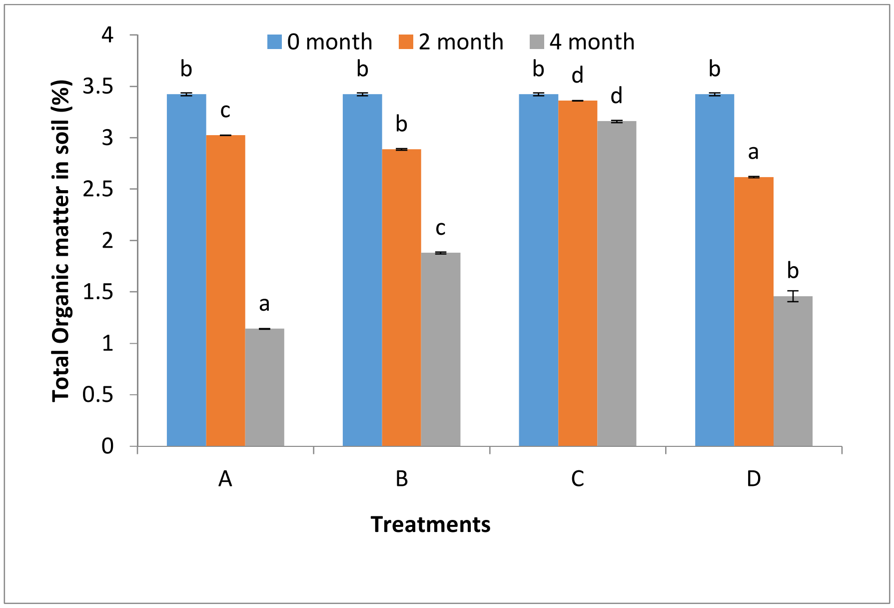

Fig. 7. Effects of treatments on total organic matter in soil

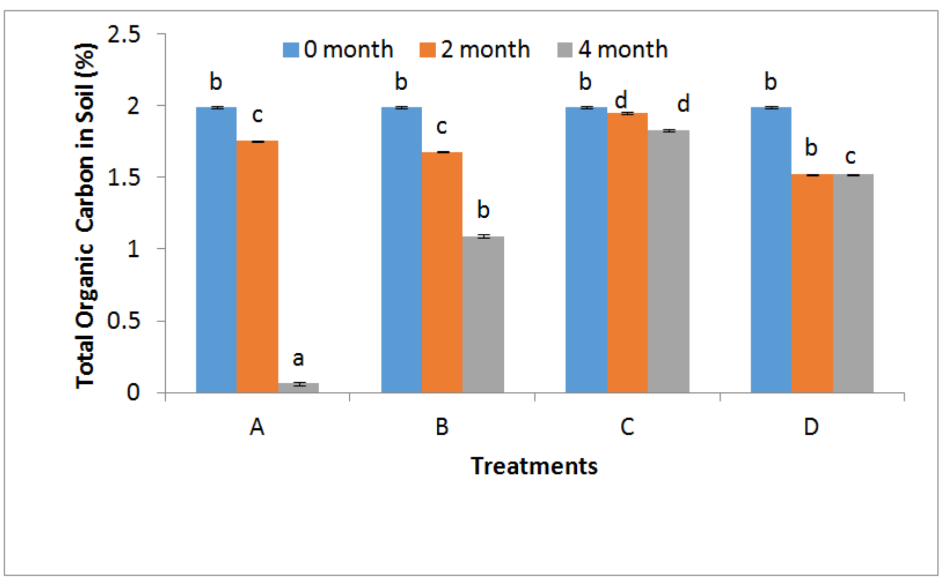

Fig. 8. Effects of treatments on total organic carbon in soil

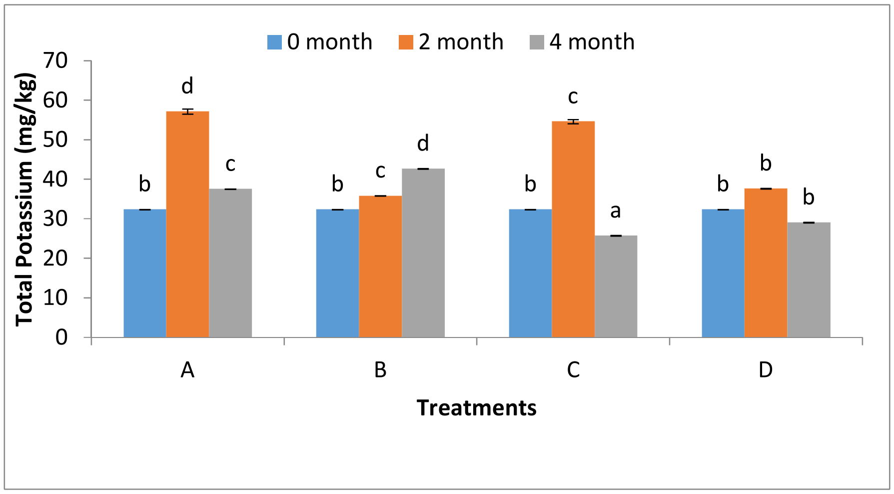

Fig. 9. Effects of treatments on total potassium in soil

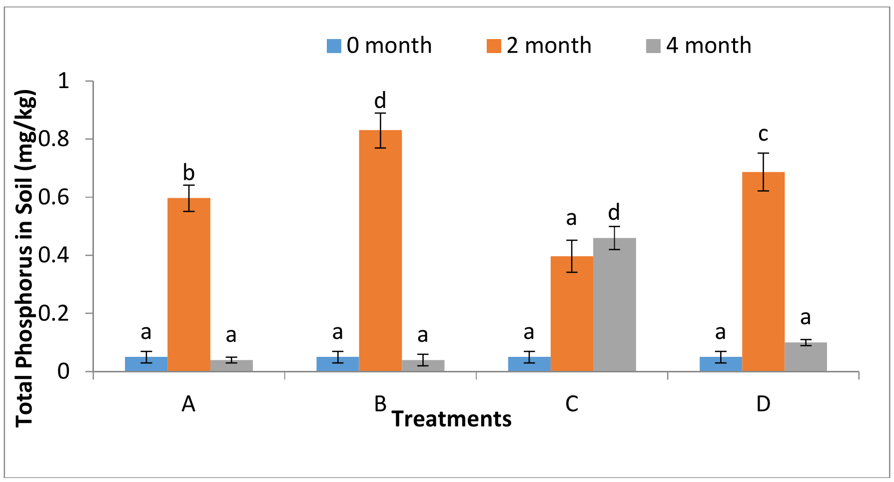

Fig. 10. Effects of treatments on total phosphorus in soil

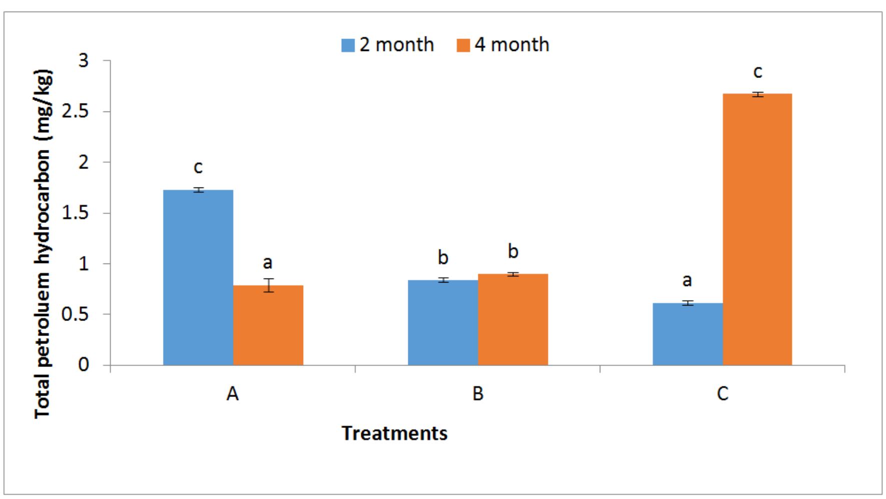

Fig. 11. Effects of treatments on total petroleum hydrocarbon in plant

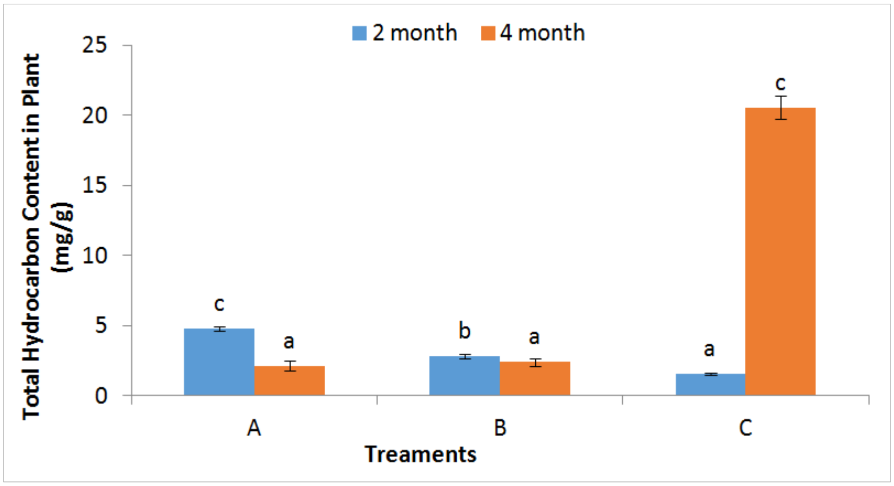

Fig. 12. Effects of treatments on total hydrocarbon content in plant

4. DISCUSSION

Soil pollution caused by crude oil brings about undesirable changes in the physicochemical and biological properties of affected soil which in turn negatively impact plants [29,30]. These undesirable changes can be mitigated by phytoremediation. Result showed reductions in Total petroleum hydrocarbon and total hydrocarbon content of soil in all the treatments. Higher reductions were obtained in the phytoremediated treatments (A, B, C) while least reduction was obtained in the control (D). The reduction observed in *Chromolaena odorata* phytoremediated soil (treatment A) could be attributed to the ability of *Chromolaena odorata* to thrive (grow) in the contaminated soil and also remove the contaminant [31]. This also infers that the phytoremediation plant was tolerant to the contaminant and has the potential for phytoremediation of hydrocarbon contaminated soil. This agrees with the report of Harrison [32] that C*hromolaena odorata* can tolerate relatively elevated concentrations of oil in the environment. Tolerance of plant to contaminant is a major determinant in the selection of plant for phytoremediation [33-35]. It has also been reported that resistance of plant to crude oil toxicity has direct influence on their phytoremediation potential [36,37].

Higher reduction observed in stimulated phytoremediated treatments may be due to the addition of urea to crude oil polluted soil. The urea might have supplied growth limiting nutrient to the plant for metabolic activities thus enhancing (stimulating) the growth and phytoremediation potential of *Chromolaena odorata* in the polluted soil [38-40]. Shtangeeva et al. [41] also report that the addition of urea to crude oil polluted soil enhanced the growth of wheat in such soil. It can thus be deduce that the plant provided an enabling environment for microbial increase at the root area of the plant thus encouraging microbial degradation of the hydrocarbon pollutant [42,43]. Agbede [44] has also reported an increase in microbial population in amended soil. This is feasible as a result of the symbiotic relationship between test plant and the microorganisms, which is usually simulated by the distinctive features of the plant [45,46]. Raymond and Harrison [31] opined that the biological action of plant has demonstrated an encouragement for organic contaminant reduction. Reduction in TPH and THC observed in treatment D (the control) is attributed to natural attenuation. The polluted soil undergoes self-recovery that requires long time for complete removal of the pollutant. Natural attenuation is slow thus the low reduction of pollutant observed [47,48]. Significant increase in soil pH observed in treatment C (*Chromolaena odorata* +40 g/m^2, 20 g/m^2 urea) is attributed to the combination of urea (biostimulation) and phytoremediation. Urea addition provided nutrient in the soil, improved soil properties and subsequently increase soil pH. The increase pH stimulated the growth of indigenous hydrocarbon degraders which led to the observed reduction in concentration of the pollutant (petroleum hydrocarbon) [49,50]. Similar result has been reported by Angelova et al. [51] who also observed increase in soil pH in biostimulated soil leading to availability of other soil nutrients. Increase in soil electrical conductivity observed in treatment C at 2 month could be attributed to the addition of urea as a stimulant in the polluted phytoremediated soil. This is line with Atiyeh et al. [52] who observed an increase in conductivity due to addition of organic and inorganic amendments. Increase in soil nitrogen in treatment C could be tied to input from the urea added. The stimulant (urea) enhanced the availability of nitrogen in soil for plant growth and all microbial activities resulting in biodegradation of hydrocarbon [53].

The decrease in TOC and TOM could be attributed to increase plant activities (which include utilization of the soil nutrients for their growth and development) thus producing compounds that allow microbial growth and increased microbes activities [54,55] within the rhizophere resulting in the microbe utilizing the carbon as energy source in the degradation of the pollutant [56].

The increase in phosphorus in the polluted and phytoremediated soils may have resulted from reduction in TPH and THC of the soil; an indication that soil properties previously altered by crude oil creating anaerobic environment in soil and negatively affected soil microbial communities [57-59] can be improved by phytoremediation stimulated with urea. The presence of TPH and THC in test plant indicates that the test plant has the potential for uptake of pollutant. This infers that the plant can extract and metabolize hydrocarbon pollutant from soil thus the plant is a candidate for phytoextraction of hydrocarbon contaminated soil. Higher accumulation of TPH and THC observed in stimulated phytoremediated soil (treatment C) could be attributed to the urea which improved nutrient composition of the soil leading to increase growth and tolerance of the plant to pollutant toxicity through the release of stress enzymes secreted in exudates into the soil and subsequent phytodegradation of pollutant mediated outside the plant before uptake of resultant compound within the plant [32,60].

5. CONCLUSION

The study has revealed that *Chromolaena odorata* has the potential for phytoremediating crude oil polluted soil either with or without a stimulant; with the reduction of total petroleum hydrocarbon (TPH) and total hydrocarbon content (THC) observed. However, better performance of the phytoremediation plant in reduction of TPH and THC was observed in the contaminated soil stimulated with urea especially at low concentration. This indicates that Urea amendment stimulated the rate of degradation of petroleum hydrocarbon by *Chromolaena odorata.* The high amount of THC accumulated in the plant suggests potential of *Chromolaena odorata* as accumulator.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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