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Remediation of Glyphosate Polluted Soil Using *Commelina Erecta and Triton X-100*

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KEYWORDS

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ABSTRACT: This study was carried out to investigate the potentials of *Commelina erecta* and triton X-100 in remediating glyphosate polluted soil. Humus soil sample was collected from a vegetable garden in Alakahia community in Rivers State, Nigeria and was subsequently divided into different treatment groups I-VI each containing 2 kilograms of the soil sample mixed with 50ml of glyphosate in a polypropylene bag. The different treatment groups were incubated at 28-30 ^{o}C for 60 days and thereafter analysed for pH, conductivity, heavy metals, mineral elements, organic ions, total organic carbon (TOC) and nitrogen (TON) contents of the soil. The least pH value (7.01) was recorded in group III while the highest conductivity value (4173.33µs/cm) was observed in group IV. Copper, nickel and cadmium levels in all the amended groups were reduced when compared with the control. Generally, the mineral levels were increased in the amended groups when compared with the control. The TOC and TON contents of the amended soil did not exhibit significant difference when compared with the control except for group IV.

INTRODUCTION

Man's unwholesome activities which are most times geared towards the provision of a better life for him have created severe imbalances in our ecosystem. The need to feed the world's increasing population has prompted the use of agrochemicals meant to increase food production and ensure the continuation of human race. Such agrochemicals include pesticides like glyphosate. Glyphosate, a post-emergence nonselective broad-spectrum herbicide and glyphosate containing herbicides are the most extensively used herbicides in agriculture for the control of many annual and perennial weeds [1, 2]. The widespread use of glyphosate may result in weed resistance or alter the biological functions of soil. Glyphosate can have extensive unintended effects on nutrient availability and disease severity [3] resulting from direct glyphosate-induced weakening of plant diseases and increased pathogen population and virulence [1].

Currently there are a number of possible mechanisms for the clean-up of pesticides in soil, such as chemical treatments, volatilization and incineration. Chemical treatment and volatilization although feasible are problematic as large volumes of acids and alkalis are produced and subsequently must be disposed. Incineration, which is a very reliable physico-chemical method for destruction of these compounds, has met serious public opposition, because of its potentially toxic emissions, and its elevated economic cost [4]. Overall, most of these physico-chemical technologies are expensive and rather inefficient [5] because the contaminated soil has to be excavated at a site and moved to a storage area where it can be processed.

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Due to environmental concerns associated with the accumulation of pesticides in food products and water supplies, there is a great need to develop safe, convenient and economically feasible methods for pesticides remediation [4]. For this reason, several biological techniques involving biodegradation of organic compounds by micro-organisms have been developed [6]. Studies have shown that glyphosate can be degraded by micro-organisms and plants. The most active glyphosate-degrading micro-organisms were isolated from soils polluted by organophoshates [7]. The development of an affordable and environmentally friendly bioremediation method using glyphosate-degrading bacteria is a promising approach for cleaning and restoring soils contaminated with these herbicides [8]. Several bacteria produce biosurfactant that may be used to enhance biodegradation rates of hydrophobic organic contaminants during soil remediation. Because of many advantages over the synthetic counterparts, biosurfactants are widely used in various industrial processes such as pharmaceuticals, cosmetics, petroleum, food production, enhanced oil recovery and cleaning of oil tanks and soil remediation.

Expansion of agricultural and industrial activities in recent decades has led to the pollution of soil and ground water with pesticides and many treatment processes have been developed to reduce the environmental impacts of these contaminants. Physical and chemical methods for soil clean-up are very expensive, and for this reason it is of great interest to assess the potential use of biological method in the bioremediation of glyphosate contaminated soil. Therefore, the aim of this research was to remediate glyphosate (pesticide) contaminated soil using *Commelina erecta* (a phyto-surfactant) and triton X-100 (a chemical surfactant).

MATERIALS AND METHODS

The soil samples used in this study were obtained from a vegetable garden in Alakahia community of Obio-Akpor Local Government Area of Rivers State, Nigeria. Humus soil sample was collected in the garden with aid of a clean shovel to the depth of 10cm and was immediately transferred into a clean black polypropylene bag and stored in an air tight container. The plant *Commelina erecta* was collected with the leaves fresh from a garden in Alakahia community, Obio-Akpor Local Government Area, Rivers State and was duly identified by a plant scientist in the Department of Plant Science, University of Port Harcourt, and Rivers State, Nigeria.

Experimental design

Exactly 2 kg of humus soil samples were placed in six polypropylene bags and labelled I, II, III, IV, V and VI respectively. In each of the bags, 50 ml of the herbicide (glyphosate) was added, mixed properly and left for 14 days. At the end of the 14 days, the glyphosate polluted soil was treated as follows:

Sample I (Polluted and not remediated) polluted 2kg humus soil; no remediation treatment was performed on it and therefore served as the control.

Sample II (Polluted and treated with 5ml of triton X-100)

Sample III (Polluted and treated with 50g of *Commelina erecta*)

Sample IV (Polluted and treated with 100 ml of triton X-100)

Sample V (Polluted and treated with 100g of *C. erecta*)

Sample VI (Polluted and treated with 5ml of triton X-100 and 50g of *C. erecta*)

The content of each bag was properly mixed and left for 60days prior to analysis

Determination of soil pH

The determination of pH was carried out according to the method of [9]. Twenty grams of the soil sample was mixed with de-ionized water using a clean glass rod. The sample was allowed to stand for 1hr for the stabilization of temperature. The sample temperature was measured and the temperature regulator of the pH meter was set to be as that of the sample temperature. Standardization of the pH meter was performed by dipping the electrodes into the standard solution provided at a pH of 7.01. The electrodes of the meter were allowed to make good contact with the soil samples and left for 30 seconds before reading to allow the meter to stabilize. The pH value was read and recorded.

Determination of soil conductivity

Soil conductivity was determined according to the method of [10]. Exactly 20g of air-dried soil was

weighed into a 50ml beaker and 20ml of distilled water was added and occasionally stirred with a glass rod. The sample was allowed to stand for 30 minutes after which the electrodes of the conductivity meter was inserted and the reading taken.

Analysis of metals present in the samples

Analysis of metals present in the samples was according to [11]. Five grams of the soil samples were weighed into 100ml teflon beakers and 20ml of 1.0M HCl acid was added and thereafter transferred unto a hot plate where it was heated to near-dryness. The acid digest was filtered into a 50ml measuring cylinder via a filter paper and the residue rinsed thoroughly to allow for further washing through of the metals. The volume of the filtrate in the measuring cylinder was made up to 50ml with distilled water. End determination of the metal was performed using Atomic Absorption Spectrophotometer after calibrating using standard solutions of the respective metals of interest.

Determination of total organic carbon in soil

Total organic carbon determination was according to the method of [12]. Two grams of soil samples were weighed into a 250ml Erlenmeyer flask and 10ml of $K_2Cr_2O_7$ solution was added to it while swirling gently. To the content in the flask, 20ml of Conc. H_2SO_4 was added and the flask swirled to allow for proper mixing of the reagents with the soil. The flask was allowed to stand for 30 minutes after which 100ml of distilled water was added and filtered, 3 drops of ferroin indicator was added to the filtrate and titrated with 0.5N ferrous sulphate solution until the end point was reached. Percentage organic carbon was obtained using the formula:

$$\%\, Total\ Organic\ Carbon = \frac{Blank - volunme\ (sample\ titre) \times 0.195}{Weight\ of\ soil\ sample\ (g)} \times 100$$

Determination of total organic nitrogen in soil

This analysis was performed using the regular micro-kjeldahl method [13]. Ten grams of air-dried soil sample was weighed into a dried 500 ml micro-Kjeldahl flask and 20 ml of distilled water was added. It was then allowed to stand for 30 minutes after which 1 tablet of mercury and 10g of K_2SO_4 (Kjeldahl catalyst) was added to the content of the flask. The micro-kjeldahl method was employed in the determination of the total organic nitrogen in the soil samples.

Determination of available phosphate in the soil

Available phosphate in soil samples was determined according to the method of [14]. One gram of air-dried soil sample was weighed into a 15ml test tube and 7ml of the extraction solution (15ml of 1.0N NH_4F and 25ml of 0.5N HCl added to 460ml distilled water) added to the sample. The test tube and content was shaken by means of a mechanical shaker and was ten centrifuged at 2000rpm for 15 minutes. Two millilitres of the supernatant was pipetted into a 20ml test tube, 5ml distilled water and 2ml of 0.5 ammonium molybdate solution were added. The content was mixed properly and thereafter, 1ml of 0.1M $SnCl_2.2H_2O$ dilute solution was added and mixed. After 5 minutes, the percentage absorbance was measured using a spectrophotometer at 660nm wavelength.

Determination of sulphate in the soil

Soil sulphate was determined according to the method of [15]. Exactly 10ml of the soil extract (the extracting medium was $CaCl_2$) was pipetted into a 25ml volumetric flask and the volume was made to 20ml by adding distilled water. One millilitre of 5.0M gelatine-barium chloride reagent was added to the flask and the content mixed thoroughly. The flask was left to stand for 30 minutes and thereafter the percentage absorbance and optical density (OD) were measured at 420nm using a spectrophotometer. The concentration of sulphate in the soil sample was obtained using the formula:

$$Sulphate\ concentration\ \left(\frac{mg}{kg}\right) = \frac{Total\ conc.of\ solution}{molar\ conc.of\ solution} \times \%\ absorbance$$

Determination of nitrate in the soil

This analysis was carried out according to the method of [16]. The soil sample (5.0g) was transferred into a shaking bottle. Exactly 0.25g of activated carbon and 20ml of extracting solution (30ml of 99.58% acetic acid + 100 sodium acetate dissolved in 500ml of distilled water). The bottle was hand shaken for 1 minute and filtered using a filter paper. Thereafter, 1ml aliquot of the soil extract was transferred to a vial. Exactly 0.5ml of the brucine reagent and 2ml of 2M sulphuric acid was added and mixed thoroughly. The vial was allowed to stand for 5 minutes after which 2ml of distilled water was added and mixed again. The samples were transferred into a test tube placed in cold water for 5 minutes. The absorbance was measured at 470nm.

RESULTS

pH and conductivity values of the soil samples

Results in Tables 1 and 2 shows the pH and conductivity values in soil samples analysed in this present study. The pH values ranged from 7.01 to 7.87 in all sampling groups, with the least and highest values recorded for groups III (soil sample + 50g macerated plant) and group V (soil sample + 100g macerated plant) respectively. The conductivity values ranged from 2146.67 to 4173.33 (µs/cm) with group IV and V recording the least and highest values respectively.

Table 1. pH values of soil samples.

Groups	Soil Samples	pH
I	Soil sample without Treatment (Control)	7.43 ± 0.02^{a}
II	Soil sample + triton X-100 (5ml)	7.66 ± 0.02^{b}
III	Soil sample + 50g macerated plant	7.01 ± 0.05^{c}
IV	Soil sample + triton X-100 (10ml)	7.77 ± 0.02^{b}
V	Soil sample + 100g macerated plant	7.87 ± 0.03^{c}
VI	Soil sample + triton X-100 (5ml) + 5g macerated plant	7.42 ± 0.01^{a}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with differentsuperscript in the same column are significantly different at ($p\leq0.05$) when compared with the control, while values with same superscript are not statistically significant.

Table 2. Conductivity values of soil samples

Groups	Soil Samples	Conductivity (µs/cm)
I	Soil sample without Treatment (Control)	3496.67 ± 12.02^{a}
II	Soil sample + triton X-100 (5ml)	2260.00 ± 70.24^{b}
III	Soil sample + 50g macerated plant	3383.33 ± 61.19^{a}
IV	Soil sample + triton X-100 (10ml)	2146.67 ± 37.12^{c}
V	Soil sample + 100g macerated plant	4173.33 ± 29.06^{d}
VI	Soil sample + triton X-100 (5ml) + 50g macerated plant	2320.00 ± 100.66^{e}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with different superscript in the same column are significantly different at ($p\leq0.05$) when compared with the control, while values with same superscript are not statistically significant.

Heavy metal levels of soil samples

The mean concentration of heavy metals obtained in the present study are as shown in Table 3. The results obtained for lead (Pb) ranged from 0.06 to 8.13 mg/kg with the least and highest values recorded for groups III and II respectively. The values for copper (Cu) ranged from 0.56 to 1.49 mg/kg with groups IV and I recording the least and highest values respectively. Similarly, nickel (Ni) recorded the least and highest values in groups IV and II respectively while the least and highest values for cadmium (Cd) were recorded in groups IV and I respectively.

Table 3. Heavy metal levels of soil samples.

Groups	Soil Samples	Pb(mg/kg)	Cu (mg/kg)	Ni(mg/kg)	Cd(mg/kg)
I	Soil sample without Treatment (Control)	4.65 ± 0.03^{a}	1.49 ± 0.02^{a}	3.02 ± 0.21^{a}	0.33 ± 0.04^{a}
II	Soil sample + triton X-100 (5ml)	3.87 ± 0.18^{b}	0.78 ± 0.06^{b}	2.38 ± 0.29^{a}	0.09 ± 0.01^{b}
III	Soil sample + 50g macerated plant	0.06 ± 0.00^{c}	0.99 ± 0.06^{c}	1.13 ± 0.03^{b}	0.09 ± 0.01^{b}
IV	Soil sample + triton X-100 (10ml)	2.24 ± 0.39^{d}	0.56 ± 0.10^{d}	0.40 ± 0.01^{c}	0.04 ± 0.01^{b}
V	Soil sample + 100g macerated plant	0.84 ± 0.30^{c}	0.66 ± 0.04^{c}	0.51 ± 0.03^{d}	0.05 ± 0.01^{b}
VI	Soil sample + triton X-100 (5ml) + 50g macerated plant	1.57 ± 0.23^{d}	0.91 ± 0.02^{f}	1.80 ± 0.05^{e}	0.07 ± 0.01^{b}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with different superscript in the same column are significantly different at ($p\leq0.05$) when compared with the control, while values with same superscript are not statistically significant.

Mineral element levels of soil samples

The mineral element levels obtained in this study are as presented in Table 4. Sodium had the least and highest values in groups V and IV respectively whereas the least and highest values for magnesium (Mg) were recorded in groups V and II respectively. Also, potassium (K) recorded the least and highest values in groups II and V respectively and which was statistically significant ($p\leq0.05$). Calcium recorded the least and highest values in groups V and VI respectively which was also statistically significant ($p\leq0.05$).

Table 4. Mineral element levels of soil samples.

Groups	Soil Samples	Na(mg/kg)	Mg (mg/kg)	K(mg/kg)	Ca(mg/kg)
I	Soil sample without Treatment (Control)	130.37 ± 1.63^{a}	127.57 ± 1.89^{a}	48.53 ± 0.73^{a}	957.60 ± 10.52^{a}
II	Soil sample + triton X-100 (5ml)	143.57 ± 6.80^{a}	151.17 ± 4.97^{b}	26.50 ± 1.33^{b}	1027.73 ± 33.21^{b}
III	Soil sample + 50g macerated plant	123.83 ± 2.62^{a}	145.10 ± 3.64^{b}	87.83 ± 0.73^{c}	910.93 ± 7.83^{a}
IV	Soil sample + triton X-100 (10ml)	148.37 ± 1.05^{b}	122.70 ± 2.24^{a}	44.03 ± 1.30^{a}	788.97 ± 3.85^{b}
V	Soil sample + 100g macerated plant	108.37 ± 1.77^{c}	98.73 ± 1.29^{c}	163.07 ± 3.28^{d}	457.33 ± 11.00^{c}
VI	Soil sample + triton X-100 (5ml) + 50g macerated plant	109.57 ± 1.39^{d}	138.93 ± 1.15^{f}	63.23 ± 2.22^{e}	1048.63 ± 35.80^{b}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with different superscript in the same column are significantly different at ($p\leq0.05$) when compared with the control, while values with same superscript are not statistically significant.

Exchangeable anion level of soil samples

The results of the exchangeable ions are as presented in Table 5. Results obtained indicate that phosphate had the highest value (34.77 mg/kg) recorded for group I while the least value (21.27 mg/kg) was recorded for group V which was significant at $p \leq 0.05$. Conversely, sulphate had the highest and lowest values recorded for group V and I respectively and was statistically significant ($p \leq 0.05$). The highest and least values for nitrate were recorded in groups V and IV respectively which was also statistically significant ($p \leq 0.05$).

Table 5. Exchangeable anion levels of soil samples.

Groups	Soil Samples	Phosphate(mg/kg)	Sulphate(mg/kg)	Nitrate(mg/kg)
I	Soil sample without Treatment (Control)	34.77±0.32^{a}	32.33±1.45^{a}	37.33±0.88^{a}
II	Soil sample + triton X-100 (5ml)	23.47±1.16^{b}	45.33±3.28^{b}	15.67±1.45^{b}
III	Soil sample + 50g macerated plant	29.53±0.60^{c}	44.67±1.45^{b}	32.00±1.53^{a}
IV	Soil sample + triton X-100 (10ml)	22.07±1.01^{b}	39.67±1.45^{a}	10.33±0.88^{b}
V	Soil sample + 100g macerated plant	21.27±0.58^{b}	142.00±2.52^{c}	48.67±1.45^{c}
VI	Soil sample + triton X-100 (5ml) + 50g macerated plant	27.00±1.15^{c}	44.00±2.65^{b}	22.00±1.73^{d}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with different superscript in the same column are significantly different at ($p \leq 0.05$) when compared with the control, while values with same superscript are not statistically significant.

Total organic carbon and nitrogen of soil samples

Results in Table 6 shows the total organic carbon and nitrogen analysis obtained in this study. Groups II and IV recorded the least and highest values respectively for TOC and TON contents and was statistically significant ($p \leq 0.05$).

Groups	Soil Samples	TOC(%)	TON(%)
I	Soil sample without Treatment (Control)	1.76±0.02^{a}	0.15±0.00^{a}
II	Soil sample + triton X-100 (5ml)	1.68±0.03^{a}	0.14±0.00^{a}
III	Soil sample + 50g macerated plant	1.77±0.02^{a}	0.16±0.00^{a}
IV	Soil sample + triton X-100 (10ml)	2.08±0.03^{b}	0.18±0.00^{b}
V	Soil sample + 100g macerated plant	1.76±0.03^{a}	0.15±0.00^{a}
VI	Soil sample + triton X-100 (5ml) + 50g macerated plant	1.73±0.03^{a}	0.16±0.01^{a}

Values are expressed as mean± standard error of mean (SEM) of triplicate determinations, (n=3). Values with different superscript in the same column are significantly different at ($p \leq 0.05$) when compared with the control, while values with same superscript are not statistically significant.

DISCUSSION

pH and conductivity properties of the soil samples

The pH and conductivity values of soil samples polluted with glyphosate and the ability of *Commelina erecta* and triton X-100 to remediate such soils were investigated in this study. It was observed that group v had the highest PH (7.87±0.03) when compared with the control and other groups and this value was statistically significant ($P \leq 0.05$). The pH values obtained in the present study corroborates the report of Greenfield [17] and Anacletus *et al.,* [18]. They reported that for a favourable mineralization of organic pollutants, a pH range of 6.5-8.0 is required. The pH correlates with accumulated acid formation and the breakdown of such acids to produce heat and CO_2 [19]. As reported by Anacletus *et al.,* [20], when the rate of acid formation is faster than the rate at which it is degraded, acids build up and the pH falls and when the rate of acid formation and degradation are equal, the pH attains equilibrium. Hence the pH values in present study for all the test groups were suitable for soil micro-organisms to thrive and thus enhance bioremediation. This study corroborates the report of Dibble and Bartha [21]. However, the pH trend in present study was not in agreement with the reports of Ayotamuno *et al.,* [22] and Merkl *et al.,* [23]. These scientists reported that soil pH decreased due to the degradation of organic pollutants. Since soil microbes thrive better in neutral than in acid soils [24], results in this study indicates that *C. erecta* enhance the microbial population in glyphosate polluted soil and thereby lead to higher degradation of this pesticide in the soils [25]. There was an increase in the electrical conductivity of the soil sample treated with 100g of macerated plant (group V) when compared with the control (group I) which may be an indication that *C. erecta* enhanced the electrical conductivity of the soil. This could also be attributed to the pH of the soil.

Heavy metal levels of the soil samples

The heavy metal content of the soil sample in this study was significantly decreased in all the treatment groups as compared with the control (group I). This significant reduction especially in group III and IV is an indication that some microbial and enzymatic reactions contributed by addition of *C. erecta* may have been responsible for the reduction in metal level. This may be attributed to the fact that the salts of these metals are soluble and thus are accumulated by the plant. Several authors have reported the ability of plants to accumulate toxic heavy metals in their tissues and are therefore used for bioremediation. Clemence *et al.*,[26] and Grispen *et al.,* [27] suggested that ideal plants for remediation purposes should possess properties such as fast growing high biomass and should be able to accumulate a range of heavy metals in their tissues. Results in this study agrees with the findings of Ekwumemgbo *et al.*,[28] who carried out a phytoremediation of heavy metals contaminated soil using *Bryphyllum pinnatum*. At maximum bioaccumulation of heavy metals, their concentrations followed the order: Pb > Ni > Cu > Cd. This is seen in the difference between the control (group I) and the least values in the treatment groups. This trend agrees with the findings of Ekwmemgbo *et al.*,[28] who reported similar trend. The effect of the surfactant used (triton X-100) was observed to have effectively clean up the soil and thus provided a suitable atmosphere for microbial growth which enhanced the reduction of the heavy metals in the polluted soil. This finding is in agreement with the report of Anacletus *et al.* [20].

Mineral element levels of the soil samples

The mineral elements analysis of the soil samples in this study showed variation in their distribution pattern with Calcium (Ca) having the highest value followed by magnesium (Mg), sodium (Na) and potassium (K). There was an observed increase in the mineral element concentration in groups II and IV as compared with the control (group I) for sodium. Magnesium recorded an increase in groups II, III and IV when compared with the control. Potassium recorded an increase in groups III, V and VI while Ca recorded an increment in groups II and VI when compared with the control (group I). This may be an indication that *C. erecta* and triton X-100 was able to enhance the mineral elements of the soil which is

essential for plant growth. Result in present study is in agreement with the research conducted by Anacletus *et al.,* [19].

Exchangeable anion of the soil samples

The phosphate and nitrate levels in this study indicate that the treatment groups (groups II to IV) except nitrate (group V) showed decreased amounts as compared with the control (group I). this may be attributed to an uptake of this organic ion by soil microbes for growth during their incubation period [20]. However, sulphate was observed to be increased when compared to the control which may indicate that they were not utilized by the soil microbial community for growth during the incubation period.

Total organic carbon and nitrogen of the soil samples

The total organic carbon and nitrogen of the soil sample analysed in all the treatment groups did not have any significant difference when compared with the control (group I) except for TOC content of group IV which was observed to have a significant increase as compared to the control. This may be an indication of rapid consumption of both the organic carbon and nitrogen content of the soil by microbial activity which may have utilized them for growth during the incubation period. Atlas and Bartha [29] asserted that in an oil contaminated soil, there could be a nitrogen deficiency which retards the growth of bacteria as well as deficiency in certain nutrients which may be growth rate limiting.

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