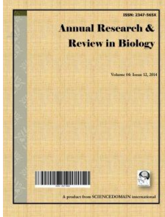

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Variance in Protease, Dehydrogenase, Phosphatase and Respiratory Activities during Phytoremediation of Crude Oil Polluted Agricultural Soil Using *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CCC, JCI, MOM and CE designed the study. Authors CCC and JCI wrote the protocol, performed the statistical analysis and managed the analyses of the study. Author CCC wrote the first draft of the manuscript and managed the literature searches. Author CE identified the plant species. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the variation in the activities of some soil enzymes and microbial respiration during phytoremediation of crude oil polluted agricultural soil.
Study Design: Indigenous plants of a crude oil polluted agricultural farmland were harvested and identified. Two species (*Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f.) were

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selected for this study. Nursery was set up using sterile soil and mature and viable seeds of selected species, and germinated seedlings were transplanted into an 8 kg potted homogenised polluted soil for remediation.
Place and Duration of Study: Polluted agricultural soil from Ogoniland Nigeria, University of Port Harcourt ecological garden, between May 2017 and February 2018.
Methodology: Protease activity was determined based on the amino acids released after incubation of soil with sodium caseinate. Phosphatase activities determination was based on determining the degradation extent of ρ-nitrophenol phosphate (PNPP) by the samples. Dehydrogenase activity was determined based on estimation of 2,3,5- triphenyltetrazolium chloride (TTC) reduction to triphenyl formazan (TPF) in soils after incubation. The substrate induced method was adapted to estimate the respiratory activity. Organic matter was determined by weight loss on ignition method.
Results: Dehydrogenase activities in remediated groups increased after 4 weeks but decreased at the end of the remediation period. Protease and phosphatase activities, and soil organic matter of remediated groups reduced over time while the soil microbial respiratory activity reduced at the end of 12 weeks remediation. There was a restoration of the polluted soils by the treatments towards normalcy with regards to activities of proteases, acid and alkaline phosphatases, and dehydrogenases.
Conclusion: Soil microbial activities can reflect soil quality, and soil enzyme activities can directly reflect the metabolic need and nutrient availability of soil microorganisms. The extracellular enzymes (protease, dehydrogenase, acid and alkaline phosphatase) were shown to vary with crude oil pollution relative to time thus indicating ameliorative effects.

Keywords: Dehydrogenase; phosphatase; respiratory activity; phytoremediation; Schwenkia americana; Spermacoce ocymoides.

1. INTRODUCTION

Soil is an abode for life [1] and its contamination by petroleum hydrocarbons emanating from commercial exploration and spillage [2] from petroleum industry activities (including oil exploration, drilling, production, transportation, processing and storage), well blow-outs, pipeline rupture, tanker accidents, and pipeline vandalisation by saboteurs and hoodlums poses a risk challenge in many oil producing areas ascribable to their environmental consequences to man [3].

Oil pollution dreadfully affects soil ecosystem through adsorption and surface assimilation of soil particles purveying of an excess carbon which might be unattainable for microbial use and the investiture of a constraint in soil nutrients [4]. During oil spillages, non-organic compounds, carcinogens, and growth inhibiting chemicals obtainable in crude oil are introduced to the environment [3], and protracted exposure to acute oil contamination could result to the instigation of kidney and liver diseases, bone mutilation marrow and intensified risk of cancer [5]. There is a proportional reduction in contaminant extraction and biodegradation because the interaction between particles of soil and pollutants increase [6]. Biodegradation makes use of bacteria, fungi or various biological means to disintegrate materials. Microorganisms possess a great ability to metabolise degradable contaminants by employing them as the energy source and/or converting them to non-toxic product such as carbon dioxide, biomass and water. This relies on the nature and amount of hydrocarbons present [7].

Soil microbial and enzymatic activities of soil can reveal succinctly the soil quality [8]. The soil enzymes activities of soil enzymes can be used to reveal metabolic need and nutrient availability of soil microorganisms which are essential in processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter [9]. Extracellular enzymes such as proteases, dehydrogenases and phosphatases are involved in organic matter decomposition process and cycling of key elements such as carbon, nitrogen and phosphorus [10]. Studies have revealed that enzyme activities in the soil are related to heavy metal contamination. Almost all enzyme activities in soils are significantly reduced by 10 to 50 times with heavy metals concentration increase at soil [11]. Heavy metal toxicity affects microbial population size, diversity, and activity and also affects their genetic structure. It also alters nucleic acid structure, disrupts cell membrane, and causes

functional disturbance thereby inhibiting the enzyme activity and oxidative phosphorylation [12]. This study thus assays for the presence of some soil enzymes in crude oil polluted agricultural soil and their activities with respect to remediation of the soil using *S. americana* and *S. ocymoides*.

2. METHODOLOGY

A polluted agricultural farmland located in Ogoniland, Nigeria was identified in Bodo community, Gokana L.G.A. of Rivers State and assessed to ascertain contaminants types involved and to determine most appropriate technologies to its restoration. In this assessment, the site was mapped to determine its physical characteristics and contaminants' size and location as well as plant ecological community. Thereafter, indigenous plants from polluted site were harvested and taken to Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria for identification. Two species (*S. americana* and *S. ocymoides*) were selected for the study based on existing reports on their ability to thrive in polluted environments. The polluted soil sample that was remediated in this study was collected from crude oil polluted site while unpolluted soil sample was collected from an agricultural farmland within University of Port Harcourt. These soil samples were collected following method described by Motsara & Roy [13]. Prior to remediation of polluted soils, viable seeds of selected plants were used to set up a nursery, using sterile unpolluted soil. Three to four weeks after germination of seeds to seedling levels, 4 seedlings each of the plants were transplanted into an 8 kg potted homogenised polluted soil set up in triplicate. Two sets of pots were set up in triplicate, one with polluted soil alone and the other with unpolluted soil alone, without vegetation in order to serve as controls. For laboratory analyses, soil samples were first collected before potting for baseline analyses and subsequently at 4th, 8th and 12th week. Fresh soil samples were collected and taken immediately to the laboratory. The activities of acid and alkaline phosphatases, dehydrogenases and proteases were assayed. The organic matter content of the soil was also determined.

The method of Alef & Nannipieri [14] with modification was employed for all enzyme assays and respiratory activity performed in triplicate and compared to controls while soil organic matter was determined by loss of weight on ignition method [13].

The assay of protease activity is based on determining the amino acids released after soil incubation with sodium caseinate for 2 hours at 50°C using Folin-Ciocalteu reagent. Two grams of moist, sieved (2 mm) soil was weighed into a 15 mL centrifuge tubes designated as test and control. Aliquot (5 mL) of 1% substrate, prepared at night before and kept in a refrigerator, was added to test tubes. For controls, only 5 mL of TRIS HCl buffer at pH 8.1 was added. The tubes were shaken for 2 hours at 50°C and cooled immediately in cold water. An aliquot of 2 mL 17.5% trichloroacectic acid was added into test and control tubes and centrifuged at 3000 rpm for 2 minutes. The supernatant (2 mL) was dispensed into test tubes, and 3 mL 1.4 M $NaSO_4$ was added in both test and control tubes. The tubes were shaken thoroughly and 1 mL of dilute Folin-Ciocalteu reagent (prepared by diluting three times) was added, and the content of the tubes centrifuged at 200 rpm for 2 minutes. An aliquot from each tube was taken and read on a spectrophotometer at a wavelength of 578 nm. TRIS HCl buffer at pH 8.1 was used as blank and calibration curves were prepared.

Enzyme activity (mg tyrosine kg^{-1} dry matter h^{-1}) = (C x 10.5) /dw

Where, C = measured tyrosine concentration; dw = dry weight of 2 g moist soil.

Acid and alkaline phosphatase activities were assayed based on determination degradation extent of ρ-nitrophenol phosphate (PNPP) by the samples. One gram each of air-dried and homogenised soil samples was placed in centrifuge tubes and 0.25 mL toluene was added and placed in a fume chamber for 10 minutes. Thereafter, 4 mL of ACP (or ALP) buffer solution was added followed by addition of 1 mL of ACP (or ALP) PNP substrate. The tubes contents were incubated for 1 hour at 37°C. Aliquots of 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to the tubes, whose contents were shaken with a rotator mixer for 3 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant was read spectrophotometrically at 485 nm using ACP (or ALP) buffer solution as blank. The controls were prepared similarly but without substrate while calibration curve was prepared using standard ρ-nitrophenol solution. The ρ-nitrophenol per mL of filtrate was calculated by reference to the calibration curve.

The ρ-nitrophenol released after incubation was used to calculate the enzyme activity as follows:

Enzyme activity (mmol PNP kg^{-1} dw h^{-1}) = (C x V) / (dw x SW x t)

Where, C = measured concentration of ρ-nitrophenol in μg ml^{-1} filtrate; V = total volume of soil suspension in ml; dw = dry weight of 1 g moist soil; SW = weight of the soil sample used; and t = incubation time in hours.

The assay of dehydrogenase (DH) activity is based on the estimation of 2,3,5-triphenyltetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) in soils after incubation at 30°C for 24 hours. Five grams of field-moist soil was prepared in centrifuge tubes designated as test and control tubes. Using Eppendorf pipette, 5 mL of the 1% TTC was added to the test tubes while 5 mL of TRIS HCl buffer at pH 7.4 was added to control tubes. The tubes contents were mixed thoroughly and incubated at 30°C for 3 d. After incubation, 20 mL of methanol was added in all tubes and shaken for 5 minutes on a turnover shaker at room temperature and then centrifuged at 300 rpm for 3 minutes. An aliquot from each tube was taken and analysed using a spectrophotometer at a wavelength of 485 nm, using TRIS HCl at pH 7.4 as blank. The ρ-nitrophenol contents of filtrates were extrapolated from the calibration curve. The amount of TPF formed was used to evaluate enzyme activity as follows:

Enzyme activity (mg TPF kg^{-1} d^{-1}) = (C x v) / (dw x SW x t)

Where, C = measured concentration of TPF in mg ml^{-1} filtrate; v = total volume of soil suspension in ml; dw = dry weight of 1 g moist soil; SW = weight of the soil sample used; and t = incubation time in hours.

The substrate (glucose) induced method was adapted to estimate the respiratory activity. A 10 g screened (2 mm sieve) soil sample was added into outer jar of respiratory flask. An aliquot of 2.5 mL 0.2 M NaOH was added into inner jar. Glucose solution (1 mL) was added to soil. For the control flasks, screened (2 mm) 10 g of soil sterilised in an autoclave at 121°C for 20 minutes was used. An aliquot of 2.5 mL 0.2 M NaOH was added with 1 mL 40% glucose as describe above. The flasks were allowed to stand for 24 hours at 25°C. Afterwards, all the NaOH was transferred to a 25 mL beaker containing 1 mL 1 M $BaCl_2$ and the solution was titrated using 0.1 M HCl and phenolphthalein indicator. The titre values were noted for calculation.

To calculate the mass of CO2 generated:

CO_2.C (mg) = [(HCl_b – HCl_s) / 1000 mL/L] × HCl molarity (mol/l) × 12g C/mol × 1000 mg/g

Where, HCl_b = ml HCl used in titration of blank; HCl_s = ml HCl used in titration of sample; CO_2•C = mass of CO_2-carbon generated (mg).

This simplifies to:

CO_2.C (mg) = (HCl_b – HCl_s) × 12

To determine the organic matter content of soil samples, five grams of sieved (2 mm) soil samples were weighed into crucibles. The crucibles with soil samples were placed in a drying oven, set at 105 °C and allowed to dry. After 4 hours, the crucibles were removed from drying oven and placed in a dry atmosphere. When cooled, the crucibles with soil samples were weighed to nearest 0.01 g. Again, the crucibles with dried soil samples were placed in a muffle furnace, set at 400 °C. After 4 hours of ashing, they were removed from muffle furnace, cooled in a dry atmosphere, and reweighed to the nearest 0.01 g.

The percentage organic matter is given by:

% OM = [(W_1- W_2) / W_1] × 100

Where, W_1 = the weight of soil at 105°C; W_2 = the weight of soil at 400°C.

2.1 Statistical Analyses

Results of all the studies are expressed as means ± standard deviation of triplicate determination. To detect a significant difference between the groups, statistical analysis was carried out using one way analysis of variance (ANOVA). Data between groups were analysed by the Bonferroni test using Statistical Package for the Social Science (SPSS®) Version 20 statistics software at 95% (P = 0.05) confidence level, while data between periods were analysed by the Student t-test.

3. RESULTS AND DISCUSSION

The protease activities of various soil samples are presented in Table 1. Compared to baseline values, the protease activities of remediated

groups reduced over time. This may be due to inhibitory influence of remediating plants on soil microorganisms. It may however be due to limiting effect of nutrients in the pots, since they have been depleted over time, with resultant reduction in microbial activity. The later argument may account for the reduction observed at the unpolluted group. The former contention can be substantiated by the findings of Adeniyi et al. [15] that plant extracts inhibited the growth of certain fungi and bacteria such as the fungi *Penicillium chrysogenum* and bacteria *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Percentage recovery of the remediated soils, calculated as: % recovery = {[Parameter in consideration] × [Test (polluted) Control] / [Normal (unpolluted) control] × [Test (polluted) Control]} × 100, showed that by week 4, the treatments restored the polluted soils towards normal values (12.82% and 9.74%), however, by weeks 8 and 12, the values nose dived, indicating a failure in restoration, especially with regards to the activities of proteases.This study also showed a correlation between organic matter versus enzyme activities, and organic matter versus respiratory activities. The Pearson's correlation coefficient of -1.00, -0.98 and -0.80 (Table 7) for protease activity in unpolluted control, polluted control and *S. americana* remediated groups respectively, showed substantial negative correlations with organic matter (OM) where as soil remediated with *S. ocymoides* showed fair positive correlation (+0.47 PCC).

The dehydrogenase activities of various soil samples are presented in Table 2. Compared to baseline values, the dehydrogenase activities of remediated groups showed a significant (*P= 0.05*) rise in activities after 4 weeks but reduced at the end of remediation. The increase may have been a result of an initial increase in microbial population within the first 4 weeks which afterwards reduced with depletion of carbon source or available nutrients, since they have been depleted over time, with the resultant reduction in microbial activity. Though there might be available nutrients in the unpolluted group, the absence of a carbon source may account for the insignificant activities observed. Zhuang et al. [16] reported an undesirable reduction in dehydrogenase activity and associated that with the low activities of microorganisms in the polluted soil. Nath & Samanta [17] further reported that both the microbial population, activity of microbial population and the kind of microbe present in the soil determine the enzyme activity. This trend as observed in Table 3 follows similar trends reported by some authors [18,19,20]. By week 4, the treatments restored the polluted soils to normal values (164.95 % and 106.89 %), while by week 12, the treatment with *S. spermacoce* restored the polluted soil to 2144. 49 %. However, the values by week 12 for *S. americana* treatment nose dived, indicating a failure in restoration, especially with regards to the activities of dehydrogenases. Furthermore, substantial positive correlation (+0.96 PCC) for dehydrogenase activity and OM was observed only in soil remediated with *S. americana.* While unpolluted control and soil remediated with *S. ocymoides* showed a fair positive correlation of +0.36 and +0.55 respectively, the polluted control soil showed a substantial negatively correlation (-0.90 PCC) (Table 7).

The acid and alkaline phosphatase activities of various soil samples are presented in Tables 3 and 4. Compared to baseline values, the acid phosphatase activities (Table 3) of remediated groups reduced over time. This may be due to

Table 1. Protease activity (in mg tyrosine kg^{-1} dry matter h^{-1}) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 4	Week 8	Week 12	% R Week 4	% R Week 8	% R Week 12
Unpolluted Control	32.90±3.86^{a}	38.62±0.77^{a,*}	20.34±1.59^{a,*}	11.90±1.00^{a,*}	NA	NA	NA
Polluted Control	44.44±0.77^{b}	22.00±8.06^{b,*}	13.66±43.47^{a,b,*}	2.69±2.88^{b,*}	NA	NA	NA
S. americana	44.44±0.77^{b}	24.13±3.32^{b,*}	10.54±1.18^{b,*}	1.38±0.47^{b,*}	12.82	-46.67	-14.26
S.ocymoides	44.44±0.77^{b}	23.62±16.81^{a,b,*}	6.28±5.56^{b,*}	2.40±1.19^{b,*}	9.74	-110.43	-3.15

Values are mean ± standard deviations of triplicate determinations, Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

Table 2. Dehydrogenase activity (in mg TPF kg^{-1} d^{-1}) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 4	Week 12	% R Week 4	% R Week 12
Unpolluted Control	1.18±0.95^{a}	7.67±6.72^{a}	0.61±0.08^{a}	NA	NA
Polluted Control	0.15±0.21^{b}	11.41±6.66^{a,*}	0.63±0.11^{a}	NA	NA
S. americana	0.15±0.21^{b}	5.25±2.72^{a,*}	0.92±0.40^{b}	164.95	-1285.46
S. ocymoides	0.15±0.21^{b}	7.42±0.61^{a,*}	0.15±0.06^{c}	106.89	2144.49

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

Table 3. Acid phosphatase activity (mmol PNP kg^{-1} dw h^{-1}) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 4	Week 8	Week 12	% R week 4	% R week 8	% R week 12
Unpolluted Control	1.52±1.06^{a}	1.64±0.15^{a}	7.58±1.22^{a,*}	0.05±0.06^{a}	NA	NA	NA
Polluted Control	5.47±1.74^{b}	1.57±1.00^{a}	2.84±4.61^{a,b,c}	0.15±0.04^{a,*}	NA	NA	NA
S. americana	5.47±1.74^{b}	4.54±1.21^{b}	6.13±1.72^{c}	0.21±0.39^{b,c,*}	4797.25	69.41	-56.44
S. ocymoides	5.47±1.74^{b}	6.53±1.51^{c}	0.18±0.50^{b*}	0.21±0.25^{a,c,*}	8020.23	-56.04	-61.46

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

Table 4. Alkaline phosphatase activity (mmol PNP kg^{-1} dw h^{-1}) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 4	Week 8	Week 12	% R Week 4	% R Week 8	% R Week 12
Unpolluted Control	3.29±1.11^{a}	0.18±0.16^{a,b,*}	3.22±1.20^{a}	0.63±0.43^{a,*}	NA	NA	NA
Polluted Control	4.36±1.06^{a}	0.59±0.26^{a,c,*}	3.36±1.05^{a}	1.21±0.96^{a,*}	NA	NA	NA
S. americana	4.36±1.06^{a}	0.49±0.07^{c,*}	0.18±0.04^{b,*}	0.63±0.31^{a,*}	23.86	2193.17	100.63
S. ocymoides	4.36±1.06^{a}	0.28±0.64^{b,*}	11.41±2.44^{c,*}	0.84±0.69^{a,*}	77.69	-5547.31	64.21

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

inhibitory influence of remediating plants on soil microorganisms. It may however be due to the limiting effect of nutrients in the pots, since they have been depleted over time, with resultant reduction in microbial activity. The later argument may account for the reduction observed for the polluted and unpolluted groups, since the pots were unvegetated. However, the increase observed at week 8 for unpolluted group may indicate a rise in peak in microbial activity which may have reduced owing to depletion in available nutrients. Likewise, compared to the baseline values, alkaline phosphatase activities of remediated groups, as shown in Table 4, reduced over time albeit a recorded increase in *S. ocymoides* remediated group at week 8. The reduction may be due to hampering influence of remediating plants on soil microorganisms. Nonetheless, it may be due to limiting effects of nutrients in the pots as depletion may have taken place over the period of time, thus resulting to reduction in microbial activity. If the later argument is true, it may therefore account for the reduction observed for unpolluted and polluted groups. However, the population and/or the presence of certain microorganisms specific for alkaline phosphatase secretion may have influenced the increase in activity recorded in *S. ocymoides* remediated group at week 8. This finding is supported by the report [17], that microbial population, activity and the kind of microbe present in the soil determine the enzyme

activity. By week 4, the treatments restored the polluted soils to normal values (4797.25% and 8020.23 %) while by week 8, only the treatment using *S. americana* restored the polluted soil to 69.41 %. However, the values by week 12 nose-dived, indicating a failure in restoration, especially with regards to the activities of acid phosphatases. For treatment using *S. americana*, there was a restoration of the polluted soils towards normalcy (23.86%, 2193.17% and 100.63%) by week 4, 8 and 12 while treatment using *S. ocymoides*, there was restoration towards normalcy (77.69% and 64.21%) by week 4 and 12, even though the value by week 8 nose-dived with regards to activities of alkaline phosphatases. Acid phosphatase activity showed a substantial positive correlation with OM for unpolluted control and *S. americana* remediated soil (+0.84 and +0.80 PCC, respectively). However, whilst soil remediated with *S. ocymoides* showed a fair positive correlation (+0.55 PCC); the polluted control soil indicated almost no correlation (+0.05 PCC). On the other hand, alkaline phosphatase activity revealed a fair positive correlation with OM for unpolluted control, polluted control and soil remediated with *S. americana* (+0.61, +0.52, and +0.36 PCC, respectively), and its correlation with OM for *S. ocymoides* remediated soil revealed a fair negative correlation of -0.57 (Table 7).

Assessment of organic matter oxidation by aerobic microorganisms, known as respiration, confirmed microbial activity in all the soils. According to Wolińska et al. [21], soil respiratory activities and microbial abundance are sensitive to contamination with petroleum derivatives. Stotzky & Norman [22] associated decline of respiratory activity, similar to what is represented in Table 5, to depleted available carbon substrates. Additionally, respiration declines in soils that lack nutrients and other supporting factors for microbial and other biological activities [23]. While respiratory activity showed a substantial negative correlation with OM for the unpolluted control (-0.96 PCC), its correlation with OM for polluted control showed arguably no correlation (-0.07 PCC). Nonetheless, a fair positive correlation was recorded in *S. americana* remediated and *S. ocymoides* remediated soils (+0.51 and +0.54 PCC, respectively) (Table 7).

As shown in Table 6, the soil organic matter in remediated and polluted control groups, when compared with baseline values, reduced over time. Organic matter is the major source of plant nutrients [24] and its mineralisation depends on interaction between chemicals present in soil [4]. Brussard [25] reported that organic matter decomposition is largely a biological process that occurs naturally and determined by soil

Table 5. Respiratory activity (CO_2.C) (in mg) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 12
Unpolluted Control	1.4400±0.01^{a}	0.800±0.37^{a,*}
Polluted Control	1.9200±0.01^{b}	0.24±0.12^{a,*}
S. americana	1.9200±0.01^{b}	0.40±0.37^{b,*}
S. ocymoides	1.9200±0.01^{b}	0.28±0.18^{c,*}

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

Table 6. Organic matter (in %) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

Group	Before	Week 8	Week 12
Unpolluted Control	2.4900±0.01^{a}	2.4700±0.22^{a}	2.2267±0.19^{a}
Polluted Control	4.800±0.10^{b}	4.0200±0.09^{b,*}	3.7767±0.14^{b,*}
S. americana	4.800±0.10^{b}	3.8467±0.24^{b,*}	3.7267±0.11^{b,*}
S. ocymoides	4.800±0.10^{b}	3.8067±0.25^{b,*}	3.5767±031^{b,*}

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment*

Table 7. Pearson's correlation coefficient (PCC) of observed enzyme activities versus organic matter (OM)

Enzyme activity	Unpolluted control	Polluted control	*S. americana*	*S. ocymoides*
Protease	-1.00*	-0.98	-0.80	+0.47
Dehydrogenase	+0.36	-0.90	+0.96	+0.55
Acid phosphatase	+0.84	+0.05	+0.80	+0.55
Alkaline phosphatase	+0.61	+0.52	+0.36	-0.57
Respiratory	-0.96	-0.07	+0.51	+0.54

organisms, physical environment and quality of the organic matter. The reduction in organic matter in the groups may therefore be associated with its utilisation by microorganisms to release nutrients for use by plants and microorganisms.

4. CONCLUSION

Crude oil spillages evoke deleterious effects on environment. Both microbial activities of soil can reflect sensitively the soil quality, and soil enzyme activities can directly reflect the metabolic need and nutrient availability of soil microorganisms which are key in nutrients' processing and recovery from detrital inputs and accumulated soil organic matter. Microorganisms secrete degradative enzymes which can counter the effect induced by spillage thus effecting amelioration of polluted soil. The activities of extracellular enzymes: proteases, dehydrogenases, acid and alkaline phosphatases are shown to vary with crude oil pollution relative to time thus indicating ameliorative effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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