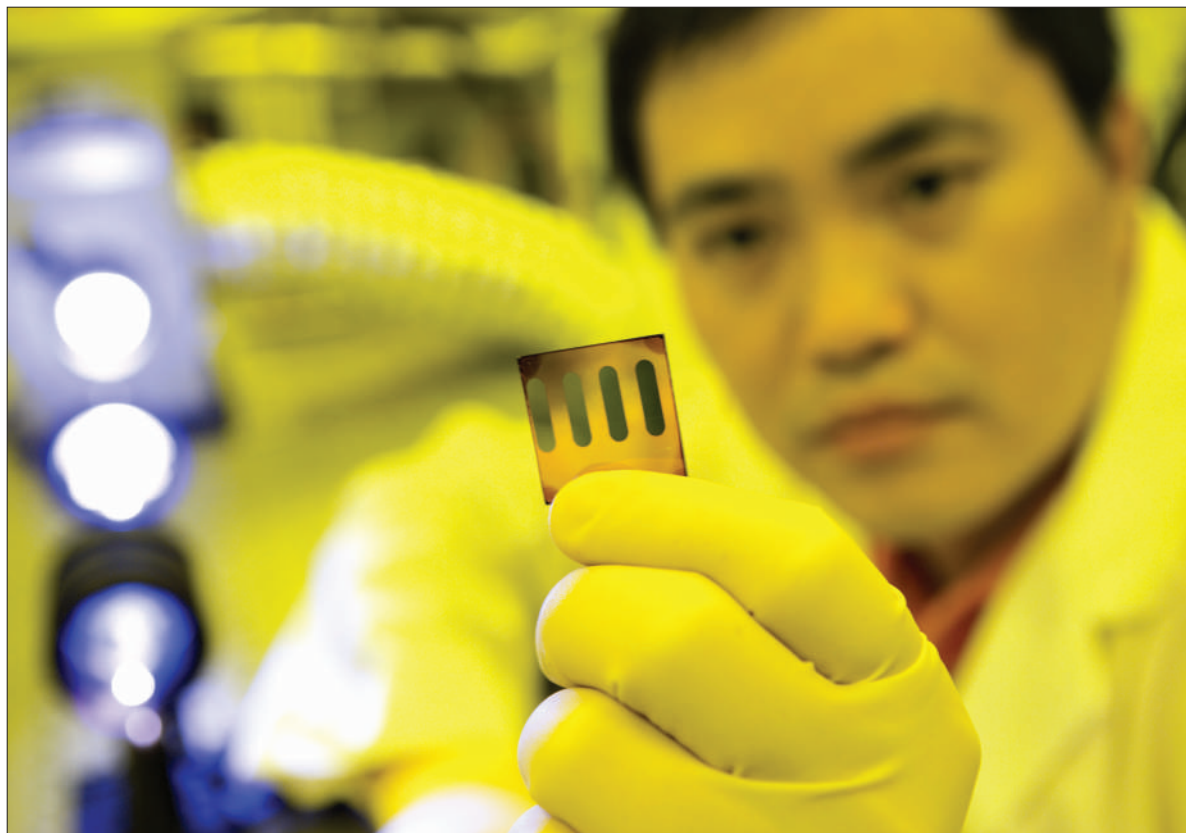




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Research Article

Soil Heavy Metals Remediation Using *Mariscus alternifolius*, *Fimbristylis ferruginea*, *Schwenkia americana* and *Spermacoce ocymoides*

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Abstract

Background and Objective: The successful use of plants to effectively remediate crude oil contamination of the ecosystems has gained prominence over the years. This study was undertaken to evaluate the soil heavy metals remediation using *Mariscus alternifolius*, *Fimbristylis ferruginea*, *Schwenkia americana* and *Spermacoce ocymoides*. **Materials and Methods:** Remediation of the contaminated soil was carried out for a 12 week period. Pot experiment was adopted for the study. Standard field and laboratory procedures were duly followed. **Results:** The heavy metals concentrations of the polluted soil before remediation were $6.27 \pm 0.10 \text{ mg kg}^{-1}$ Cd, $390.37 \pm 5.00 \text{ mg kg}^{-1}$ Pb and $143.66 \pm 1.00 \text{ mg kg}^{-1}$ Cr. Results after 12 weeks remediation, revealed amongst the plant species used for the treatment, *F. ferruginea* was the most effective species for the remediation of cadmium and chromium with 70.3 and 93.80% removal, respectively. For lead, however, *M. alternifolius* boosted as the best performing with 89.0% removal. As per restoration, *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides* restored the polluted soil towards normalcy. For lead, only treatment using *S. ocymoides* restored the polluted soil towards normalcy (65.07%) while in regard to chromium, treatments using *M. alternifolius*, *F. ferruginea* and *S. americana* restored the polluted soils at 145.47, 27.18 and 353.36% recoveries, respectively. **Conclusion:** The application of these plants has demonstrated to be efficient for the removal of heavy metals in crude oil polluted soils. These plants are, therefore, recommended for use in the remediation of heavy metals contaminated soils.

Key words: Phytoremediation, heavy metals, *Mariscus alternifolius*, *Fimbristylis ferruginea*, *Schwenkia americana*, *Spermacoce ocymoides*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crude oil, a raw material for the production of petroleum and other chemicals, is one of the most vital energy sources worldwide¹. However, the contamination of soil by crude oil resulting from anthropogenic activities introduces hazardous chemicals to the ecosystem². Nevertheless, these hazardous contaminants can effectively be removed by bioremediation to provide a long-term rehabilitation of the residual oil contamination³.

The successful use of plants to effectively remediate crude oil contamination of the ecosystems has been reported in many studies⁴⁻⁶. Sourcing for the most potent remediation species for the removal of heavy metals and other specific compounds has been a critical step in bioremediation trials. Mathematical modelling has been employed to establish appropriate plant species⁷ yet the selection of bioremediation depends on preliminary results from pot experiments⁸⁻¹⁰.

Some phytoremediation studies have been carried out in the Niger Delta, Nigeria. Most studies have reported the use of weeds. Some weeds reported include *Pennisetum purpureum*¹¹, *Talinum triangulare*, *Panicum maximum*¹², *Centrosema pubescens*⁵, *Phyllanthus amarus* Schum and Thonn., *Hyptis spicigera* Lam., *Sida rhombifolia* L.¹³, *Chromolaena odorata*, *Synedrella nodiflora*, *Talinum triangulare*¹⁴, *Axonopus compressor*, *Cyperus rotundus*¹⁵, *Tithonia diversifolia*¹⁶, *Fimbristylis littoralis*, *Hevea brasiliensis*, *Cymbopogon citratus*, *Vigna subterranea*⁶, *Phragmites australis*¹⁷, *Eleusine indica* and *Chromolaena odorata*¹⁸.

It has been implicit that the focal benefits of grass species compared to other species is the extensive fibrous root systems, which boast a significantly greater root surface area (per m⁻³ of soil) that can penetrate the soil up to the depth¹⁹ of 3 m. The goal of this experiment was to evaluate the suitability of *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides* for use in the bioremediation of heavy metals present in crude oil polluted soil.

MATERIALS AND METHODS

Assessment and mapping of the polluted site: This study was carried out between May, 2017 and February, 2018. The site for the study was a crude oil contaminated farmland situated in Ogoniland, Rivers State, Nigeria. Assessment of the farmland was effectuated and the best appropriate technique for the remediation of the soil was established. During the assessment, the site was mapped and the physical characteristics, size of the farmland, location of the pollutants and presence of plant ecological community were determined.

Collection and identification of plants: The plants used for the study were indigenous plants collected from the crude oil spill site. After collection, the plants were taken to the herbarium for identification at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.. The identified plants were *Schwenkia americana* L. (UPH/V/1295), *Fimbristylis ferruginea* (UPH/V/1296), *Spermacoce ocymoides* Burm. f. and (UPH/V/1297) and *Mariscus alternifolius* Vahl. (UPH/V/1298). Samples of the plant species were deposited in the herbarium for reference purposes.

Seed viability test: The seeds of *S. americana*, *F. ferruginea*, *S. ocymoides* and *M. alternifolius* were assembled from the wild within the University of Port Harcourt. To determine the viable seeds before use for nursery, a wet paper germination test method as described by Abedin and Meharg²⁰ was adopted. Twelve Petri dishes were rinsed with ethanol to ensure sterility. To each of the Petri dishes, 2 pieces of Whatman No. 1 filter paper measuring 10 cm in diameter were carefully placed and thereafter moistened until thoroughly damp while ensuring that runoff or dripping did not occur.

Thirty seeds each of the plant species were used for the test, totalling 120 seeds for all the selected species. All the seeds used were first surface-sterilized by soaking in NaOCl (1%) for 1 min and thereafter rinsed 3 times for another 1 min using distilled water. The dishes were set up in triplicates per species with each dish containing 10 randomly selected seed placed in a way that they were not in close proximity. The filter paper was folded in half sandwiching the seeds between the two layers and gently pressed down to ensure seeds were in contact with the damp paper. The Petri dishes were finally positioned away from direct sunlight. Soon after germination, the dish lid and paper covering the seeds were carefully removed to ensure that fragile shoots were not destroyed. The seeds with plumule and radicle extending 2 mm from the juncture were denoted as germinated.

Nursery: The seeds ascertained as viable were used for nursery. The soil for the nursery was collected from the agricultural farmland of the University of Port Harcourt without any history of pollution. To ensure sterility and the growth inhibition of unwanted competing seeds, the moist soil was first sterilized by dry heat following the method described by Baker²¹ where moist humus soil was amassed and sterilized at 82 °C for 30 min using an oven. The sterile dry soil was thereafter potted in cool condition and labelled. Seeds were then propagated after moistening the dry soil and thereafter, the germination of the seeds was monitored.

Seedlings transplant: The polluted (soil from the spill site) and unpolluted (soil with no history of pollution from an agricultural farmland of University of Port Harcourt) soil samples were amassed the method as described by Motsara and Roy²² where soil auger was used to collect soil samples between the depths of 1 and 15 cm, bagged with sterile unused plastic bags sealed with rubber band and transported to the ecological centre of University of Port Harcourt for pot experiment. Before potting of the soil samples, sieved (2 mm) and homogenized soil samples were collected from both the polluted and unpolluted soils for baseline analysis. Afterwards, a total of 24 pots were divided and set up into 2 groups. The first group contained 12 pots representing in triplicates, *M. alternifolius*, *F. ferruginea*, polluted control 1 and unpolluted control 1 while the second group, likewise, represented *S. americana*, *S. ocymoides*, polluted control 2 and unpolluted control 2 triplicate. Each of the vegetated pots contained 4 seedlings as per the plant species designated for the pot.

Analytical methods: All chemicals employed in this study were of analytical grade with high purity and acquired from Sigma-Aldrich Co., USA. The heavy and trace metals (cadmium, lead, chromium, copper, zinc, iron and manganese) were determined by microwave digestion method as described by Mwegoha and Kihampa²³ and Rashid *et al.*²⁴. Fine powdered sieved soil (2.5 g) was weighed into a crucible and mixed with aqua regia (10 mL) consisting of HCL and HNO₃ (3:1). The mixture was thereafter digested for 1 h at 95°C. The digest after cooling was diluted to 50 mL using distilled water and allowed to settle overnight. The ensuing

solution was filtered through Whatman No. 1 filter paper. The concentrations of the heavy and trace metals were determined with atomic absorption spectrometry (AAS) (SensAA).

Statistical analysis: Results are expressed as means $\pm$ standard deviation of triplicate determination. Statistical analysis was carried out using one way analysis of variance (ANOVA). The data were analyzed by the Turkey HSD test using Statistical Package for the Social Science (SPSS®) Version 20 statistics software at 95% ($p < 0.05$) confidence level.

RESULTS

The results of the heavy and trace metals before remediation (Table 1-14), revealed significantly higher ($p < 0.05$) concentrations of Cd, Pb, Cr, Cu, Fe, Mn and Zn in the polluted soil when compared to unpolluted soil.

Heavy metals

Cadmium: The Cd concentration of the polluted soil (6.27 ± 0.10 mg kg⁻¹) significantly decreased ($p < 0.05$) after remediation using in *M. alternifolius* (2.65 ± 0.29 mg kg⁻¹), *F. ferruginea* (1.86 ± 0.51 mg kg⁻¹), *S. americana* (3.22 ± 1.70 mg kg⁻¹) and *S. ocymoides* (3.59 ± 1.02 mg kg⁻¹) as shown in Table 1 and 2. While a 57.74, 70.33, 48.64 and 42.74% decrease, were respectively recorded in soils remediated with *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides*, the percentage recovery² of 136.04, 16.80, 1802.19 and 2302.73% were correspondingly noted.

Table 1: Cadmium levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	3.21 ± 0.10^a	2.41 ± 1.20^a	57.60	NA
Polluted control 1		$1.75 \pm 2.11^{a,b,*}$	72.09	NA
<i>M. alternifolius</i>	6.27 ± 0.10^b	$2.65 \pm 0.29^{a,*}$	57.74	136.04
<i>F. ferruginea</i>		$1.86 \pm 0.51^{b,*}$	70.33	16.80

Values are mean $\pm$ standard deviation of triplicate determination, ^{a,b}Significantly different at $p < 0.05$, * $p < 0.05$ compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 2: Cadmium levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	3.21 ± 0.10^a	1.98 ± 1.77^a	38.32	NA
Polluted control 2		$1.91 \pm 1.36^{a,*}$	69.54	NA
<i>S. americana</i>	6.27 ± 0.10^b	$3.22 \pm 1.70^{a,*}$	48.64	1802.19
<i>S. ocymoides</i>		$3.59 \pm 1.02^{a,*}$	42.74	2302.73

Values are mean $\pm$ standard deviation of triplicate determination, ^{a,b}Significantly different at $p < 0.05$, * $p < 0.05$ compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 3: Lead levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	104.45 ± 1.00 ^a	18.67 ± 14.44 ^{a,*}	82.13	NA
Polluted control 1		31.71 ± 45.80 ^{a,*}	91.88	NA
<i>M. alternifolius</i>	390.37 ± 5.00 ^b	43.06 ± 34.48 ^{a,*}	88.97	-87.04
<i>F. ferruginea</i>		51.28 ± 46.83 ^{a,*}	86.86	-150.08

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 4: Lead Levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	104.45 ± 1.00 ^a	102.77 ± 37.69 ^a	1.61	NA
Polluted control 2		205.88 ± 181.74 ^{a,*}	47.26	NA
<i>S. americana</i>	390.37 ± 5.00 ^b	228.12 ± 140.89 ^{a,*}	41.56	-21.57
<i>S. ocymoides</i>		138.79 ± 37.92 ^{a,*}	64.45	65.07

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 5: Chromium levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	71.50 ± 1.00 ^a	13.28 ± 7.55 ^{a,*}	81.43	NA
Polluted control 1		7.32 ± 6.14 ^{a,*}	94.90	NA
<i>M. alternifolius</i>	143.66 ± 1.00 ^b	15.99 ± 3.84 ^{a,*}	88.87	145.47
<i>F. ferruginea</i>		8.94 ± 3.54 ^{a,*}	93.78	27.18

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not Applicable

Table 6: Chromium levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	71.50 ± 1.00 ^a	70.08 ± 25.29 ^a	1.99	NA
Polluted control 2		65.47 ± 36.90 ^{a,*}	54.43	NA
<i>S. americana</i>	143.66 ± 1.00 ^b	81.76 ± 25.36 ^{a,*}	43.09	353.36
<i>S. ocymoides</i>		56.13 ± 34.88 ^{a,*}	60.93	-202.60

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Lead: Similarly, there was a significant decrease (p<0.05) from the initial Pb concentration of 390.37 ± 5.00 mg kg⁻¹ in the polluted soil to 43.06 ± 34.48, 51.28 ± 46.83, 228.12 ± 140.89 and 138.79 ± 37.92 mg kg⁻¹ in soils remediated with *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides*, respectively, thus, correspondingly accounting for 88.97, 86.86, 41.56 and 64.45% reductions as shown in Table 3 and 4. While most of the remediated soils nosedived as regards to the percentage recovery, soil remediated with *S. ocymoides* recorded 65.07% recovery.

Chromium: Chromium level decreased from the initial 143.66 ± 1.00 mg kg⁻¹ level to 15.99 ± 3.84, 8.94 ± 3.54, 81.76 ± 25.36 and 56.13 ± 34.88 mg kg⁻¹ in *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides* remediated soils accounting for 88.87, 93.78, 43.09 and 60.93%, respectively as shown in Table 5 and 6. While the soil

remediated with *S. ocymoides* nosedived as regards the percentage recovery, soils remediated with *M. alternifolius*, *F. ferruginea*, *S. americana* recorded 145.47, 27.18 and 353.36%, respectively.

Trace metals

Copper: Copper concentration, after the 12 weeks remediation, significantly increased (p<0.05) from the initial concentration of 12.81 ± 0.10 mg kg⁻¹ in the polluted soil to 55.90 ± 0.23 and 37.27 ± 7.40 mg kg⁻¹ in the soils remediated with *S. americana* and *S. ocymoides* as shown in Table 7 and 8. However, no significant difference (p>0.05) was observed in soils remediated with *M. alternifolius* and *F. ferruginea*. While the soil remediated with *S. ocymoides* plummeted as regards to the percentage recovery of the soil, those remediated with *M. alternifolius*, *F. ferruginea* and *S. americana* recorded 42.80, 18.48 and 23.29% recovery, respectively.

Table 7: Copper levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	1.48±0.10 ^a	42.65±10.07 ^{a,*}	-2781.80	NA
Polluted control 1		10.41±0.55 ^{b,*}	18.74	NA
<i>M. alternifolius</i>	12.81±0.10 ^b	24.21±13.18 ^{a,b}	-89.00	42.80
<i>F. ferruginea</i>		16.37±13.64 ^b	-27.80	18.48

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 8: Copper Levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	1.48±0.10 ^a	75.69±15.05 ^{a,*}	-5014.20	NA
Polluted control 2		49.89±22.92 ^{a,c}	-289.46	NA
<i>S. americana</i>	12.81±0.10 ^b	55.90±2.23 ^{a,*}	-336.38	23.29
<i>S. ocymoides</i>		37.27±7.40 ^{b,c,*}	-190.94	-48.91

Values are mean ± standard deviation of triplicate determination, ^{a,c}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 9: Iron levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	436.01±10.00 ^a	1212.69±47.06 ^{a,*}	-178.13	NA
Polluted control 1		1101.00±55.81 ^{a,*}	-34.17	NA
<i>M. alternifolius</i>	820.60±10.00 ^b	858.55±30.99 ^{b,*}	-4.62	-217.07
<i>F. ferruginea</i>		961.65±144.41 ^{a,b,*}	-17.189	-124.77

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 10: Iron Levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	436.01±10.00 ^a	318.63±34.63 ^{a,c,*}	26.92	NA
Polluted control 2		422.16±59.88 ^{a,*}	48.55	NA
<i>S. americana</i>	820.60±10.00 ^b	388.72±67.00 ^{b,*}	52.63	32.30
<i>S. ocymoides</i>		331.49±66.30 ^{c,*}	59.60	87.58

Values are mean ± standard deviation of triplicate determination, ^{a,c}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Iron: Iron concentration significantly increased (p<0.05) from the initial concentration of 820.60±10.00 mg kg⁻¹ in the polluted soil to 858.55±30.99 and 961.65±44.41 mg kg⁻¹ in soils remediated with *M. alternifolius* and *F. ferruginea* respectively, while significantly decreased (p<0.05) in soils remediated with *S. americana* (388.72±67.00 mg kg⁻¹) and *S. ocymoides* (331.49±6.30 mg kg⁻¹), thus accounting for 52.63 and 59.60% decrease, as well as 32.30 and 87.58% recovery, respectively according to Table 9 and 10.

Manganese: For the manganese concentrations of the remediated soils, a significant decrease (p<0.05) was recorded in soils remediated with *M. alternifolius* (54.84±14.40 mg kg⁻¹), *F. ferruginea* (49.57±12.51 mg kg⁻¹), *S. americana* (105.38±48.12 mg kg⁻¹) and *S. ocymoides*

(119.00±17.73 mg kg⁻¹) which accounted for 93.32, 93.96, 87.16 and 85.50% decrease, respectively as shown in Table 11 and 12.

Zinc: Similarly for zinc, a significant decrease (p<0.05) was recorded in the soils remediated with *M. alternifolius* (72.12±6.31 mg kg⁻¹) and *F. ferruginea* (67.35±10.28 mg kg⁻¹), accounting for 54.62 and 57.63% decrease, respectively, while a significant increase (p<0.05) was recorded in soils remediated with *S. americana* (180.93±9.52 mg kg⁻¹) as shown in Table 13 and 14. However, no significant difference (p>0.05) was recorded in the soil remediated with *S. ocymoides*. Soils remediated with *S. americana* and *S. ocymoides* recorded 35.36 and 77.07% recovery, respectively.

Table 11: Manganese levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	43.31 ± 10.00 ^a	658.42 ± 422.09 ^a	-1420.25	NA
Polluted control 1		59.57 ± 25.58 ^{a,*}	92.74	NA
<i>M. alternifolius</i>	820.78 ± 10.00 ^b	54.84 ± 14.40 ^{a,*}	93.32	-0.79
<i>F. ferruginea</i>		49.57 ± 12.51 ^{a,*}	93.96	-1.67

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 12: Manganese Levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	43.31 ± 10.00 ^a	384.03 ± 14.14 ^{a,*}	-786.70	NA
Polluted control 2		162.13 ± 89.60 ^{b,*}	80.25	NA
<i>S. americana</i>	820.78 ± 10.00 ^b	105.38 ± 48.12 ^{b,*}	87.16	-25.57
<i>S. ocymoides</i>		119.00 ± 17.73 ^{b,*}	85.50	-19.44

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 13: Zinc Levels (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 1	55.85 ± 1.00 ^a	151.06 ± 65.13 ^a	-170.47	NA
Polluted control 1		83.05 ± 32.30 ^{a,*}	47.75	NA
<i>M. alternifolius</i>	158.94 ± 1.00 ^b	72.12 ± 6.31 ^{a,*}	54.62	-16.07
<i>F. ferruginea</i>		67.35 ± 10.28 ^{a,*}	57.63	-23.08

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

Table 14: Zinc levels (mg kg⁻¹) of *S. americana* and *S. ocymoides* remediated soils

Groups	Before transplant	12 WAP	Decrease (%) (12 WAP)	Recovery (%) (12 WAP)
Unpolluted control 2	55.85 ± 1.00 ^a	150.08 ± 14.99 ^{a,*}	-168.72	NA
Polluted control 2		197.81 ± 106.68 ^{a,b}	-24.46	NA
<i>S. americana</i>	158.94 ± 1.00 ^b	180.93 ± 9.52 ^{b,*}	-13.84	35.36
<i>S. ocymoides</i>		161.02 ± 29.12 ^{a,b}	-1.31	77.07

Values are mean ± standard deviation of triplicate determination, ^{a,b}Significantly different at p<0.05, *p<0.05 compared to the corresponding values before transplant, WAP: Week(s) after planting, NA: Not applicable

DISCUSSION

The results showed that the heavy metals concentrations were significantly (p<0.05) higher in the polluted soils compared to the unpolluted soil. This finding corroborates Akubugwo *et al.*²⁵, who reported an increase in the heavy metal concentrations of crude oil polluted soils. Such a higher concentration is tantamount to the presence of heavy metals in crude oil which is deposited to the soil after contamination. Kakulu *et al.*²⁶ had previously opined that crude petroleum contributes to metal pollution in the Niger Delta, Nigeria. Shukry *et al.*²⁷ associated such an increase with increase in crude oil pollution. The higher concentration is, therefore, tantamount to the presence of heavy metals in crude oil which is deposited to the soil after contamination of the soil. According to Ezeaku and Egbemba²⁸, pollution by crude oil makes some nutrients that are toxic to plants more available.

It has been indicated that crude oil in soil makes the condition of the soil unfavourable for plant growth because of the reduction in the level of available plant nutrients or rise in toxic levels of certain elements^{29,30}. Thus, the elevated concentration of the heavy and trace metals recorded in the polluted soil compared to the unpolluted soil before transplant substantiated the effect of the crude oil pollution.

Heavy metals: Amongst the treatment plant species, *F. ferruginea* was the most effective for the removal of cadmium accounting for 70.33% reduction of cadmium. The order of cadmium removal by the species is *F. ferruginea* > *M. alternifolius* > *S. americana* > *S. ocymoides*. Remediation of cadmium using these plant species, thus, was effective after the 12 weeks period to the levels that fall below the 5 mg kg⁻¹ limit³¹.

Similarly, *M. alternifolius* boosted as the most effective species for the removal of Pb in the order: *M. alternifolius* > *F. ferruginea* > *S. americana* > *S. ocymoides*.

For chromium, *F. ferruginea* was the best performing species with 93.8% removal and trailed by *M. alternifolius*, *S. ocymoides* and *S. americana*.

Nevertheless, the significant decrease ($p < 0.05$) in the heavy metals of the polluted {Cd [polluted control 1 (72.1%) and polluted control 2 (69.5%)], Pb [polluted control 1 (91.9%) and polluted control 2 (47.3%)] and Cr [polluted control 1 (94.9%) and polluted control 2 (54.4%)] and unpolluted {Pb [unpolluted control 1 (82.13%) and Cr [unpolluted control 1 (81.43%)] control soils may be due to environmental factors, essentially microorganisms which Malik³² previously observed that they can trap heavy metal ions and subsequently sorb them onto the binding sites of their cell walls. If this is true, it may indicate that the treatment plants retarded the growth and or activities of the soil microorganisms subsequently decreased their potential to absorb the heavy metals. As reported by Ayangbenro and Babalola³³, the quantity of metal absorbed depends on the kinetic equilibrium and composition of the metal at the cellular surface. This mechanism encompasses several processes including electrostatic interaction, ion exchange, precipitation, the redox process, surface completion and bioaccumulation or active uptake. Organisms capable of accumulating heavy metals should have tolerance to one or more metals at higher concentrations and should exhibit enhanced transformational abilities, transforming toxic chemicals to harmless forms that allow the organism to lessen the toxic effect of the metal and at the same time, keep the metal contained³⁴.

Trace metals: The significant increase ($p < 0.05$) in the copper content of the soils after remediation may be attributed to copper response to the soil conditions such as acidification as the soil reaction may have turned more acidic over time³⁵. Reed and Martens³⁶ had also observed that copper levels in soil decrease as the quantity of organic matter increase. This may, however, insinuate that the increased soil copper level may be due to decrease in organic matter of the soil. Engel and Kirkby³⁷ had opined that organic matter hampers copper availability possibly by reducing soil mineral fixation and leaching. However, once the organic matter has adequately decomposed, sufficient copper can be released into the soil which is available for plant uptake.

The increased Fe levels after remediation as recorded in *M. alternifolius* and *F. ferruginea* remediated soils may be due to the change in soil condition. It has been reported that the concentration of soluble Fe in soils is extremely low in comparison with the total Fe concentration. In well-aerated

soils, however, Fe^{2+} contributes little to the soluble inorganic Fe except under high soil conditions, with soils being relatively higher in soluble inorganic Fe. Additionally, when soils are waterlogged, there is a reduction from Fe^{3+} to Fe^{2+} which is accompanied by an increase in Fe solubility. By this process, insoluble Fe^{3+} compounds become soluble and Fe^{2+} is dissolved in the soil solution³⁷. This reduction may have been brought about by anaerobic bacteria which use Fe oxide as electron acceptors in respiration³⁸.

Probably, the significant decrease ($p < 0.05$) in concentration as shown in the trace metals, such as chromium, after remediation may be due to degradation and subsequent utilization of the nutrients by plants and associated microorganisms³⁴. Thus, the results obtained for both heavy and trace metals validate previous findings of Chukwuma *et al.*^{2,39} that the use of *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides* can effectively remove hydrocarbons, oil and organic carbon contents of crude oil polluted soils which was further substantiated by the soil enzymes, respiratory and microbial activities of the soil.

CONCLUSION

The application of *M. alternifolius*, *F. ferruginea*, *S. americana* and *S. ocymoides* has demonstrated to be efficient for the removal of heavy metals in crude oil polluted soils. Amongst the plant species, *F. ferruginea* was the most efficient for the removal of Cd and Cr, while *M. alternifolius* boosted as the best performing species for the removal of Pb. These plant species are recommended for use in the remediation of heavy metals (cadmium, lead and chromium) contaminated soils.

SIGNIFICANCE STATEMENT

This study discovered that *Mariscus alternifolius*, *Fimbristylis ferruginea*, *Schwenkia americana* and *Spermacoce ocymoides* can be suitable for the remediation of heavy metals contaminated soil. The findings from this study will help researchers uncover the potential of these plants in the phytoremediation of polluted soils.

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