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CAPACITY FOR BIOREMEDIATION OF LABORATORY-INDUCED CRUDE OIL POLLUTION BY BOWSTRING-HEMP (*SANSEVIERIA LIBERICA)*

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Abstract

The objective of this work was to investigate the capability of bowstring-hemp (Sansevieria liberica) to degrade crude oil pollution. This was determined using 0.3, 1.3 and 6.3% v/w concentrations of crude oil which were employed to pollute the soil planted with the stem cuttings of the plant. These treatments were repeated in non-vegetated soils while the control had no crude oil pollution. Total Petroleum Hydrocarbons (TPH) were determined for both vegetated and non-vegetated soils as well as the leaves, stem and roots using Gas Chromatography–Flame Ionization Detector (GC-FID). TPH degraded in the vegetated soils were 95.8, 88.5 and 68.1% while those of non-vegetated soils were 94.93, 85.58 and 65.81% for 0.3, 1.3 and 6.3% v/w crude concentrations, respectively. S liberica alone degraded 0.87, 2.92 and 2.29% for the same treatments. Percentage accumulations of 0.3% v/w crude oil pollution for the leaf, stem and root were 0.002, 0.036 and 0.209%, respectively, those for 1.3% v/w were 0.004, 0.067 and 0.315%, respectively while those of 6.3% v/w were 0.008, 0.085 and 0.43%, respectively. Most degradation took place in 0.3 % v/w crude oil concentration, while the highest percentage accumulation of hydrocarbons occurred in the root for same concentrations.

Key words: Total Petroleum Hydrocarbons (TPH), degradation and accumulation.

Introduction

Hydrocarbon spills from petroleum products both on land and in water, have been a problem since the discovery of crude oil as a fuel source. Oil spillage on soil has many detrimental effects on the composition, structure and functioning of terrestrial ecosystems, including loss of biodiversity (Osuji *et al*., 2004). Soil contamination arising from oil spills is one of the most limitingfactors to soil fertility. It affects growth of plants thereby causing negative impacts on food productivity and this has attracted much attention in recent decades(Onwurah*et al*., 2007).

Hydrocarbon degradation is believed to occur through a rhizosphere effect; in this case, plants exude organic compounds through their roots, which increase the density, diversity and activity of specific microorganisms in the surrounding rhizosphere, which in turn degrade hydrocarbons.Phytodegradation involves the degradation of organic contaminants directly, through the release of enzymes from roots, or through metabolic activities within the plant tissues. In phytodegradation organic contaminants are taken up by roots and metabolized in plant tissues to form less toxic substances. Dixit *et al.* (2011) observed that transgenic tobacco plant expressing a *Trichoderma virens* showed tolerance and degraded anthracene-A (a 3 benzene ring compound) to naphthalene (a 2 benzene ring compound) when compared to the wild type plants.

Sansevieria liberica is a member of the Asparagaceae family and is popularly called Bowstring-hemp. It is an evergreen perennial plant with creeping rhizome which is sometimes above ground, sometimes underground with fibrous roots. Its stiff leaves grow vertically from a basal rosette.

(Chahinian, 2005). A study by National Aeronautics and Space Administration (NASA) found that it is one of the best plants for improving indoor air quality by passively absorbing toxins such as nitrogen oxides and formaldehyde (Wolverton *et al*., 1989).

Materials and methods

Ninety six black perforated polythene bags were each, filled with 12 kg of the soil media. The bags were well labelled and displayed in a completely randomized design under the sun in the Botanic Garden, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Rhizomesof *Sansevieria liberica* of equal length (10 cm each) with circumference of 5 to 6 cm (collected from same garden) were sown in 48 soil bags. One piece was sown in each bag. The remaining 48 bags were left non-vegetated. Vegetated and non-vegetated bags were watered sparingly when necessary. On emergence of the plant shoots,plant height (cm) was measured from the soil surface to the apex of the longest leaf. When all the plants had grown to a minimum height of 10 cm, both vegetated and non-vegetated bags were polluted with raw or unused crude oil. Each of the 24 soil bags (12 with plants and 12 without plants) were polluted with 30 ml (0.3%v/w) of crude oil. This was repeated using 150 ml (1.3%v/w) and 750 ml (6.3%v/w) instead of 30 ml. The control was not polluted with crude oil and the experiment was carried out in 3 replicates. Sixty days after pollution, both vegetated and non-vegetated soils, stems, leaves and roots of plants treated with different concentrations of crude oil (as well as the control) were randomly collected and subjected to Gas Chromatography-Flame Ionization Detector (GC-FID) to determine the Total Petroleum Hydrocarbons (TPH). The unused crude oil was also analysed to determine the TPH composition which served as the reference standard.

For TPH determination, anhydrous sodium sulphate was added to 5.0 g of each soil sample and mixed until a dry mixture was obtained. To the dried mixture, Analar grade Dichloromethane (DCM), Hexane and Acetone (3:1:1, v/v/v) were added in an extraction bottle and covered tightly with Teflon cap and shaken gently for 30 minutes using a mechanical shaker. The two phases were separated by filtration using a 100 ml capacity sintered funnel to remove any particulates present and poured into a clean 100 ml conical flask. The extracted organic phase was cleaned using activated neutral alumina and concentrated to 0.5 ml using a rotary vacuum evaporator and transferred into 2.0 ml volumetric flask. Randomly collected plants treated with 0 ml, 30 ml, 150 ml and 750 ml were separately dissected into leaves, stem and roots. These different parts from each treatment level was crushed separately and put in beakers one at a time. Each crushed sample was carefully mixed thoroughly using a glass rod and the same procedure used for the soil was then followed.

One microliter of the final extract was injected in an already calibrated GC-FID Varian 3400. Nitrogen was used as a carrier gas. The column temperature was set at 60^{o}C for 10 min, followed by a linear increase of 10^{o}C per minute to 280^{o}C, and then the temperature was held for 8.17 min. Detector temperature was maintained at 300^{o}C while injector temperature was 200^{o}C and pressure program (set point) was 14.0 psi. Sample concentrations were calculated by comparing sample response data with the initial calibration
(USEPA, 1996).

Total TPH for the treatments sum for each column (Tables 1, 2 and 3) was obtained by subtracting

the total sum in each column from the total sum of the unused crude oil. Percentage TPH degraded for each treatment was obtained by dividing the total TPH degraded for each treatment by total TPH in the standard and multiplying by 100.Percentage TPH degraded by the plant alone was obtained by subtracting percentage TPH degraded in non-vegetated soil from percentage TPH in vegetated soil (Nwadinigwe and Obi-Amadi, 2014).

Table 1: Total petroleum hydrocarbon distribution (mg/kg) in non-vegetated soil polluted with different concentrations of crude oil.

Straight chain group	conc. of hydrocarbon in unused crude oil	0.3 %v/w			1.3 %v/w			6.3 %v/w		
		A	B	C	A	B	C	A	B	C
C_{11}	5009	247	254	261	248	259	255	534	532	530
C_{12}	5473	183	188	193	348	340	338	446	458	455
C_{13}	7834	190	187	190	455	450	451	870	877	869
C_{14}	9983	195	202	203	428	432	436	839	825	832
C_{15}	7843	204	201	195	197	189	214	557	563	569
C_{16}	8734	324	320	319	323	321	319	718	720	731
C_{17}	8932	301	286	313	299	306	295	618	594	588
C_{18}	7098	349	357	362	363	358	347	721	729	719
C_{19}	7771	356	351	349	351	351	354	777	762	792
C_{20}	9812	325	321	326	765	759	762	768	753	765
C_{21}	5891	228	242	232	346	342	338	653	647	671
C_{22}	11141	311	307	309	2395	2392	2404	7876	7885	783
C_{23}	12015	455	459	454	2546	2540	2537	7664	7679	7682
C_{24}	10872	662	651	649	2898	2894	2899	6675	6680	6679
C_{25}	12458	760	754	748	2548	2553	2522	6892	6887	6891
C_{26}	10587	789	793	791	3521	3517	3540	7911	7913	7876
C_{27}	13221	673	677	672	3276	3263	3271	8013	8001	7986
C_{28}	13897	894	896	898	2414	2404	2427	6752	6749	6761
C_{29}	12048	828	832	836	2341	2362	2356	5568	5592	5574
C_{30}	14771	642	648	639	3544	3539	3543	7657	7661	7653
C_{31}	13562	736	735	731	2982	2967	3000	5010	5011	5006
C_{32}	10589	871	854	867	2000	2008	2001	7667	7684	7683
C_{33}	13487	759	762	771	2518	2523	2519	3415	3429	3419
C_{34}	10486	981	917	802	607	592	595	1323	1327	1322
C_{35}	8124	428	440	422	622	633	614	1328	1320	1321
C_{36}	7592	655	649	658	632	624	640	1346	1353	1357
C_{37}	8723	538	545	546	719	722	725	1759	1752	1748
C_{38}	7624	234	237	231	429	436	431	890	906	910
C_{39}	5623	193	204	203	447	450	459	891	894	885
Total TPH (mg/Kg)	**281200**	**14311**	**14269**	**14170**	**40562**	**40526**	**40592**	**96138**	**96183**	**96105**

A, B and C represents replicates

Table 2: Total petroleum hydrocarbon distribution (mg/kg) in soil, vegetated with *Sansevieria liberica*, polluted with different concentrations of crude oil (%v/w).

Straight chain group	conc. of hydrocarbon in unused crude oil	0.3 %v/w			1.3 %v/w			6.3 %v/w		
		A	B	C	A	B	C	A	B	C
C_{11}	5009	136	130	142	252	261	249	950	956	968
C_{12}	5473	98	100	99	341	343	342	760	761	753
C_{13}	7834	80	90	97	452	449	455	872	836	887
C_{14}	9983	101	104	101	438	430	428	6533	6601	6429
C_{15}	7843	159	165	141	203	189	208	767	760	747
C_{16}	8734	208	197	213	334	322	307	624	621	630
C_{17}	8932	214	201	213	233	251	218	426	431	418
C_{18}	7098	223	254	246	340	345	341	565	571	556
C_{19}	7771	261	239	208	298	301	301	989	1023	994
C_{20}	9812	234	250	251	684	679	689	547	539	558
C_{21}	5891	174	152	148	210	215	214	359	354	361
C_{22}	11141	252	248	250	2018	1900	1809	4603	4571	4530
C_{23}	12015	397	389	411	1116	1113	1101	4903	4885	4681
C_{24}	10872	423	494	460	2011	2006	2010	7660	7489	7591
C_{25}	12458	620	658	678	2453	2500	2400	5459	5501	5444
C_{26}	10587	791	766	786	2835	2992	3134	4598	4560	4522
C_{27}	13221	540	544	542	2894	2899	2898	5312	5215	5223
C_{28}	13897	487	489	491	2010	2008	2006	4537	4611	4472
C_{29}	12048	658	636	668	1730	1729	1746	4559	4563	4564
C_{30}	14771	792	808	800	1899	1898	1891	4348	4411	4315
C_{31}	13562	543	541	542	2597	2701	2798	5642	5618	5603
C_{32}	10589	576	562	569	1622	1608	1645	5678	5686	5703
C_{33}	13487	648	658	656	998	972	1030	3256	3257	3249
C_{34}	10486	895	887	891	591	576	570	2562	2560	2558
C_{35}	8124	463	456	449	601	577	571	2661	2512	2801
C_{36}	7592	320	334	312	588	581	592	2588	2601	2569
C_{37}	8723	459	452	457	680	672	682	2654	2699	729
C_{38}	7624	186	178	194	395	386	389	1298	1300	1308
C_{39}	5623	119	121	120	284	296	320	891	895	899
Total TPH (mg/Kg)	**281200**	**11057**	**11103**	**11140**	**31107**	**31199**	**31144**	**86601**	**86387**	**86062**

A, B and C represents replicates

Table 3: Total petroleum hydrocarbon distribution (mg/kg) of leaf, stem and root of *Sansevieria liberica*, polluted with different concentrations of crude oil.

Straight chain group	conc. of hydrocarbon in unused crude oil	Leaf (%v/w)			Stem (%v/w)			Root (%v/w)		
		0.3	1.3	6.3	0.3	1.3	6.3	0.3	1.3	6.3
C_{11}	5009	0	0	0	0	0	0	0	0	0
C_{12}	5473	0	0	0	0	0	0	0	0	0
C_{13}	7834	0	0	0	0	0	0	0	0	0
C_{14}	9983	0	0	0.6	2.1	3.56	0	0.9	2.1	8.5
C_{15}	7843	0.11	0.23	0.5	5.03	8.25	10.5	2.1	3.2	6.9
C_{16}	8734	0.11	0.21	1.2	3.12	5.64	8.21	2.43	0.92	24.6
C_{17}	8932	0.11	0.22	1.2	2.12	4.23	9.24	1.2	3.2	4.5
C_{18}	7098	0.07	0.15	1.3	3.25	4.23	8.3	0.3	0.36	5.6
C_{19}	7771	2.54	4.5	6.5	26.4	64.64	88.4	532.6	820.1	1073.6
C_{20}	9812	0.23	0.45	0.6	5.6	8.9	8.3	4.5	6.2	7.1
C_{21}	5891	0.24	0.45	0.3	5.1	6.5	8.12	3.2	4.5	5.5
C_{22}	11141	0.07	0.14	0.8	1	3.2	0	1.8	0.54	6.5
C_{23}	12015	0.21	0.42	0.5	5.9	9.2	9.25	4.2	4.2	6.5
C_{24}	10872	0.1	0.37	1.6	6.2	11.2	10.25	3.2	7.3	6.2
C_{25}	12458	0.25	0.45	0.8	1.2	4.5	0	1.6	4.3	9
C_{26}	10587	0.4	0.8	0.5	5.3	10.25	13.46	4.6	3.85	4.5
C_{27}	13221	0.155	0.33	0.5	5.2	8.35	11.3	4.5	4.2	5.3
C_{28}	13897	0.1	0.2	0.5	5.2	9.2	11.3	3.6	4.1	6.5
C_{29}	12048	0.3	0.6	0.6	5.3	9.3	13.2	3.5	4.2	6.9
C_{30}	14771	0.25	0.5	0.6	5.1	6.32	8.34	2.3	5.6	6.2
C_{31}	13562	0.25	0.5	0.5	5.1	9.32	8.15	5.6	0	5.6
C_{32}	10589	0	0	0.4	1	2.1	0	0	2.3	0
C_{33}	13487	0.1	0.26	1.38	1.8	0	4.96	3.3	0.3	9.5
C_{34}	10486	0.15	0.3	0.6	0	0	0	0.9	0	0
C_{35}	8124	0.15	0.3	0	0	0	0	0	0	0
C_{36}	7592	0.43	0.1	0	0	0	0	0	0	0
C_{37}	8723	0	0	0	0	0	0	0	0	0
C_{38}	7624	0	0	0	0	0	0	0	0	0
C_{39}	5623	0	0	0	0	0	0	0	0	0
Total TPH (mg/Kg)	**281200**	**6.325**	**11.48**	**21.48**	**101.02**	**188.95**	**231.28**	**586.33**	**880.07**	**1210.1**

0, means absence of hydrocarbons

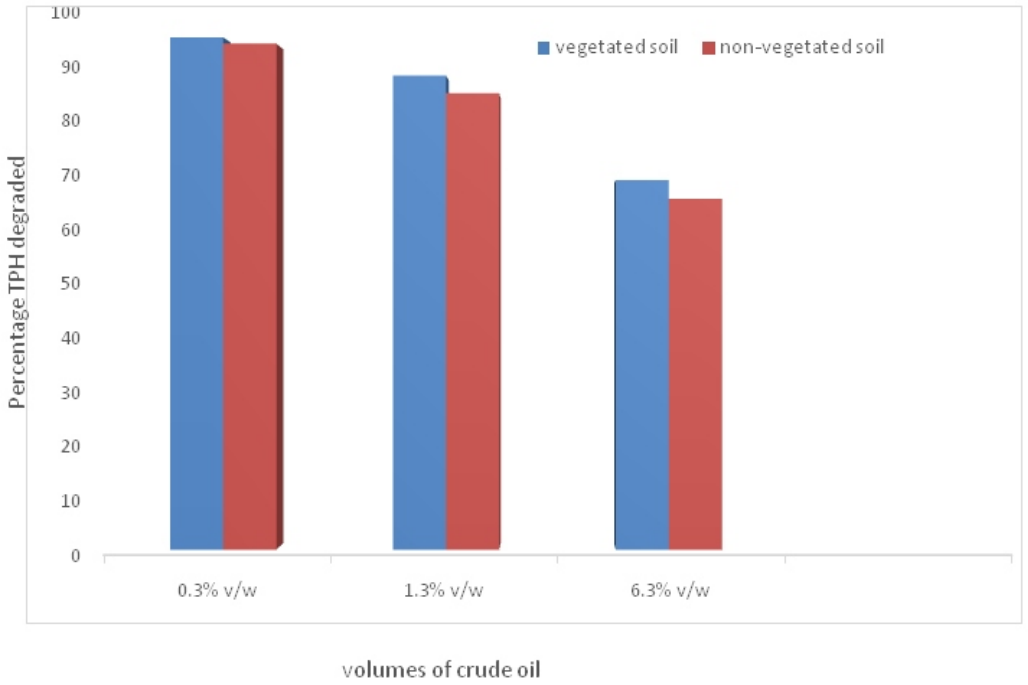

Figure 1: Percentage Total Petroleum Hydrocarbons (TPH) degraded in soil non-vegetated and vegetated with *Sansevieria liberica* polluted with different volumes of crude oil.

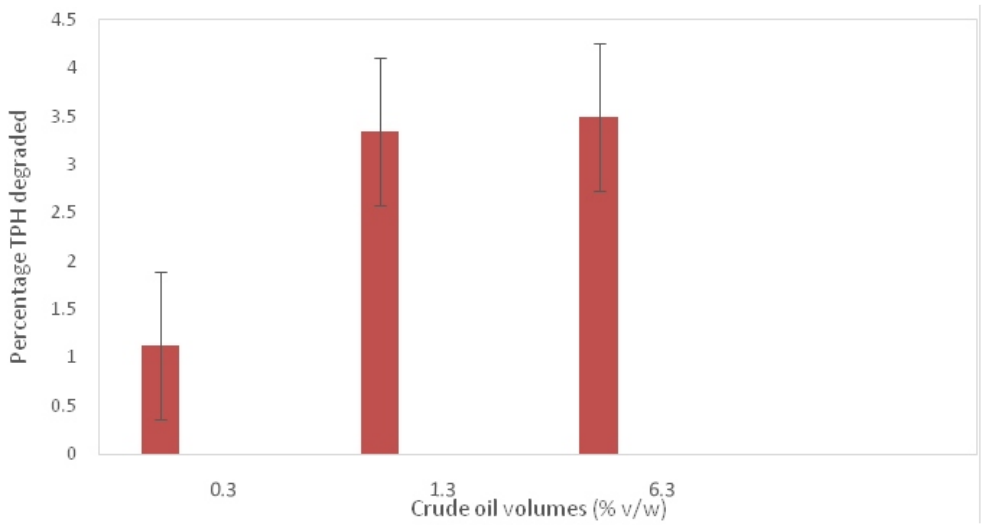

Figure 2: Percentage Total Petroleum Hydrocarbon (TPH) degraded by plant alone for different concentrations of crude oil pollution.

Results

Total petroleum hydrocarbons (TPH) degraded in the soil

Results of GLC analysis for the unused crude oil sample, which is the reference (unused crude oil), showed the presence of high concentrations of straight chain hydrocarbons of $C^{1} – C^{40}$ but $C^{11} – C^{39}$ where the only hydrocarbons detected in the soil and plant samples (Tables 1,2 and 3) and so will be the only once to be considered for manageable table arrangement. Hydrocarbons $C^{11} – C^{39}$ were detected in all the polluted soil samples (vegetated and non-vegetated) although in small numbers when compared with the unused crude oil. No hydrocarbons were detected in the control. Percentage TPH degraded in vegetated soil polluted with 0.3, 1.3 and 6.3 % v/w crude oil treatments were 96.1%, 88.9% and 69.3%, respectively while the percentage TPH degraded in non-vegetated soil polluted with the same quantities of crude oil were 94.9%, 85.6% and 65.8%, respectively (Figure 1). Percentage TPH degraded by the plants alone was obtained by subtracting the percentage TPH degraded in non-vegetated soil from the percentage TPH degraded in vegetated soil. Thus, for 0.3% v/w crude oil pollution, the plant degraded 1.12%, for 1.3% v/w, it degraded 3.34% and for 6.3% v/w, it degraded 3.49% (Figure 2).

Hydrocarbons $C^{11} – C^{13}$ and $C^{37} – C^{39}$ (Table 2) were not detected in any of the plant organs (leaves, stem and roots) even though they were found in the standard. No hydrocarbons were detected in the unpolluted leaves, stem and roots (control). Hydrocarbon C^{14} was detected in all volumes of crude oil pollution in the root but in the stem it was detected in 0.3 and 1.3% v/w and absent in 6.3% v/w concentrations. The reverse was the case in the leaf where in 0.3 and 1.3% v/w hydrocarbon C^{14} was not detected but present in 6.3% v/w. Hydrocarbons $C^{15} - C^{31}$ and C^{33} were detected in all the plant parts and across the various volumes of crude oil pollution, except in the stem at 6.3% v/w concentration where C^{22} was not detected and in the root at 1.3% v/w concentration, C^{31} was not detected. Hydrocarbon C^{32} was only present in 6.3% v/w of crude oil pollution in the leaves and present in the stem of 0.3 and 1.3% v/w concentrations but in the root it was present in only 1.3% v/w, while C^{34} was detected in the leaves across all the volumes but completely absent in the stem and was detected in the root at 0.3% v/w concentration only. Hydrocarbons C^{35} and C^{36} were only detected in the leaf of 0.3 and 1.3% v/w concentrations.

The leaf accumulated 0.002%, 0.004% and 0.007% for 0.3, 1.3 and 6.3% v/w concentrations, respectively. The stem accumulated 0.036%, 0.067% and 0.082% for 0.3, 1.3 and 6.3% v/w concentrations, respectively. Similarly, the root at same volumes of crude oil pollution accumulated 0.209%, 0.313% and 0.43%, respectively (Figure 3). The root had the highest accumulation of hydrocarbons when compared with those of the stem and leaves. The leaves had the least accumulation level of hydrocarbons.

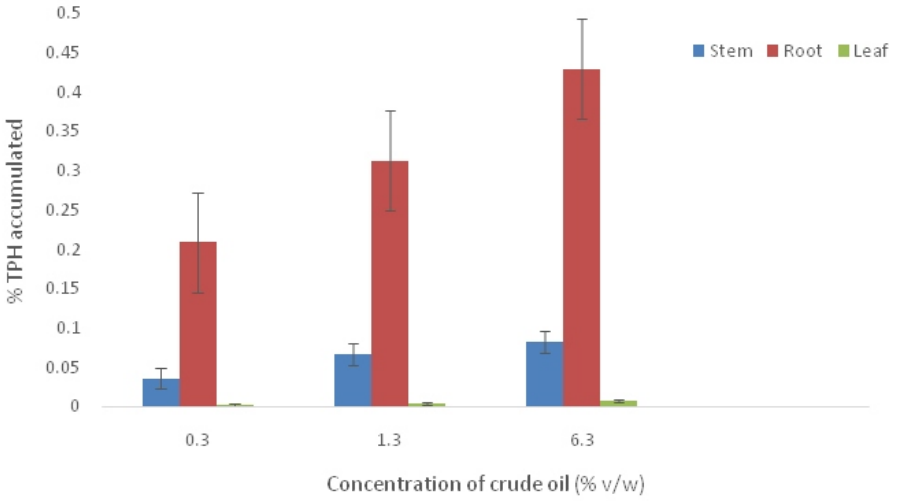

Figure 3: Percentage accumulation of Total Petroleum Hydrocarbons (TPH) in plant organs polluted with different concentrations of crude oil.

Discussion

Some straight chain groups of hydrocarbons like $C^{1} – C^{10}$ were perhaps volatile and so could not be detected by GC- FID. No hydrocarbons were detected in the control (0 ml) for all plant and soil samples since there was no crude oil pollution. For the polluted vegetated and non-vegetated soils, C^{11} to C^{39} hydrocarbons were found but in smaller quantities when compared with the unused crude oil sample. This showed that phytodegradation took place in this work. The plant metabolized some of the hydrocarbons and used them for growth as evident in their survival after pollution. In this study, more degradation of hydrocarbons took place in the presence of *Sansevieria liberica* than in its absence. However, the difference in the degradation levels between vegetated and non-vegetated soil was minimal. This indicates that other unseen factors in the soil (perhaps microorganisms) were responsible for some of the degradation of crude oil in the absence of the plant. This phenomenon is in agreement with the report of Wenzel (2009) who stated that, "the efficiency of phytoremediation relies on the establishment of vital plants with sufficient shoot and root biomass growth, active root proliferation and/or root activities that can support a flourishing microbial consortium assisting phytoremediation in the rhizosphere". *Sansevieria liberica* nourished the rhizosphere very well in support of microbial consortium for phytoremediation. The findings of the present work agree with the work of Nwadinigwe and Obi-Amadi (2014) who reported that *Pennisetum glaucum* phytodegraded crude oil polluted soils contaminated with varying volumes of crude oil, and they showed that viable microbial count in vegetated soils was significantly higher than that of non-vegetated soil, indicating that the plant enhanced the biodegradation of crude oil by stimulating the proliferation of microorganisms in the soil. In this present work, percentage TPH degraded in vegetated soil polluted with 0.3, 1.3 and 6.3% v/w were 96.1%, 88.9% and 69.3%, respectively, while percentage TPH degrade in non-vegetated soil polluted with the same quantities were 94.93%, 85.58% and 65.81%, respectively, 60 days after pollution. Edema *et al.* (2011) in their work with mushroom, cowpea and algae reported that degradation of PAHs in crude oil contaminated soils of varying concentrations was achieved by these plants at the following levels; 98.93% degradation for mushroom, 97.90% for cowpea and 97.07% for algae. Also Banks *et al.* (2003) in their experiment with four varieties of *Sorghum bicolor* observed higher degradation of TPH

associated with vegetated soil (69%) than in unvegetated soils (35%). Euliss *et al*. (2008) in a period of one year recorded 70% loss of TPH in crude oil polluted soil planted with *Carex exigua, Panicum virgatum* and *Tripsacum dactyloides.* Diab (2008) observed that *Vicia faba* degraded 47% of TPH in petroleum contaminated soil in a 60 day period.

TPH accumulation in the plant organs

In this work, after a period of 60 days the percentage accumulation in the roots contaminated with various volumes of crude oil pollution was highest followed by that of the stem, while the leaves had the lowest level of accumulation. This finding is similar to that of Sakineh *et al.* (2013) who reported that *Avicennia marina* showed higher level of TPH accumulation in the root than the leaf in a period of three months. Denise *et al.* (2013) observed that *Heteranthera callifolia* in a period of 4 weeks bioaccumulated TPH in the roots, petioles and leaves, but in this case the leaves had the highest concentration of 0.434 ± 0.170 mg l^{-1} followed by the petioles (0.202 ± 0.116) mg l^{-1}, while the roots had the least uptake of 0.096 ± 0.080 mg L^{-1}. In like manner, Onwuka *et al*. (2012), working with *Cynodon dactylon* showed that there was significant difference in accumulation of TPH in the stem between polluted and unpolluted plants within a period of two months. The stem accumulated more TPH than the leaves and roots. Edwin-Wosu and Albert (2010) reported that two leguminous species, *Leucaena leucocephala* and *Bauhinia monandra* accumulated TPH from the crude oil contaminated soil the plants were sown. Atagana (2011) reported that *Chromolaena odorata* L. degraded more TPH in soils contaminated with used engine oil (40 g kg^{-1}) in 90 days. Plabita *et al*. (2013) recorded higher accumulation of TPH in plant shoots than in the roots, which is in contrast with this work. They further observed that higher accumulations were achieved in the second year (50,000 ppm) when compared with the first year (10,000 ppm). This is an indication that the longer the plants are allowed to grow on the pollution site, the higher the level of accumulation, which is in agreement with the report of Kamath *et al.* (2004) who *stated that "*degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase and this may become the rate determining step. Therefore, phytoremediation may require more time to achieve clean-up standards than other more costly alternativessuch as excavation or ex-situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles. In many cases, phytoremediation mayserve as a final 'polishing step' to close sites after more aggressive clean-uptechnologies have been used to treat the hot spots".

Conclusion

The results of this work confirmed that *Sansevieria liberica* was able to degrade petroleum hydrocarbon. Though percentage degradation of hydrocarbons by the plant alone was minimal, it indicates that the plant stimulated the activities of the microorganisms in their phytoremediative work. The work also showed that *S. liberica* could accumulate hydrocarbons in its organs, and so there is the need to allow the remediative period to take longer time for greater accumulation in future research. It was also observed that this plant is more tolerant to small levels of crude oil contamination as such should not be exposed to highly contaminated areas, possibly it could be used for secondary phytoremediation.

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