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Comparative effects of pretreatment of stem cuttings of Chromolaena odorata (Siam weed) with sodium azide and hydroxylamide on the survival and phyoremediative performance in an oil-polluted soil

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Abstract

The method of phytoremediation was applied to clean up heavy metals and polyaromatic hydrocarbon contents of a waste engine oil (WEO)-polluted soil, using Chromolaena odorata exposed to sodium azide (NaN_3) and hydroxylamine hydrochloride ($NH_2OH.HCl$) solutions respectively. WEO was poured into sun-dried top soil, and thoroughly mixed to obtain 5% w/w concentration in soil. After one month, soil was sown with sodium azide and hydroxylamine hydrochloride pretreated stem cuttings of C. Odorata. Concentrations of either of the mutagenic agents were 0.016%, 0.064%, and 0.25% respectively. Results showed that there was significant decrease in heavy metal and polyaromatic hydrocarbon (PAH) components of soil. Remediation of Cu and Pb by C. Odorata was best at 0.25% $NH_2OH.HCl$ treatment. Remediation efficiencies for PAH and heavy metals were better enhanced with the pretreatment of Chromolaena odorata with mutagenic agents. Results also showed that with the increase in concentration of mutagenic solutions required for presoaking, total heavy metal concentration in soil decreased. PAH remediation efficiency was highest (89.88 - 90.99%) when soils were remediated with sodium azide-treated plants than their hydroxylamine hydrochloride counterparts (72.54 - 81.14%). Similar observations were made in heavy metal reduction rates.

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Introduction

The world is one global village with industrialization the building block or backbone of most nations' economy. In Nigeria, industrialization is powered by the petroleum industry, while creating economic boom it has led to environmental and socio-economic problems (Raven et al., 1993; Ibekwe et al., 2006). Spills from oil are devastating to plants and animals found in the soil or which inhabit soils, because of their toxicity and also because the presence of hydrocarbon reduce oxygen tension and increase anaerobiosis in the soil which is damaging to plant roots (Bossert and Bartha, 1984).

One of the major products of petroleum is engine oil or lubricating oil. Engine oil helps to reduce friction between moving parts of auto machines and engines, prevents corrosion of these auto machines parts. It contains additive chemical substance such as amines, benzene, phenols, barium, lead, zinc, sulphur, magnesium and phosphorus and polyaromatic hydrocarbons and synthetic poly-chlorinated biphenyls. According to Wang et al. (2002), engine oil got from automobile (covering up to 3,000km) contains significantly higher concentrations of polyaromatic hydrocarbon (PAH), compared to new lubricating oil which contains only low concentration of polyaromatic hydrocarbon. A major source of oil pollution in the environment is as a result of disposal or discharge of used engine oil from vehicles. Extintion or total death of soil ecosystem and its habitat of living organism is an effect of oil pollution on soil (Akoachere et al.,2008).

The common practice by most automobile operators and service men of disposal of used engine oil into watercourse,

gutters, farmland and open vacant plots has increased the incidence of oil contamination to agricultural lands. Heavy metals such as vanadium, lead, nickel and iron, present in spent engine are of high value while that present in unused or new engine oil is relatively low (Whisman et al., 1974). These oil contaminated soils are serves as potential source for surface and ground water contamination, beside the fact that they are unsuitable for agricultural and recreational purposes.

Lands or soils exposed to oil pollution are damaged and made infertile. Crude oil, as well as its products, in soil results in poor soil fertility (Odu, 1972). oil polluted soil has been reported to have great impact on plant. Soil pollution is the introduction of contaminant into the soil matrix, which hinder or reduce the activites of organism present in the soil. The most common type of contaminants are chemicals which could be organic such as hydrocarbon, polyaromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs), chlorinated compounds, detergents and pesticides. Inorganic such as nitrates, phosphates and heavy metals such as cadium, lead and vanadium.

Different remediation methods exist for the removal of these contaminants. However, most of these methods are not enviromental suitable as they release or add contaminant to the environment. Among these methods for remediation of oil pollution is phytoremediation i.e the use of plant to clean up or degrade contaminant from the environment. Phytoremediation is an effective, non-environmental contaminanting process and low cost as most plants used for used for phytoremedation is easily accessible with one or two food crop. All plants which have phytoremediative capabilities are usually tolerant to the contaminants which they remediate. A number of researchers conducted, stated that Chromolaena odorata has the ability to tolerate soil acidity, aluminum saturation and oil (Anoliefo and Vwiko, 2001). Exampls of plant species used for phytoremediation are Willow, Poplar, Soybean, Sunflower, Indian mustard, Red clover, Chromolaena odorata e.t.c. Chromolaena odorata (commonly called siam weed, Awolowo or shell plant in Nigeria) has been found to have phytoremediation properties, it degrade contaminant from soil when cultivated or found naturally growing as weeds on a polluted site. The use of plants in the reclamation of contaminated lands is called phytoremediation. This process maintains soil structure, is environmental friendly and is low cost (Khan et al., 2000). Pollutants which can be degraded by phytoremediation are ot two types, the elemental pollutants and the organic pollutants (Meagher, 2000).

Evidently, phytoremediative capabilities of most plants is a factor of its improved morphological presentations, it is suffices to say therefore, that any process that is adopted to enhance the growth, performance and morphological presdentations of the plants, would also enhance phytoremediation. One of such methods is the use of chemical mutagens (Mshembulla et al., 2012; Ikhajiagbe et al., 2013). The addition of certain chemical substance such as hydroxylamide and sodium azide, which have mutagenic properties has been known to increase plant growth and yield. There is significant increase in crop production due to mutation induction (Kharkwal and Shu, 2009) and the induction of desired attributes. This research, therfore aims to access the effect of these chemical reagents (hydroxylamide, sodium azide) on Chromolaena odorata as a phytoremediation agent. This outcome of this findings will be useful in the growing or cultivation of Chromolaena odorata on contaminated soils in Nigeria and other countries where Chromolaena species thrive.

Materials and Method

Top soil (0-10 cm) was collected, sun dried to constant weight and a measured 20 kg of the sun-dried soil was measured into experimental boxs. Waste engine oil was measured (1 kg/bucket, specific gravity = 0.846) and poured into the soil, it was mixed thoroughly to obtain a 5%w/w oil- in-soil concentration.

NaN_3 and $NH_2OH.HCl$ solutions: Different concentrations of sodium azide and hydroxylamine solutions were prepared and used in the experiment. Percentage concentrations for sodium azide and hydroxylamide solutions included; 0.016%, 0.064%, and 0.25%w/v, for both chemicals (designated in the study as PS1, PS2, and PS3 for sodium azide treatments and PX1, PX2, and PX3 for hydroxylamine treatments respectively).These respectives concentrations were obtained by weighing 0.16g, 0.64g and 2.5g each of sodium azide (NaN_3) and hydroxylamine hydrochloride ($NH_2OH.HCL$), and dissolved in 1000 mlof distilled water (pH 7) respectively.

Pretreatment of stem cuttings of Chromolaena odorata: Stem cuttings were collected from a fallow land located within the University of Benin Ugbowo Campus, Benin City, Nigeria. Thirty (30) centimeter-long stems, of relatively equal sizes were obtained. Twelve stem cuttings (i.e 3 stem cuttings per treatment for 4 replicates) were placed in bowls containing each of the mutagenic solutions already prepared. Stems were completely submerged in the solutions for 1 hour with regular stirring every 15 minutes, to ensure equal exposure of stems.

Sowing: The research set up was made up of 8 treatments, each of which was replicated 4 times, making total of 32 experimental units. The experimental boxs were labeled and arranged according to treatment used for identification ease. After exposure, pretreated stems were immediately sown in the designated treatment experimental boxs, having priorly exposed the soil to 1 month of oil polution. This was to allow for natural attenuation of the soil before sowing. The set up was left for additional 2 months, after which soil was analysed for heavy metal and PAH contents of soil, whereas some plant growth parameters were also observed.

Extraction of Micronutrients in Soils by Hydrochloric Acid Method: Ten (10) g of soil was weighed into a 250 ml plastic bottle. 100 ml of 0.1 m HCl was added, stoppered, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42. Following this – iron, copper, manganese, cadmium, chromium, lead, nickel and vanadium were determined in the filtrate by Atomic Absorption Spectrometery, Model Solaar 969 Unicam Series. An air-acetylene flame was used.

Determination of Polyaromatic Hydrocarbon Contents of Polluted Soil by Gas Chromatography (GC): A 10 g sample was extracted with methylene chloride (DCM). The extract was filtered through anhydrous sodium sulphate to remove any trapped water molecule. This was followed by a clean- up/ fractionation of the sample extract into aromatic (PAH) components. Finally, the components were concentrated using a rotary evaporator for GC analysis, using FID as detector. GC results used was calculated as follows:

Sample (mg/kg) = $\dfrac{Area \ x \ F.vol \ x \ 1000}{Rf \ x \ Wt}$

Where,

Rf = Response factor = Total Area / Total Concentration, obtained from instrument calibration with standards.
Area is obtained from the chromatogram output.
F.vol is the final volume of the concentrated extract (in ml)
Wt is the initial weight of the homogenized sample (in grams)

Identification of Soil Microorganisms: The soil samples were air-dried and sieved through a 2 mm mesh to remove undwanted material. One gram (1 g) of the soil was transfered to nine (9 ml) millimetres of sterile distilled water in sterile glass containers to serve as serial dilution. The glass containers were shaken for 5 minutes and was taken as l0-1 dilution factor, 10 ml were then transferred from the 10-1 dilution into another 9 ml blank to obtain a 10-2 dilution and same process of transfer was repeated twice to obtain a dilution factor of 10-4. The spread plate method was employed in taking the heterotrophic bacteria counts. One (1) ml of the serially diluted portion of 10-4 of each soil sample was inoculated onto nutrient agar plates for bacteria and Potato dextrose agar plates for fungal counts. The plates were inoculated at room temperature for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation colonies were then counted and the colony forming unit (cfu/g) of the soil samples determined.

Isolation of Bacterial and Fungal Oil Degraders: Bushnell- Haas (BH) medium ($MgSO_4$, 0.20 g/l; $CaCl_2$, 0.02 g/l; K_2H,PO_4, 1 g/l; NH_4NO_3, 1 g/l; $FeCl_3$, 0.05 g/l; KH_2PO_4, 1 g/l; pH 7.0, was used as the enrichment medium with 8 % (v/v) filter sterilized oil as the sole carbon source. The medium was dispensed into in 100 ml Erlenmeyer flasks and autoc1aved at 121 ^{O}C for 15 minutes. Thereafter, 5 g of each soil sample was inoculated into each flask of the medium and incubated at 130 rpm at room temperature in a HY-4 multifunctional shaker (B. Bran Scientific and Instrument Company, England). After 10 days, 1 ml of enriched media was transferred into freshly prepared enrichment media and incubated under the same conditions as described above. Serial dilutions from the third enrichment process were inoculated onto nutrient agar plates and potato dextrose agar plates for oil-degrading bacterial and funga1 counts respectively using the methods described by Cowan and Steel (1974) and Cheesebrough (1998).

Determination of Growth and Yield Parameters: Six measurable growth parameters were obsereved in the study, including shoot height, number of sprouted branches, total number of leaves per plant, average length of sprouted branch, as well as leaf area.

Statistics: Analysis of variance in completely randomized design was done using the SPSS-15 statistical software, and means were separated by using the Least Significant Difference. Other statistical determinations for ecotoxicological assessments included Hazard Quotient (HQ) and Toxic Equivalency (TEQ) for PAH.

HQ = <u>Measured concentration</u>

Toxicity reference value or selected screening benchmark.

When HQ > 1: Harmful effects are likely due to contaminant in question

When HQ = 1: Contaminant alone is not likely to cause ecological risk

When HQ < 1: Harmful effects are not likely

TEQ = Ti x PEF

Where TEQ = Toxic Equivalency

Ti = PAH concentration in soil

PEF = Potency Equivalency factor

Results

The chemical composition of materials used for the experiment is indicated in Table 1. Heavy metal concentration of Mn, Ni and V were below detection levels in all the treatments (Table 2). Similarly, soils with sodium azide-treated plants were totally remediated of Cr, compared to a range of 7.29 – 16.51 mg/kg in other treatments. Results also showed that with the increase in concentration of mutagenic solutions required for presoaking, total heavy metal concentration in soil decreased. Total heavy metal was least in PS3 (16.17 mg/kg), compared to 21.04 mg/kg as total in PS1, 28.43 mg/kg in PX1 and 20.43 mg/kg in PX3.

PAH components of soil exposed to experimental treatments is presented on Table 3. Total PAH at 1 month after pollution and just before plants were sown was 916.91mg/kg. PAH remediation efficiency of the sown NaN_3-treated stem cuttings (i.e. PS) in the oil-polluted soils was highest (89.38 - 90.99%), compared to those remediation efficiency of $NH_2OH.HCl$-treated stem cuttings (PX-soils) (72.54 to 81.14%). Naphthalene, acenaphthylene, 2-bromonaphthalene, acenaphthene, benzo(a)anthracene and chrysene were all remediated below detection (< 0.0001 mg/kg) in all PS-soils. However PX-soils treatment contained detectable concentrations of the respective PAH components (0.03 - 9.69 mg/kg). There was also total remediation of naphthalene, acenaphthylene, 2-bromonaphthalene, acenaphthene, benzo(a)anthracene and chrysene in both PX1 and PX2. Comparatively PAH remediation were better enhanced in the soil sown with PS-treated plants.

Hazard quotients (HQ) to accertain ecological toxicity of residual concentration of heavy metal and PAH fractions are presented on Table 4. HQ was less than1 through out the duration of the experiment for Cu, Mn, Ni, V and Pb, an indication that ecological toxicity may not be indicated as a result of the presence of these heavy metals. However, HQ for Cr was greater than1 just before chromoleana odorata was sown (18.33) and at 2 MAP after untreated plant was sown. HQ was zero in PS-soils, this signified a significant remediation of Cr from toxic level to ecologically harmless level. Pretreatement of $NH_2OH.HCl$ plants did not enhance phytoremediation of Cr after two months, compared to pretreatment with NaN_3. HQ to accertain toxicity of heavy metals to soil microorganism and microbial process was less than 1 for Cu, Mn, Ni, V and Pb through out the experiment (Table 4). When HQ is greater than 1, ecotoxic effects were due to that particular contaminant. HQ was greater than 1 in the PAH components evaluated for PX3 soil as well as in the soil that had the untreated plants.

The TEQ concentration of Benzo(a)anthracene was 5.38mg/kg at Day 1, and 5.52 at 1 MAP. However, this reduced to zero in PS-soil as well as PX1 and PX2 after 2 months (Table 5). Total TEQ at Day 1 was 238.72mg/kg compared to 243.79mg/kg in the soil sown with untreated plant at 3 MAP/2 MAS. However, TEQ in PS-soil ranged from 19.58 to 21.33mg/kg and from 32.74 to 61.74mg/kg in PX-soil. When TTEQ for concentration of PAH mixture exceeds the method B clean up levels, the clean up level for Benzo(a)pyrene(0.137) was no meant for the level.

Table 1: Chemical composition of materials used for the experiment.

Parameters	Soil (mg/kg)	Waste oil (mg/kg)
Naphthalene	Bd	26.58
Acenaphthylene	Bd	7.98
2-bromonaphthalene	Bd	29.54
Acenaphthene	Bd	26.32
Fluorine	Bd	42.04
Phenanthrene	0.85	3.68
Anthracene	Bd	21.57
Fluoranthene	Bd	32.68
Pyrene	Bd	23.98
Benzo(a)anthracene	Bd	42.05
Chrysene	Bd	106.54
Benzo(b,j,k)fluoranthene	Bd	41.68
Benzo(a)pyrene	40.28	129.87
Indeno(1,2,3-cd)pyrene	5.24	129.54
Dibenzo(a,h)anthracene	12.25	34.68
Benzo(g,h,i)perylene	19.24	63.25
Copper, Cu	Bd	7.92
Manganese, Mn	Bd	Bd
Nickel, Ni	Bd	Bd
Vanadium, V	Bd	Bd
Chromium, Cr	0.08	16.85
Lead, Pb	Bd	9.96

Bd – below detectable limit of 0.0001 mg/kg.

Table 2: Heavy metal composition of soil subjected to various treatments

Heavy metals (mg/kg)	Polluted soil at Day 1	1 month after pollution	3 months after pollution								LSD (0.05)
			Unpolluted	Polluted soil	PS1	PS2	PS3	PX1	PX2	PX3	
			(mg/kg)								
Cu	16.23	14.03	Bd	12.21	7.18	6.02	5.19	7.03	6.38	5.02	1.87
Mn	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	NA
Ni	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	NA
V	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	NA
Cr	20.36	18.33	0.02	16.51	Bd	Bd	Bd	10.56	9.21	7.29	2.63
Pb	18.39	16.85	Bd	13.68	13.86	13.05	10.98	10.84	9.08	8.12	4.32
Total	54.98	49.21	0.02	42.40	21.04	19.07	16.17	28.43	24.67	20.43	-

Bd – below detectable limit of 0.0001 mg/kg. NA – not available. WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN_3-treated stem cutting, PS2- 0.064% NaN_3, PS3- 0.250% NaN_3, PX1- soil on which was sown 0.016%$NH_2OH.HCl$-treated stem cuttings, PX2- 0.064% $NH_2OH.HCl$, PX3- 0.250% $NH_2OH.HCl$. Means on the same row that are separated by their corresponding LSD values are significantly different from each other ($p<0.05$).

Table 3: Polyaromatic hydrocarbon contents of oil- polluted soil after exposure to mutagenic treatments.

PAH components	Polluted soil at Day 1	1 month after pollution	3 months after pollution									LSD (0.05)
			Unpolluted	Polluted soil	PS1	PS2	PS3	PX1	PX2	PX3		
			(mg/kg)									
Naphthalene	29.25	25.33	Bd	22.53	Bd	Bd	Bd	Bd	Bd	3.66		3.14
Acenaphthylene	10.54	9.58	Bd	8.63	Bd	Bd	Bd	Bd	Bd	3.69		2.04
2-bromonaphthalene	35.21	29.03	Bd	29.03	Bd	Bd	Bd	15.98	16.68	4.01		3.74
Acenaphthene	35.46	30.51	Bd	28.33	Bd	Bd	Bd	Bd	Bd	3.92		3.43
Fluorene	45.22	40.58	Bd	29.70	15.54	BDL	BDL	19.54	16.52	3.78		3.22
Phenanthrene	5.62	3.68	0.28	1.29	0.08	2.68	1.77	0.96	0.29	0.03		2.31
Anthracene	29.24	28.65	Bd	26.32	16.18	2.68	Bd	16.95	17.22	4.36		5.23
Fluoranthene	42.52	41.62	Bd	39.99	Bd	Bd	Bd	Bd	Bd	4.11		2.64
Pyrene	36.20	35.98	Bd	32.70	15.55	Bd	Bd	21.93	16.66	6.26		4.93
Benzo(a)anthracene	53.87	55.21	Bd	47.21	Bd	Bd	Bd	Bd	Bd	9.69		2.03
Chrysene	129.54	119.52	Bd	102.04	Bd	Bd	Bd	Bd	Bd	4.04		8.62
Benzo(b,j,k)fluoranthene	59.48	52.98	Bd	43.12	Bd	28.28	19.34	8.56	1.88	9.75		6.03
Benzo(a)pyrene	209.16	185.67	23.86	183.72	19.17	18.33	20.34	54.92	30.08	31.02		11.23
Indeno(1,2,3-cd)pyrene	169.54	139.54	1.24	118.42	15.74	12.54	8.93	68.20	26.85	35.05		12.69
Dibenzo(a,h)anthracene	52.20	50.67	1.33	49.81	3.07	2.65	1.89	39.25	26.61	28.38		6.52
Benzo(g,h,i)perylene	72.65	68.42	9.50	66.38	22.52	24.52	23.35	32.54	28.52	42.87		8.02
Total	1015.70	916.97	36.21	829.22	107.85	91.68	91.55	278.83	181.31	194.62		-
Efficiency (%)	-	9.74	96.44	18.36	89.88	90.99	90.98	72.54	81.14	80.83		-

Bd- Below detectable limit. WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN_3-treated stem cutting, PS2- 0.064% NaN_3, PS3- 0.250% NaN_3, PX1- soil on which was sown 0.016%$NH_2OH.HCl$-treated stem cuttings, PX2- 0.064% $NH_2OH.HCl$, PX3- 0.250% $NH_2OH.HCl$. Means on the same row that are separated by their corresponding LSD values are significantly different from each other (p<0.05).

Table 4: Hazard quotient to show toxicity of heavy metal and PAH components of the polluted soil

Heavy metals (mg/kg)	Polluted soil at Day 1	1 month after pollution	3 months after pollution							
			Unpolluted	Polluted soil	PS1	PS2	PS3	PX1	PX2	PX3
				(mg/kg)						
HQ for toxicity of heavy metals to ecosystem										
Cu	0.41	0.35	0	0.30	0.18	0.15	0.13	0.17	0.16	0.13
Mn	0	0	0	0	0	0	0	0	0	0
Ni	0	0	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0	0	0
Cr	20.36	18.33	0.02	16.51	0	0	0	10.56	9.21	7.29
Pb	0.37	0.33	0	0.27	0.28	0.26	0.22	0.21	0.18	0.16
HQ for toxicity of heavy metals to microbial activities and processes										
Cu	0.16	0.14	0	0.12	0.07	0.06	0.05	0.07	0.06	0.05
Mn	0	0	0	0	0	0	0	0	0	0
Ni	0	0	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0	0	0
Cr	2.04	1.83	$2*10^3$	1.66	0	0	0	1.05	0.92	0.72
Pb	$2*10^{-2}$	$1.8*10^{-2}$	0	0.02	0.02	0.01	0.01	$1.2*10^{-2}$	$1.0*10^{-2}$	$9.0*10^{-3}$
HQ for toxicity of polyaromatic hydrocarbon components to ecosystem										
Naphthalene	292.5	253.30	0	225.30	0	0	0	0	0	36.60
Acenaphthene	1.77	1.53	0	283.30	0	0	0	0	0	0.19
Fluorene	1.51	1.35	0	297.00	0.52	0	0	0.65	0.55	0.13
Phenanthrene	5.62	36.80	2.80	12.90	0.80	26.80	177.00	9.60	2.90	0.30
Anthracene	292.40	286.50	0	263.20	161.80	26.80	0	169.50	172.2	43.60
Fluoranthene	425.20	416.20	0	399.90	0	0	0	0	0	41.10
Pyrene	538.70	359.80	0	327.00	155.50	0	0	219.30	166.60	62.20
Benzo(a)pyrene	209.16	1856.70	233.80	1837.20	191.70	183.30	203.40	549.20	300.80	310.20

HQ > 1 indicates toxicity. Toxicity benchmarks for whch HQ were obtained are provided by Efroymson et al. (1997). WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN_3-treated stem cutting, PS2- 0.064% NaN_3, PS3- 0.250% NaN_3, PX1- soil on which was sown 0.016%$NH_2OH.HCl$-treated stem cuttings, PX2- 0.064% $NH_2OH.HCl$, PX3- 0.250% $NH_2OH.HCl$.

Table 5: Toxicity equivalency concentration of the polyaromatic hydrocarbon contents of polluted soil after exposure to mutagenic treatments.

PAH components	Polluted soil at Day 1	1 month after pollution	3 months after pollution							
			Unpolluted	Polluted soil	PS1	PS2	PS3	PX1	PX2	PX3
			(mg/kg)							
Benzo(a)anthracene	5.38	5.52	0	47.21	0	0	0	0	0	9.69
Chrysene	1.29	1.19	0	1.02	0	0	0	0	0	0.04
Benzo(b,j,k)fluoranthene	209.16	185.67	23.86	183.72	19.17	18.33	20.34	54.92	30.08	31.02
Benzo(a)pyrene	16.95	13.95	0.12	11.84	1.57	1.25	0.89	6.82	2.66	2.84
Indeno(1,2,3-cd)pyrene	238.72	206.33	23.98	243.79	20.74	19.58	21.23	61.74	32.74	43.59
TOTAL TEQ	471.5	412.66	47.96	487.58	41.48	39.16	42.46	123.48	65.48	87.18

WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN_3-treated stem cutting, PS2- 0.064% NaN_3, PS3- 0.250% NaN_3, PX1- soil on which was sown 0.016%$NH_2OH.HCl$-treated stem cuttings, PX2- 0.064% $NH_2OH.HCl$, PX3- 0.250% $NH_2OH.HCl$.

Achromobacter sp, Micrococcus varians and P.aeruginosa were relatively dorminant bacteria species in that order (Table 6). Total heterotrophic bacteria count was 7.6 x 10^5 cfu/g in the unpolluted soil after sowing Chromoleana. In PS-soils, heterotrophic bacteria count ranged from 4.2 - 6.5 x 10^5 cfu/g, and 3.8 - 5.8 x 10^5 cfu/g in PX-soils. Comparatively increase concentration of the mutagenic agent decreased heterotrophic bacteria composition. However, increase concentration of mutagenic solution increased percentage composition of hydrocarbon degrading fungi from 244.7% in PS1 to 71.4%

in PS3, and from 46.2% in PX1 to 61.9% in PX3. Example of hydrocarbon degrading fungi found in this study were Aspergillus niger (most prevalent), A.Flavus, Penicillium sp, Fusarium Solani.

Number of sprouted branches per plant stand at 2 months after sowing (MAS) for all treatment was highest at PX1 with 5 branches and lowest at PS3 with 2 branches (Table 6). Total number of leaves was 68 leaves per plant at PX1 and 27 leaves at PS3. Whereas necrotic leaves per plant at 2 MAS were 6 leaves at PS1 and 4 leaves at PX3.

Table 6: Microbial composition of soil at 3 months after pollution

PAH components	Control		PS1	PS2	PS3	PX1	PX2	PX3
	Unpolluted	Polluted soil						
Bacterial species								
Achromobacter sp	+	+	+	+	-	+	+	-
*Micrococcus varians	+	+	+	+	+	+	+	+
M. roseus	-	+	+	+	-	+	-	-
*Bacillus pumilis	+	-	+	+	-	+	+	-
B. subtilis	+	+	+	-	-	+	-	-
Pseudomonas sp	-	-	+	-	+	+	+	-
*P. aeruginosa	+	+	+	+	+	+	-	-
Heterotrophic bacteria (x 10^5 cfu/g)	7.6	5.6	6.5	5.6	4.2	5.8	4.5	3.8
Hyd. Deg. bacteria (x 10^5 cfu/g)	4.9	3.9	3.8	3.2	2.6	3.4	2.5	1.7
% Hyd	64.5	69.6	58.5	57.1	61.9	58.6	55.6	44.7
Fungal species								
*Aspergillus niger	+	+	+	-	+	+	-	-
*A. Flavus	+	+	+	-	-	+	-	-
*Penicillium sp	+	-	+	+	-	+	+	-
*Fusarium solani	-	-	-	+	+	-	-	+
Mucor sp	+	+	-	-	-	+	-	+
Geotrichum sp	+	+	+	-	-	+	-	-
Trichoderma sp	+	+	-	-	-	-	-	-
Heterotrophic Fungi (x 10^5 cfu/g)	4.9	3.8	3.8	3.2	2.8	3.9	3.2	2.1
Hyd. deg. Fungi (x 10^5 cfu/g)	2.7	1.8	1.7	1.5	2.0	1.8	1.6	1.3
% Hyd	55.1	47.4	44.7	46.9	71.4	46.2	50	61.9

+present, -absent, *hydrocarbon degraders. WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN_3-treated stem cutting, PS2- 0.064% NaN_3, PS3- 0.250% NaN_3, PX1- soil on which was sown 0.016% $NH_2OH.HCl$-treated stem cuttings, PX2- 0.064% $NH_2OH.HCl$, PX3- 0.250% $NH_2OH.HCl$.

Table 6: Selected plant parameters at 2 months after sowing

Plant parameter	Control		NaN$_3$ solution			NH$_2$OH.HCl solution			LSD (0.05)
	Unpolluted	Polluted soil	PS1	PS2	PS3	PX1	PX2	PX3	
No. Of sprouted branches	3.2	4.1	3.1	2.8	2.2	5.3	3.2	4.3	0.7
Leaf area (cm^2)	9.4	3.2	4.3	5.3	7.0	6.6	4.9	9.8	2.1
Length of sprouted branch (cm)	32.2	15.5	13.5	12.3	16.0	17.6	16.0	16.2	5.1
*Total No. Of leaves	48.0	37.0	28.1	19.2	27.0	68.0	42.0	45.0	4.8
No. Of necrotic leaves per plant	0	0	6.3	0	0	0.	0	4.2	1.6
Total No. of senesced leaves	7.0	13.0	8.0	12	11.0	8.0	11.0	15.0	2.2
No. Of reddish brown leaves per plant	0	4.0	4.0	0	6.0	10.0	4.0	8.0	2.8

* This includes total number of scenesced leaves. DAS- Days after sowing, WEO-Waste engine oil, MAS –Months after sowing, PS1- soil on which was sown 0.016% NaN$_3$-treated stem cutting, PS2- 0.064% NaN$_3$, PS3- 0.250% NaN$_3$, PX1- soil on which was sown 0.016%NH$_2$OH.HCl-treated stem cuttings, PX2- 0.064% NH$_2$OH.HCl, PX3- 0.250% NH$_2$OH.HCl. Means on the same row that are separated by their corresponding LSD values are significantly different from each other (p<0.05).

Discussion

Reseults of this study showed that pollution of soil by WEO brought about increased concentration of heavy metals in the soil. However, introduction of plants resulted significantly in the reducution of heavy metal concentrations of the soil.Results also showed that with the increase in concentration of mutagenic solutions required for presoaking, total heavy metal concentration in soil decreased. Total heavy metal was least in PS3 (16.17 mg/kg), compared to 21.04 mg/kg as total in PS1, 28.43 mg/kg in PX1 and 20.43 mg/kg in PX3.

Metals are amongst mineral nutrients needed by plant, but at higher concentration all metal are toxic due to the fact that they form free radical which causes oxidative stress. Also toxicity of metals is attributed to the ability of certain metals to be metabolism or take up by plant roots in place of necessary metals in enzymes and pigment reactions causing functtional disruption.(Henry, 2000). However, certain plants thrive in polluted environments as have been established by earlier studies (Wong and Chu, 1985; Anoliefo and Edegbai, 2001; Dede et al., 2003; Olivares, 2003; Ogboghodo et al.,2001; Vwioko and Fashemi, 2005), in Ricinus communis. Anoliefo et al (2006, 2008) also identified Chromolaena in heavy metal contaminated soils. These plants are phytoremediative and phytoremediation provides superior benefit for the remediation of heavy metal. In the present study, Chromolaena plants that had been pretreated in concentrations of sodium azide caused better heavy metal remediation than those preteated with hydroxylamine hydrochloride. For example, Cr was below detectable limit when oil-polluted soil was sown with sodium azide-preteated stem cuttings.

Chromolaena odorata has been reported from previous research to have ability to tolerate oil (Anoliefo and Vwiko, 2001), has high ash content and can therefore bioaccumulate and translocate hydrocarbon compounds and heavy metals (Belford et al.,2009). From this research it can be observed that Chromolaena odorata remediated the waste engine oil polluted soil for both poly aromatic hydrocarbon and heavy metals. Poly aromatic hydrocarbons naphthalene, acenaphthylene, acenaphthene, fluoranthene, benzo(a)anthracene, chrysene were all remediated below detectable limits in treatments with NaN$_3$ (0.016%, 0.064%, 0.25%), NH$_2$OH.HCl (0.016%, 0.064%) respectively. There was also significant reduction in the concentration of benzo[b]fluoranthene, benzo(ghi)perylene, benzo[k]fluoranthene, dibenzo[a,h]anthracene at the various treatments. These PAH components were hitherto present in the soil before pollution and increased with pollution by waste engine oil. The activities of soil microbes have been noted to help PAH remediation greatly particularly in collaboration with plant roots (Anoliefo and Ikhajiagbe, 2011).

Microbial degradation of light weight aromatic hydrocarbon, which are easily evaporated are accessible to microbes in soluble or dissolved forms (Jordan and Payne, 1980; Kappeler and Wuhrmann, 1978). Hydrocarbon degradation is not limited to few genera of microbes, but the ability to degrade has been observed in a varied group of fungi and bacteria.In the present study, Achromobacter sp., Micrococcus varians and P. Aeruginosa were the predominant bacterials species, whereas A. Niger and Penicillium sp. were predominant fungi. Occurence of P. Aeruginosa was 100% in all PS-treatments, compared to 1-in-3 PX treatments. Incidently, it was identified as an oil degrader in this study. That may perhaps explain the enhanced heavy metal and PAH reediations in the PS treatments, compared to the PX. Similarly, A. niger was present in 2-in-3 PS-treatements, as against 1-in-3 PX treatments.

There was however greater remediation in the mutagenic pretreated stems of Chromolaena odorata in all treatments and total remediation of most poly aromatic hydrocarbon with some treatment of the mutagenic agents, as seen in naphthalene, acenaphthylene, acenaphthene, fluoranthene, benzo(a)anthracene, chrysene, which were all totally remediated in treatments with NaN_3 (0.016%, 0.064%, 0.25%), $NH_2OH.HCl$ (0.016%, 0.064%). Mutagenic solutions may have enhanced the plant's capacity for remediation. Mensah and Akomeah (1992); Mensah et al. (2006) and Mshembula et al (2012) reported enhanced plant growth and development as a result of pretreatment with sodium azide and hydroxylamine hydrochloride. Ikhajiagbe et al (2013) reported that enhanced plant growth of sodium azide – treated rice exposed to waste engine oil pollution. Though the introduction of mutagens increased the growth response of stem cuttings and improved the bioaccumulation rate of Chromolaena odorata, it also increased the metabolic rate of the plant, bringing about early senescence and also death of the plant that were pretreated with the mutagens.

The leaves of Chromolaena odorata plant on the polluted soil all showed reddish-brown discoloration of leaves starting from leaf tip and progressing inward. This symptom is mainly consistent with phosphorus deficiencies which exhibits red or purple pigmentation as a result of anthocyanin pigment. Phosphorus is a component of certain enzymes and protein, adenosine triphosphate. Reddish brown coloration is also associated with N, K, Mg deficiencies in plant. Oil polluted soil are prone to nutrient deficiency, which may indirectly result from reduced water uptake from clogging of soil pore by the presence of waste engine oil in the soil. According to (Udo and Fayemi, 1975) soil contaminated by oil may be inappropriate for farming as a result of nutrient avaliability reduction or due to increase in toxic concentration of heavy metals such as manganese. The presence of these heavy metals in oil polluted soil causes inhibition of growth and disruption of metabolic processes in plants.

Conclusion

Phytoremediation is a useful and effective method of cleaning contaminants from the environment, although it is slow and usually requires a time frame to be very effective. This prolong or waiting period for efficiency makes it a tedious technique useful by environmentalise. The present study shows that the intoduction of mutagenic agent such as sodium azide and hydroxylamide to planting materials help not only to aid rapid growth of Chromolaena odorata (Siam weed), but also help the with the bioaccumulative potential of Chromolaena odorata (Siam weed), as seen in the treatment of various concentrations of sodium azide and hydroxylamide compared with the control without any treatment. There was great reduction in PAH and heavy metal component of the soil.

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