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Growth and remediation performance of *Eleusine indica* obtained from oil-polluted soils in Benin metropolis after exposure to grey water

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Abstract

As a step towards a successful reduction of environmental degradation in Benin City thus, this study was conducted to evaluate the growth and remediation performance of *Eleusine indica* obtained from four local Government Areas, Egor, Ikpoba okha, Oredo and Ovia within the Benin metropolis. Soils were collected from generator plant, within auto – mechanic workshop, auto spare parts stores, and soils around bakery. *E. indica*, was grown in soil polluted with oil and wetted with grey water daily, twice weekly, weekly, twice monthly and monthly while Control was wetted with tap water daily. Heavy metal and total hydrocarbon content of polluted soil was analyzed before and after *E. indica*, was planted. Results obtained showed that *Eleusine indica* was able to grow on the polluted soil. However, the presence of grey water improved the growth of the plant. *E. indica* wetted twice weekly showed the best morphological growth, alongside plants wetted weekly, twice weekly and twice monthly recording the highest percentage of heavy metals and total hydrocarbons reduction. The most remediated heavy metal by the test plant was chromium, which recorded up to 99.99% removal. This was followed by manganese and cadmium which had 96.77 and 89.13% respectively. Hydrocarbon reduction efficiency by grey water intervention was 81.19%. The study validated the use of grey water for improving the remediation performance of the test plant.

1. Introduction

Man uses the environment as a resource bank, a habitat and as sink for human waste [1]. As a resource bank, the environment supplies man with raw materials needed to maintain their existence, and their social and technological structures; as a habitat, people require more space per individual than any other species; and as sink for wastes, human beings produce more waste than other species. All these activities result in serious degradation of the environment, and also create a threat to the very existence of mankind [2].

Pollutants in urban areas arise from activities in residential neighbourhoods, markets, schools and central business districts [3]. The six major urban environmental challenges are: air pollution, water pollution, alteration of biogeochemical cycles, solid waste management [4]. Environmental degradation is a direct effect of uncontrolled urbanization. The main components of the environment affected by urbanization are climate, biosphere, land and water resources [5]. It has been reported that about 45 % of Nigerians live in the urban areas, and the country has the highest rate (a yearly average of 5.5 %) of urbanization in the world [2]. Consequently, this has led to an increase in the numerous problems associated with rapid urbanization. These problems include: deteriorating environment, urban decay, un-cleared refuse, flooding, erosion and pollution.

Chemicals that are known to pollute the environment include heavy metals, drugs, hydrocarbons, halogenated solvents, endocrine disrupting agents and agro-chemicals [6]. Chemical pollutants include polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, cyanide compounds, pesticides (fungicides, insecticides and herbicides) and heavy metals (such as zinc, cadmium, lead, uranium, iron, nickel, silver, thorium, radium and mercury) [7].

Soils polluted with petroleum hydrocarbons show a reduction in population and diversity of micro-organisms. Petroleum hydrocarbons also interfere with the plant-fungi relationship as biological and physicochemical relationship of the soil is altered [8][9]. These physicochemical changes are reduced soil pH, increased soil organic carbon and organic matter [10][11]. These changes have also been documented to be accompanied by increased sodium and iron content [12]. Saadoum [13] recorded a great decline in bacterial counts and diversity in soils contaminated with crude oil spill.

Bioremediation is an economically friendly way of treating hazardous pollutants. Bioremediation is an ecologically sound and state-of-the-art technique that employs natural biological processes to completely eliminate toxic contaminants [14]. It is any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition [15]. Plants like *Eleusine indica* grows well in compact, wet soils and competes successfully with warm-season and cool-season turf grasses. It is considered an invasive weed due to its vigorous growth and abundant seed production. Seeds germinate near the surface of moist soils and seed germination completely ceases if seeds are buried deeper than 3 inches [16]. The persistence of *E. indica* in the field is due to abundant seed production and tolerance to close mowing. *E. indica* can grow up to 3 feet tall [16] and spreads by reseeding itself. It is therefore the aim of this study to determine the growth and remediation performance of *Eleusine indica* obtained from oil-polluted soils in Benin metropolis after exposure to grey water.

2. Material and Methods

2.1. Collection of Soil Samples

Top soil (0 – 10 cm) was randomly collected from the within and around (within 30 cm from the periphery of the spill) spill sites and then pooled together to obtain composite samples. These were labelled appropriately, and immediately transported in Bagcosacks. The soils were then collected together and thoroughly mixed to obtain a composite sample. A composite sample was taken for analysis before use. The pooled soils were weighed and placed on bowls, consisting of 20 kg of sun-dried soils per bowl. The soil was sun-dried to constant weight prior to measurement. These were arranged on the field prior to transplantation with *Eleusine indica* (Plate 1).

2.2. Determination of Weed abundance

Before soils were collected from the designated sites, care was taken to observe the weed distribution within and around the various locations, especially those weeds that were in close association with the test plant. For the purpose of this study, an area measured 5m x 5m, which also included the polluted area of interest, was separated out for plant observation. Four different areas within the Benin Metropolis, Nigeria, were selected; soil around auto mechanic workshops, bakeries, diesel-engine driven generating plants, and auto-spare part markets. Plants, after having been physically counted within designated 25 m^2 quadrants, were collected for identification using identification manual and with help from a number staff of on ground at the Department. The Akobundu's Text [17] was also very helpful in plant identification.

2.3. Preparation of Grey Water

Grey water used in the study was obtained by washing 1 kg of dirty clothes with 20g of Premier® soap in 10 litres of tap water (pH 6.14 – 6.92). The grey water was used within one week of preparation. Initially, each soil-filled bucket was saturated with greywater on the first day; thereafter the buckets were further subjected to periodic wetting with 1500ml on a daily basis (D) after having been sown with the test plant. Other periods of wetting were twice a week (2DW), weekly (W), twice within a month (2WM), and monthly (M). The control buckets (C) were wetted with tap water.

2.4. Soil Heavy Metal Analyses

Soils were dried at ambient temperature (22-25^{o}C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of heavy metal fractions as well as total hydrocarbon content (THC) [18].

2.5. 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 HCI was added, stopped, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42. And then Fe, Mn, Zn, Cd, and Cr were determined in the filtrate by Atomic Absorption Spectrometry.

2.6. Determination of Total Hydrocarbons

Five (5) g soil was weighed into a 100 ml plastic bottle. 25 ml of n-hexane was added, and mechanically shaken for 10 minutes and let stand covered. It was then filtered, and the filtrate was read at 460 nm. THC standard stock, 1000 mg/l was done by pipetting Some 1.18 ml of Forcados Blend Crude Oil and made to 1 liter with n-Hexane. From this, 0, 10, 20, 40, 60, 80 and 100 mg/l working models were made ready. Calculation:

$$\text{THC (mg/l)} = \frac{\text{Instr.reading x Slope reciprocal x 25}}{\text{5g}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

2.7. Statistical Analyses

Considering homogeneity of experimental plot, the design adopted for this study was complete randomized design. A single factor analysis of variance was therefore adopted using the SPSS® version 20 statistical software. Means were termed significant at $p < 0.05$. For determination of weed diversity indices about the sampled plots, the Paleontological statistics software package for education and data analysis (PAST®) was used according to Hammer *et al.* [19].

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Plate 1: *Eleusine indica* at different stages of development

3. Results and discussion

Results obtained from this study showed that plants were obtained from the polluted sites in the various locations tested (Table 1). However, species distribution varied with location and nature of pollutant on the soils. Of all the species recorded in this study, *Eleusine indica* was most abundant, with a frequency of abundance of 0.11, compared to the least abundant plant (*Euphorbia hyssopifolia*) with a frequency of 0.03. *Acanthospermum hispidum* was more abundant in soils around generator plant than in the other locations; it was absent around the bakery. The most abundant plant species in the automechanic workshop was *E. indica*. The population of *Ageratum conyzoides* was <10 plants per unit area in soils around generator plant, within auto mechanic workshop, and within spare parts store in all four locations tested.

Table1: Plant distribution in areas from where soils were collected for the study

	Species identification						Species Diversity Indices								
	SGEN	SMWK	SASP	SBAK	Total	Fab	(n)	Dominance	Simpson	Shannon	Evenness	Menhinick	Margalef	Fisheralpha	BergerParker
Plant Species															
Acanthosperumhispidum	14	2	2	0	18	0.05	18	0.630	0.370	0.684	0.660	0.707	0.692	1.028	0.778
Achyranthesaspera	11	4	0	4	19	0.05	19	0.424	0.576	0.973	0.882	0.688	0.679	1.002	0.579
Ageratum conyzoides	7	7	7	4	25	0.06	25	0.261	0.739	1.363	0.977	0.800	0.932	1.344	0.280
Alternantherarepens	11	9	2	4	26	0.07	26	0.328	0.672	1.216	0.844	0.785	0.921	1.320	0.423
Andropogon tectorum	1	0	4	0	5	0.01	5	0.680	0.320	0.500	0.825	0.894	0.621	1.235	0.800
Axonopuscompressus	5	4	6	2	17	0.04	17	0.280	0.720	1.320	0.936	0.970	1.059	1.649	0.353
Eleusine indica	17	13	7	7	44	0.11	44	0.287	0.713	1.313	0.929	0.603	0.793	1.069	0.386
Euphorbia hyssopifolia	4	0	0	6	10	0.03	10	0.520	0.480	0.673	0.980	0.633	0.434	0.752	0.600
Kyllingaerecta	11	2	4	7	24	0.06	24	0.330	0.670	1.223	0.849	0.817	0.944	1.371	0.458
Leptochloacaerulescens	5	7	2	2	16	0.04	16	0.320	0.680	1.245	0.868	1.000	1.082	1.712	0.438
Mariscusalternifolios	7	7	7	7	28	0.07	28	0.250	0.750	1.386	1.000	0.756	0.900	1.277	0.250
Oldenlandiaherbacea	5	7	7	7	26	0.07	26	0.254	0.746	1.377	0.991	0.785	0.921	1.320	0.269
Panicum maximum	5	7	7	7	26	0.07	26	0.254	0.746	1.377	0.991	0.785	0.921	1.320	0.269
Paspalumscrobiculatum	13	9	4	9	35	0.09	35	0.283	0.717	1.314	0.931	0.676	0.844	1.164	0.371
Peperomiapellucida	5	2	4	4	15	0.04	15	0.271	0.729	1.340	0.955	1.033	1.108	1.785	0.333
Phyllantusamarus	5	7	7	7	26	0.07	26	0.254	0.746	1.377	0.991	0.785	0.921	1.320	0.269
Sidaacuta	7	5	7	5	24	0.06	24	0.257	0.743	1.372	0.986	0.817	0.944	1.371	0.292
Tridaxprocumbens	4	5	2	4	15	0.04	15	0.271	0.729	1.340	0.955	1.033	1.108	1.785	0.333
Diversity Indices															
Individuals (n)	137	97	79	86	399	-	-	-	-	-	-	-	-	-	-
Taxa_S	18	16	16	16	-	-	-	-	-	-	-	-	-	-	-
Dominance	0.072	0.076	0.074	0.071	-	-	-	-	-	-	-	-	-	-	-
Simpson	0.928	0.924	0.927	0.929	-	-	-	-	-	-	-	-	-	-	-
Shannon	2.746	2.658	2.675	2.701	-	-	-	-	-	-	-	-	-	-	-
Evenness	0.866	0.891	0.907	0.931	-	-	-	-	-	-	-	-	-	-	-
Menhinick	1.538	1.625	1.8	1.725	-	-	-	-	-	-	-	-	-	-	-
Margalef	3.455	3.279	3.433	3.367	-	-	-	-	-	-	-	-	-	-	-
Fisher_alpha	5.544	5.456	6.055	5.79	-	-	-	-	-	-	-	-	-	-	-
Berger-Parker	0.124	0.134	0.089	0.105	-	-	-	-	-	-	-	-	-	-	-

SGEN Soils around generating set; SMWK soils around automechanic workshop; SASP soils around autospare parts shops, Fab frequency of abundance of individual species

Table 2: Morphological parameters of *Eleusine indica* used in the study

Parameter	Value
Plant height (mm)	21.24±2.11
Flag leaf blade length (cm)	2.01±0.94
Flag leaf blade width (cm)	0.96±0.16
Internode (cm)	2.86±0.43
Number of leaves per culm	6±1
Necrosis?	No
Chlorosis?	No

Table 2 shows the morphological parameters of *Eleusine indica* tiller used from the nursery. The plant was 21.24±2.11 mm tall, with leaf blade length and width of 2.01 ±0.94 cm and 0.96 ±0.16 cm respectively. The internodes were 2.86 ±0.43 cm apart and the numbers of leaves were 21.24±2.11, the plants where chlorosis and necrosis free.

Table 3: The Physicochemical parameters of materials used for the study

Parameters	Tap Water	Grey Water
TSS (mg/l)	0.79	562.37
Dissolved Oxygen (mg/l)	0.96	9.21
Biochemical Oxygen Demand(mg/l)	0.24	11.36
Chloride (mg/l)	53.98	698.80
Phosphate (mg/l)	0.02	8.62
Nitrate (mg/l)	0.09	19.30

The physical parameters of materials tap water and grey water used for the study are shown in Table 3. The values of total suspended solids, dissolved oxygen, biochemical oxygen demand, chloride, phosphate and nitrate obtained for grey water were far higher than those recorded for tap water.

Table 4 shows the morphological parameters of the test plant, *Eleusine indica*, after three months of exposure to oil polluted soil. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote sustainable development of our society, with low environmental impact. The absence of some plant species in the different soils and locations tested in this study suggests the intolerance of the plant species to the pollutants presents in those soils and locations. The growth of the test plant in oil polluted soil demonstrates that ability of the plant to grow under environmentally stressed conditions such as those presented by heavy metals in oil – polluted soils. This is what enhances its role in the degradation of oil polluted soils, and makes it an excellent option in the bioremediation of recalcitrant pollutants in the environment [20].

Table 4: Selected morphological parameters of *Eleusine indica* after 3 months exposure to oil -polluted soil.

Plant parameters	CTR	Plant on Oil-impacted soil						p-value
		NoGW	Dly	2Wkly	Wkly	2Mthly	Mthly	
Plant Height (cm)	25.18	14.78	21.04	19.24	22.93	20.32	19.42	0.008
*No. of Leaves	38	21	32	28	34	29	26	0.012
Leaf Area (cm^2)	6.39	3.75	3.99	4.67	5.16	4.98	3.09	0.048
Leaf blade length (cm)	19.42	11.4	16.42	15.78	17.42	16.32	15.22	0.248
Internode	3.87	2.27	2.67	3.51	3.24	3.32	2.98	0.736
*No. of Tillers	18	10	15	14	16	16	13	0.204
*Total No. of Panicles	26	15	19	18	23	21	17	0.069
*No. of Roots	62	43	57	57	57	52	48	0.013

CTR control, NoGW no grey water applied, Dly Daily application, 2wkly application twice a week, 2Mthly twice a month and Mthly Monthly application.
Values with same alphabetic superscripts presented on similar columns do not differ from each other (p>0.05)
*presented to the nearest whole number

Table 5: Heavy metal and THC content of *E. indica*– sown soil after wetting with greywater

Parameters	Back-ground	Plant on Oil-impacted soil (mg/kg)												p-value
		NoGW	*Δ%*	Daily	*Δ%*	2Wkly	*Δ%*	Wkly	*Δ%*	2Mthly	*Δ%*	Mthly	*Δ%*	
Cr	4.52	0.15	*96.7*	0.04	*99.23*	0.1	*97.74*	0.03	*99.36*	<0.001	*99.99*	0.09	*97.92*	*<0.001*
Mn	32.24	11.53	*64.24*	8.34	*74.13*	7.02	*78.23*	2.11	*93.46*	1.04	*96.77*	3.85	*88.06*	*<0.001*
Zn	92.62	41.68	*55*	27.42	*70.4*	34.22	*63.05*	17.02	*81.62*	28.64	*69.08*	22.93	*75.24*	*0.028*
Fe	985.21	443.34	*55*	485.35	*50.74*	302.33	*69.31*	294.31	*70.13*	222.18	*77.45*	306.36	*68.9*	*0.056*
Cd	1.38	0.34	*75.36*	0.15	*89.13*	0.83	*39.86*	0.34	*75.36*	0.75	*45.65*	0.15	*89.13*	*0.218*
THC	1749.45	787.25	*55*	374.22	*78.61*	418.23	*76.09*	371.24	*78.78*	492.34	*71.86*	328.99	*81.19*	*<0.001*

CTR control, NoGW no grey water applied, Dly Daily application, 2wkly application twice a week, 2Mthly twice a month and Mthly Monthly application.
Δ% percentage change

Mariscus alternifolius was sparsely present in all the soils and locations tested. Generally, a total of 399 individual species were identified, 137 of which were found in soils around the diesel-engine generating set (SGEN). Eighteen (18) taxa were identified in the study; 16 were found in all sites visited except around SGEN. All taxa identified were also present in SGEN. It was based on the high frequency of abundance of *Eleusine indica* that it was adopted as the weed of choice in this study

The growth of *Eleusine indica* in oil–polluted soil which had a high concentration of heavy metals could also be due to the possession of genes responsible for resistance to environmental stress. *E. indica* has shown resistance to herbicides such as Paraquat and glyphosate [21][22]. The decrease in the plant growth parameters of *Eleusine indica* grown on oil – polluted soil indicates the pollutant was toxic to the plant [23]. It depicts the susceptibility to inhibition of the test plant by crude oil and its products [24]. The decreased growth of the test plant in oil – polluted soil concurs with the report of [25], who observed a drastic decrease in the growth of *Aspergillus niger*, during the biodegradation crude oil and its products. The decrease in the number of roots of *E. indica* sown in oil-polluted soil is in line with the findings of Gao [26], who stated that crude oil and its derived products hold the soil too compact for plant roots to penetrate. This made the number and extent of root growth, as well as absorption of nutrients by the plant to be affected.

The reduction in the content of cadmium, chromium, manganese, iron, and zinc in *Eleusine indica* – sown soil implies the bioaccumulation of these metals by the plant [27]. The variation in the rate of removal of the heavy metals tested in this study due to several factors such as soil pH, source of pollutants, bioavailability of the metals and type of plant used in remediation [28][29][30].The removal of heavy metals and petroleum hydrocarbon from polluted soils by plants can be improved through optimized agronomic practices and soil conditions [31].The improved heavy metal removal of *E. indica* in soil wetted with grey water confirms the reports of Catherine [32] and Sun [33], who stated that the efficiency of phytoextraction of heavy metals by plants in improved on soils fertilized with nutrients such as nitrogen, phosphorus, potassium and calcium, singly or in combinations.

The grey water applied to the soil increased the nutrient content of the soil, resulting in an improved growth of the test plant, *E. indica*. This in turn, led to a better remediation performance of *E. indica* [34].The higher concentration of nitrates and phosphates in the grey water used in this study could have caused an increase in the population of rhizosphere microorganisms of *Eleusine indica*, which improved the growth of the plant in the polluted soil, and led to an increased uptake of both heavy metals and hydrocarbons from the polluted soil [35].

The vast reduction in the heavy metal and total hydrocarbon content recorded in soil wetted with grey water compared to that wetted with tap water indicates that the presence of grey water enhanced the bioaccumulation of these pollutants by *Eleusine indica*. This makes grey water a catalyst in the remediation of oil polluted soils by *E. indica* [36]. Adedokun and Ataga [37] obtained a higher percentage of remediation of oil polluted soils when *Pleurotus pulmonarius* sown polluted soils were amended with sawdust and cotton waste. Other catalysts used in the remediation of oil polluted soils include poultry droppings and cow dung, shredded banana leaves, NPK fertilizer [37][38][39].

The increase in the percentage removal of heavy metals and total hydrocarbon content from polluted soil wetted with grey water compared to that wetted with tap water suggests that the presence of grey water increased the bioavailability of these hydrocarbons and heavy metals in the soil. This facilitated their uptake and subsequent removal from the soil by the test plant [40][41].

Rhizosphere microorganisms advance plant growth by improving nutrient acquisition through fixing of atmospheric nitrogen and / or liberating phosphate from organic compounds [42][43]. Rhizosphere microorganisms also increase heavy metal and hydrocarbon accumulation in plants by increasing the transport of soluble metal ions into roots, and facilitating the solubilization of non-bioavailable forms of these metals by reducing soil pH, and synthesizing chelators (organic acids) [44][45].

The 99.99% removal of chromium from contaminated soil by *E. indica* in this study makes it the most remediated element. This could imply that chromium was more readily available in the polluted soil than the other heavy metals present. However, Prokop [46] stated that cadmium was more readily bioavailable for plant uptake than other heavy metals in polluted soil. The levels of cadmium in *E. indica*-sown oil polluted soil were all below 1 mg kg-1, which is the recommended occurrence of the metal in the environment [47]. With up to 89% removal of cadmium from polluted soil, this study parades *E. indica* as on the plants useful for the phyto extraction of cadmium [48].The 89% removal of cadmium from polluted soil by *E. indica* in this study is higher than the range of cadmium removal (0.004 – 38.8%) from contaminated soils stated by Li [48]. Murakami [49] reported a 19.7% removal of cadmium from polluted soil by *Glycine max*. The reduction in the concentration of total hydrocarbon shows that *E. indica* is able to convert these compounds into carbon dioxide and water, or other secondary metabolites; hence, the reduction of the hydrocarbon content of the soil after *E. indica* was grown on it [50].

Nitrate and phosphate supplements enhance biodegradation of oil. This could also account for the higher levels of remediation recorded in soil wetted with grey water, compared to soil wetted with tap water, since the nitrate and phosphate content of grey water were lavishly higher than was recorded with tap water. The 81.19% reduction of total hydrocarbon content recorded in this study is lower than that recorded by Stamets [51] for *Pleurotus* sp. on coil polluted soil. This difference could be due to the nature of the soil and the type of bioremediation agent used. Adongbede and Sanni [52] reported a 90% decrease of petroleum hydrocarbons in *Agaricus campestris* – sown media.

Conclusion

The growth and remediation performance of the *Eleusine indica* obtained from polluted soils in the Benin City metropolis after exposure to grey water was evaluated. The study showed that polluted soils in the Benin City metropolis had high concentration of heavy metals and total hydrocarbon content. This affected the distribution of weeds in the polluted sites. The growth of the test plant in the polluted soil suggested that the plant would be useful in the successful removal of heavy metals and hydrocarbons from polluted soils. The study has been able to establish that the remediation potential of the test plant would be greatly improved by the presence of grey water.

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