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Remediation of Oil- Contaminated wetland by *Hevea brasilliensis*

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ABSTRACT

The present investigation provides data on the performance of Hevea brasilliensis at site clean - up. Effluent sample from a wetland around abandoned oil - well head in Mgbuoba, a Nigerian Niger Delta community was characterized and a 43d hydroponic experiment using H. Brasilliensis in the presence of white light and salted regime, were done to assess its performance at contaminant removal. Deionized water was used as control and all experiment was done in triplicates. Observed levels of studied heavy metals (Cd, Pb, and Fe) were statistically significant at $p \leq 0.05$ and were reduced by 98 % and 91 %) respectively for Salted and Unsalted regimes. Salt treatment showed greater root uptake and were up to 58 % (Cd) and 59 % (Pb) compared to the unsalted 42 % and 41 % respectively. Generally, results indicate excellent levels of phytoextraction of Cd and Pb leaving relatively low or non – detectable values, thus presenting H. brassilliensis as a possible candidate for phytoremediation of oil-related contaminated liquid effluent especially a salt – assisted design.

Keywords: *Oil-related effluent, Heavy metals removal, Hevea brasiliensis, Hydroponic experiment, Salt treatment.*

INTRODUCTION

Wetlands are part of the foundation of our nation's water resources and are vital to the health of waterways and communities that are downstream. Wetlands feed downstream waters, trap floodwaters, recharge groundwater supplies, remove pollution, and provide fish and wildlife habitat. Wetlands are also economic drivers because of their key role in fishing, hunting, agriculture and recreation [1]. The health of the environment and the lives of people are intertwined with the health of the water system. The food, water, and cultural identity of many local peoples are closely related to the delta ecosystem. The rivers and streams are used for drinking, bathing, fishing, harvesting, and fermenting cassava [2]. When these water sources are polluted, it may pose health risk to the people [3].Pollution is an undesirable change in the physical, chemical or biological characteristics of air, water and soil that may harmfully affect the life or create a potential health hazard for any living organism and in particular man [4]. It is well known that environmental pollution is a product of urbanization and technology, and other attendant factors of population density, industrialization and mechanization that serve to provide the necessities of the population [5,6,7]. Anthropogenic sources of pollution include those associated with fossil fuel, coal combustion, and industrial effluents [4]. Effluents discharged into rivers, which may affect aquatic animals like fish, may do so either directly or indirectly [8] and when an environment is polluted, it may require remediation. Remediation is generally used to mean the clean-up or making safe of a site or water body contaminated by toxic substances, whether they are natural or man-made [9] .The use of higher plants in remediation of metal contaminated sites, known as phytoremediation, is gaining worldwide importance due to its low cost involvement and eco-friendliness of the method [10,11]. It is therefore the objectives of this study to investigate clean – up potential of H. Brassiliensis at removal of Cadmium and Lead from polluted wetland and the influences by salting.

MATERIALS AND METHODS

Sample sourcing

Samples were collected midstream at random with sterile two (2) litres plastic containers from the wetland and immediately corked with the lid preserved in a container of at 4^0C and taken to the laboratory within twenty-four (24) hours for initial physico -chemical analysis while observing necessary protocols. *H. brasillensis* of about eight weeks old were sourced from an extension of the Delta Rubber Plantation, Elele and conveyed to Port Harcourt all in Rivers State, Nigeria while observing the necessary protocol.

Experimental Set- up

Initial characterization of relevant parameters (pH, temperature, conductivity, salinity, Total Dissolved Solid, Chemical Oxygen Demand, Biological Oxygen Demand, Dissolved Oxygen, Phenol, Cyanide, Cadmium, Mercury, Lead, Chromium, Iron, and Turbidity) were assayed immediately after collection of samples.

Initial measurements of leaf length, leaf breadth, leaf area, root and stem length were taken. The rubber plants were planted hydroponically so that only the roots with some lower parts of the stem were immersed in samples. The first set of plants was immersed in a beaker containing 1.2 litres of deionized water as a control. The second,containing effluent sample of equal volume as were in the first beaker while the third had in addition to the second, salt augumentation (50Mm of NaCl). All experiment wasperformed in triplicates and plants were exposed to florescent light at night at room temperature (43d). Illumination with florescent white light increased enzyme (peroxidase) content and the treatment with NaCl stimulated excretion of peroxidase from the cells of the plant roots [12] and this suggests that production and product recovery from plant cells can be greatly improved by application of combined treatments.

Determination of Physical Characteristics of the Sample

The pH meter (H18314-Hanna) was calibrated with phosphate buffer solutions pH determinnation. A micro thermometer'sprobe was immersed into samples and temperature measurements read off at a steady reading.In determining the chemical oxygen demand (COD), the permanganate method was employed.For each effluent sample a blank sample was prepared and 10ml of 20% H_2S0_4 was added to each sample after which, 10ml of 80N potassium permanganate was added until a pink colouration was observed. The samples and the blanks were transferred to the water bath for 30 minutes at 100^0C. Agavn, 3mg of 10% potassium iodide and 19g starch were added to each sample and their blanks. An 80N Na_2S0_3 was titrated against each sample and its blank, and the chemical oxygen demand was calculated as given: COD= (Blank - Sample (titrant) x 10) / (Blank x Sample (titrand)).

The concentration of BOD was determined by dividing the COD by a factor of 1.5

Dissolved Oxygen (DO) / Electrical conductivity/ TDS/ Turbidity Determination: Electrometric method was employed using a DO meter/ conductivity meter (Hanna combo HI 98129) / Hach 2100AN turbidimeter respectively.

Determination of Chemical Characteristics of the Sample

Salinity: Firstly, the chloride concentration of the sample was determined. This was achieved by adding two drops of dichromate (K_2CrO_4) as an indicator to the sample and Silver nitrate ($AgNO_3$) was titrated against it with stirring until the slightest perceptible reddish coloration persisted. Determination of another sample (blank) was carried out and chloride concentration calculated. The salinity of the sample is the product of chloride(Cl^-) level and the factor (1.65)

Total Suspended Solids (TSS):The UV spectrophotometer (Hach DR2400) was standardized with deionized water in the cuvette. Some of the sample was poured into a cuvette and placed in the sample compartment. The absorbance was read-off at the wavelength of 810nm.

Total Hydrocarbon (THC):Two hundred (200) milliliters of the samples was measured into a beaker and 20 ml of chloroform ($CHCl_3$) was added to it. The solution was homogenized and allowed to stand for 2min for self - separation. Cotton wool was inserted in a separating funnel placed in a beaker. Sodium sulphate (dehydrating agent) and silica gel were added to the cotton wool for purity. The solution was poured into the funnel and the tap opened to release the extract into the beaker. The total hydrocarbon was gotten by the subtraction of the volume of the chloroform from that of the extract.

Phenol Determination: The concentration of phenol was determined using phenol pillow reagent and UV spectrophotometer (Hach DR2400). The absorbance of the sample was read-off at 460nm wavelength.

Cyanide Determination: The determination of cyanide was done using UV-spectrophotometer (Hach DR2400). The device was set to cyanide-determination mode and standardized. The absorbance value was read-off at 612 nm wavelength.

Heavy metals: Plant samples were first digested to reduce organic matter interference and convert metal to a form that can be analyzed by Atomic Absorption Spectrophotometry, AAS. Analyses were done employing AAS Model GDC Avanta A6600 at corresponding wavelengths using appropriate cathode lamp and results in mg/l were recorded.

Statistical Analysis: At 95 % confidence level, means of triplicate determinations were subjected to ANOVA using Microsoft Windows Excel 2007.

RESULTS AND DISCUSSION

Physicochemistry of Wetland samples

The results for the physico-chemical properties and levels of some major contaminants in water samples from a wetland around an abandoned oil - well in Mgbuoba Community for various regimes are given in table 1. The pH of the control sample was less alkaline (6.52) than salted (8.78 and unsalted (8.86) and compares favourably with WHO [13] set limit for drinking water.Electrical conductivity (EC) is the numerical expression of an aqueous solution to carry electrical current and is a useful indicator of the mineralization in a water sample [14].The turbidity of the sample prior to treatment (245.7 NTU) decreased in the salted regime (6.56 NTU) and the unsalted (7.36 NTU). This observed value shows that the salted regime had more effect in turbidity clearance.The biological oxygen demand (BOD) for the salted regime increased to 3.242mg/l at day 23 (values not shown) when compared to the initial (1.26mg/l) but reduced at day 43 (0.42 mg/l) and in the unsalted (0.75mg/l). The salted may have encouraged microbial activity in the sample as weel as peroxidase activity [15].

Table 1: Mean levels of sample physicochemistry (before and at the end of experiment)

S/N	Parameters	Before Experiment		End of Experiment	
			control	unsalted	salted
1.	pH	8.24a ± 0.004	6.52b ± 0.01	8.86c ± 0.01	8.78c ± 0.004
2.	Temp(^{o}C)	25.6a ± 0.06	26.5b ±0.1	27.9c ±0.06	28.5d ±0.07
3.	BOD(mg/l)	1.26a ± 0.002	0.02b ± 0.006	0.745c ±0.001	0.42c±0.002
4.	DO (mg/l)	3.37a ± 0.002	5.6b ±0.007	4.90c ±0.03	5.20d ± 0.03
5.	COD (mg/l)	1.88a ± 0.006	0.03b ±0.002	1.12c ±0.001	1.27d ± 0.006
6.	Conductivity(μS/cm)	106a ±1.52	226b ± 1	187c ±1.15	14320d ± 10.8
7.	Turbidity (NTU)	245.7a ± 0.3	0.93b ± 0.003	7.36c ± 0.03	6.56d ±0.007
8.	Salinity (mg/l)	16.5a ± 0.21	16.5a ±0.06	30b ±1.15	4000c ± 20.61
9.	THC (mg/l)	10.27a ± 0.006	0.02±b0.0002	2.05c ± 0.02	6.91d ± 0.01
10.	TSS (mg/l)	510a ±4.6	2.0b ±0	8.0c ±0.15	9.0d ± 0

11.	TDS (mg/l)	54*a* ±0.91	115*b* ±1.22	95*c* ±0.50	7520*d* ± 18.71
12.	Phenol (mg/l)	0.15	0.00	<0.001	<0.001
13.	Cyanide (mg/l)	<0.001	0.00	<0.001	<0.001
14.	Cd (mg/l)	0.082±0.002	0.00	<0.001	<0.001
15.	Hg (mg/l)	<0.001	0.00	<0.001	<0.001
16.	Pb (mg/l)	0.374±0.006	0.00	<0.001	<0.001
17.	Cr^{+} (mg/l)	<0.001	0.00	<0.001	<0.001
18.	Fe (mg/l)	24.55*a* ±0.003	0.00	0.31*b* ±0.001	0. 59b

Values are means ± SEM; Means in the same row with same alphabets are not significantly different at $P \leq 0.05$)

Elevated levels of Cadmium, Lead and Iron were observed alongside Total hydrocarbons in Wetland samples. These levels were however, reduced to non – detectable or tolerable levels at the end of the experiment. Also, statistically significant decreases in COD and BOD were observed after 43d and did not conform with given Department of Petroleum Resources guideline. On the contrary, significant increases were observed for DO and that is attributable to increased removal of organics and suspended solids as shown in table 1. Respiration, activity and growth rate of fresh water organisms generally increase as temperature and dissolved oxygen increase up to a threshold [16]. Improved levels of dissolved oxygen were more marked in the salted regime (5.20mg/l) than in the unsalted (4.90mg/l),thus, suggesting that the salted regime could be more tolerable to aquatic lives.

Increased salinity, conductivity and salinity values for salt – treated regime at 43d could be attributed to salt treatment. Efficient Phenol removal was also observed for all treatments. Cyanide, Mercury and Chromium were not detected in any of the samples.The advantages of this local plant specieas reported by Malik and Biswas [10] as a remedial measureis markedly interesting.

Uptake expression by *H. brasillensis*

It is worthy of mention that plant tissue exhibited great resistance to posed phytotoxicity by all indices employed and it is typycal of toxicity described by Pourrut et al. [17] and Nafiseh et al. [18]. Mean contaminants uptake by *H. Brasillensis* is as given in table 2. Tissue analysis of root materials indicated evidence of some heavy metals uptake as shown in table 2. The higher load of Cd and Pb occurred in the roots tissues of plants in the salted regimes than in the unsalted. The study shows that salt (NaCl) induced significant positive effect in the uptake of the Cd (up to 0.05mg/kg), Pb (up to 1.82mg/kg) when compared to the unsalted; (0.06mg/kg and 0.07mg/kg) respectively.

Table 2: Mean uptake of some contaminants (mg / kg)in the root section of the three regimes

S/N	Parameters	Control Sample	Unsalted Sample	Salted Sample
1.	Cd	0.00	0.06a±0.001	0.05b±0.007
2.	Hg	0.00	<0.001	<0.001
3.	Pb	0.00	0.07a±0.01	0.18b±0.01
4.	Cr	0.00	0.02a±0.005	0.03b±0.01
5.	Fe	20.30a	74.8b±1.35	58.9c±1.59
6.	Phenol	0.00	0.03a	0.11b

Observed levels of Chromium in root tissues of H. Brasilensis may have been introduced from nursery as levels were below detectable limits for the wetland samles. However Fe concentration in the root of the salted (58.9mg/kg) was less compared to the unsalted (74.8mg/kg) and that suggests deposition of Fe in other parts of the plant like the stem and leaves and this has ecological implications [19] . Significant phenol removal by H. Brasilensis is comparable to the findings of Meikap and Rot [20].

CONCLUSION

Generally, results indicate excellent levels of phytoextraction of Cadmium, Lead, Iron and Phenol leaving relatively low values in water sample especially with salting. Following obtained results, tissue analysis should be extended to the stem and leaves for a better understanding of contaminantsdistribution. Further research should be done comparing removal of such contaminants by *H. brasillensis* with other tested and confirmed plants.

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