Biosorbent Capacity of an Indigenous *Eichhornia Crassipes* Biomass Product on Crude Oil

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ABSTRACT

Crude oil is among the natural endowments of a nation. Natural resources are often regarded as free gifts of nature. The aim of the study is to determine crude oil absorption and microbial load of water hyacinth compost to provide insights into the potential use of water hyacinth compost for environmental cleanup and remediation purposes. Water hyacinth compost (WHC) samples were collected from GOA's research group, at the University of Port Harcourt, Nigeria. The microbial load and physicochemical parameters of the dry water hyacinth compost were determined using the Manual of Methods in Environmental Microbiology. Results show the total heterotrophic bacterial count for the WHC to be 2.3× 10^8CFU/g and the hydrocarbon utilizing bacterial count as 1.12× 10^4 CFU/g. The WHC is hydrophobic by the biphasic partitioning method, hasa bulk density of 0.0225g/cc, a surface area of 6.6m^2/g and positive buoyancy in water; a unit (1g) of water hyacinth compost absorbed approximately 8x its weight of crude oil. These results show that the water hyacinth compost has the capacity as a biosorbent product for crude oil and biotechnologically beneficial microbes, and, thus, can be a remedial technique in the form of biosorbents with biodegrading potential in the mitigation of crude oil pollution in aquatic and terrestrial ecosystems

Keyword: biosorbents, absorption, crude oil, water hyacinth compost and pollution

INTRODUCTION

One invasive species that thrives and reproduces in watery environments is the water hyacinth (*Eichhornia crassipes*). This aquatic plant, which floats freely, usually thrives in rivers, lakes, reservoirs, and stagnant swamps (Jirawattanasomkul *et al.*, 2021). At the individual, genetic, and environmental levels, it threatens biological and socioeconomic diversity. Its quick development raises concerns worldwide because unchecked growth can damage power stations and choke water ways. Furthermore, water hyacinth can block sunlight from entering water bodies and reduce oxygen concentrations, which disrupt aquatic ecology by depriving the atmosphere of oxygen (Madikizela, 2021). Water hyacinth is classified as a weed or nuisance plant due to its rapid spread in aquatic situations. It persists in small quantities before growing. Particularly in lakes, ponds, reservoirs, and fish farm ponds, water hyacinth are extremely common. Although water hyacinths have many uses, research is still desperately needed to stop their spread and make them into useful products. Water bodies are home to a wide variety of invasive plant species, including mosquito fern (*Azolla pinnata*), mosquito hyacinth (*Eichhornia crassipes*), mosquito fern (*Azolla pinnata*), mosquito fern (*Azolla pinnata*), fowering pickerel weeds (*Monochoria vaginalis)*, water lettuce (*Pistia stratiotes L*.), lesser bulrush (*Typha angustifolia*), mosquito fern (*Azolla pinnata*), and yellow velvet leaf (*Limnocharis fava*).These aquatic species interact directly with the ecology since they share a niche in the water area (Pandey, 2012). The water hyacinth is among the most well-known aquatic invasive species. Its rapid invasion of the region results in significant financial losses in husbandry and agriculture. It shows up randomly everywhere and competes with cultivated plants for space, sunlight, water, and nutrients. It can end anger native plants, animals, and organisms in water bodies and disrupt their natural ecosystems. Numerous studies have reported using herbicides (Portilla & Lawler, 2020), natural enemies (e.g., *Neocetina Sp*.) (Elenwo & Akankali, 2019), and nutrient to control (Yan*et al.*, 2017; Karouach *et al.*, 2022) to manage water hyacinth in the environment. Controlling water hyacinth development is difficult, nevertheless, because of their remarkable tolerance to harsh planting circumstances and ease of reproduction. Making them consumable goods is one of the best tactics. The free-

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floating aquatic plant known as water hyacinth typically grows in lakes, reservoirs, rivers with a constant flow, and swamps. It is a biomass with a lot of potential for use. Water hyacinth has been used to make a variety of useful items, according to several reports. Due to its high crude protein content, water hyacinth was used as food for fish, pigs, geese, ducks, and ruminants (Wimalarathne & Perera, 2019).

Like other developing nations worldwide, Nigeria is focusing increasingly on ways to speed up development across diverse economic sectors. A vital component of contemporary economic activity, oil is a highly adaptable and flexible non-productive, finite natural resource (hydrocarbon) that supplies roughly half of the world's energy needs, excluding the formerly centrally controlled economy. Government budgets and foreign exchange revenues in oil-exploiting nations rely substantially on oil revenue, which typically reaches 90% or more. Crude oil, sometimes referred to as petroleum, is an oily bituminous liquid that is primarily composed of the elements hydrogen and carbon. A very minor number of non-hydrocarbon components, primarily oxygen, nitrogen, and sulfur, are also present. Crude oil exploration, production, refining, marketing, and servicing are all included in the petroleum sector. In the process of processing crude oil, these operations exploration, production, refinement, transportation, and storage may inevitably have an effect on the natural environmental media of land, water, and air. The purpose of this study is to determine the capacity of water hyacinth compost to serve as a nature-based product in the removal and clean-up of crude oil from impacted environmental media.

MATERIALS AND METHODS

Sample Collection

Water hyacinth compost was obtained from GOA's Research Group, Department of Microbiology, University of Port Harcourt, Nigeria; crude oil was obtained from Port Harcourt Oil Refinery, Nigeria.

Microbiological Analysis

Enumeration of Bacterial Population in Water Hyacinth Bio-compost:

Microorganisms were enumerated from Water Hyacinth Compost following standard procedures (Margesin *et al*, 2003). Serial dilution and plating techniques were employed to obtain the samples' countable colonies of pure cultures of isolated strains on Nutrient Agar (NA). Isolated strains were cultured on (NA) plates under controlled laboratory conditions to facilitate their growth and proliferation. Total heterotrophic bacteria in bio-compost samples were enumerated on Nutrient Agar plates, in duplicate. After the appropriate dilutions of samples, aliquots (0.1 ml) were plated on NA plates and incubated at 37^0C for 1-2 days. At the end of the incubation period colonies were counted. The population density estimate of the organisms was obtained by multiplying the average number of colonies for organisms by the dilution factor used; this is the titre, also expressed as:

Titre (CFU/g) = average number of colonies x DF(dilution factor) x (volume correction)

Where:

DF = 1/dilution and

Volume correction= 1/volume plated

Estimation of total hydrocarbon utilizing bacteria in WHC

Mean counts of total hydrocarbon utilizers were obtained by plating aliquots (0.1 ml) from10^{-2}, $10^{-3}$and 10^{-4}dilutionsof samples on mineral salts agar that contained (gl^{-1}): NaCl,10.00; $MgSO4.7H_2O$, 0.42;KCl,0.29;KH_2PO_4, 0.83;$Na_2HPO4.H2O$,1.25;NaNO3, 0.42; Agar,15.0 and deionized or distilled water to 1000 ml mark (pH, 7.2). The medium was sterilized by autoclaving at 121^0C for 15 minutes and 15psi pressure. Crude petroleum served as the sole carbon and energy source and was made available to the culture in the vapour phase. This was achieved by inverting inoculated plates over sterile filter paper moistened with the sterile crude oil held in Petri dishes 'lids. All plates were incubated at 37 ^{0}C for 24-48 hrs.

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Identification of isolates

Isolates were identified by examining their morphological properties and taxonomic characteristics using the methods in Bergey's Manual of Determinative Bacteriology (1994).

Physicochemical analysis of water hyacinth compost

The physicochemical characteristics studied were pH, moisture content, organic matter content and water holding capacity. All analyses were duplicates, and the mean values were reported following Margesin *et al.* (2003) procedure. The pH of the samples was measured after 2 hours of mixing one part of compost with 2.5 parts of 10mM of $CaCl_2$. The pH of the control (C) and the pH of the experimental were measured using Mettler Toledo MP220 pH meter. Loss on ignition as a measure of organic matter content was calculated from the weight loss of oven-dried compost after 6 hours at $250^{o\circ}$C (Margesin *et al.*,2003).Moisture content was measured from constant weight loss of oven-dried compost for 3 hours at 105^0C (Onifade, 2007). Water holding capacity (WHC) was determined by adopting the method of Jones *et al.* (2010) by placing 10g of 24-hour oven-dried compost in a plastic funnel lined with filter paper and placing it to cover the mouth of a glass measuring cylinder.

The set up was duplicated but without the compost for the second set up. An equal volume (10 ml) of distilled water was added to the setups and funnels were covered with aluminum foils to prevent evaporation of water and then left undisturbed to drain for two hours at room temperature. The volume of water retained and those that were drained out in each setup were recorded and used to determine the Water holding capacity of the compost as below:

Volume of water drained= x

Water volume held by filter paper = (weight of wet Filter paper) -(weight of dry filter paper) = y

Water held by the compost = z = 10- (y +x) Water holding capacity (ml/100g)=z x 10%

Absorption analysis

The method of Margesin*et al.*(2003) was used for the absortion analysis. Water hyacinth compost particles (10g) were put into a 250-mL beaker containing crude oil (i.e., with volumes of 100, 80, 60, 40, and20mL). The suspension was mixed (at 500 rpm for 120 min) at ambient conditions with a constant pH (approximately pH 7). An aliquot of the mixed suspension was taken and filtered through a pore size of 0.22-µm nylon membrane syringe. The filtrate was analyzed using a UV–Vis spectrophotometer (Model7205; JENWAY; Cole-Parmer; U.S.; at 250-500 nm). The UV–Vis spectrometry results were normalized and extracted using the Beer-Lambert Law:

A=aCL, where:

A=Absorbance

a= Absorption coefficient C=concentration of solute

L=optical path length of the instrument(usually 1cm)

To get the actual concentration (Pratiwi& Nandiyanto, 2021).

Oil sorption efficiency of WH-biomass

Buoyancy test: The buoyancy test should be performed on sorbents used for water spills (Rebeiro *et al.*, 2000). The static system approach performed the buoyancy test(Radetic *et al.*,2008). In a 500mL beaker, 250mL of crude oil was covered with one gram of WHC (the sorbent) and left for 15 minutes. The buoyancy percentage was then determined by measuring the floated sorbent mass.

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Hydrophobicity test: Testing the partitioning of the WHC between aqueous and hexane phases revealed the water hyacinth compost's propensity to be extracted from the water phase into a non-polar phase (Rebeiro *et al.*, 2000). A sample of about1.0g of WHC was added to a beaker with distilled water and hexane (1:1) and the mixture was swirled for three minutes. After that, the mixture was left to stand for five minutes, which is how long it takes for the phases to separate. Filtration, drying, and weighing determined the amount of material transported to the organic phase. The percentage of material held at the interface or transported to the organic phase is how the results are expressed. This value is an estimation of the degree of hydrophobicity of the WHC.

Sorption capacities of the oils onto the WHC without water: The process was followed to determine the oils' sorption ability onto the biomass when no water was present (Rebeiro*et al.*, 2000; Radetic *et al.*, 2008). Every test was run at 270 degrees Celsius. 1.0 g of water hyacinth sorbent was added to 100 ml of oil in a glass beaker to test the sorbent's ability to absorb oil in water-free oil. The oil was drained two minutes following the 60-minute sorption period, and the wet sorbent was weighed. The oil sorption capacity (Choi & Cloud, 1992) of the sorbent was determined by the following equation q = [mf-(mo+ mw)]/mo

Where, q is the sorption capacity (g/g), mf is the weight of the sorbent after 2min of drainage (g), mo is the initial weight of the sorbent (g), and mw is the weight of the water(g).It is found that oil without any water is a medium where mw is equal to zero.

Sorption capacities of the oils onto the biomass in the presence of water:

The method of Margesin *et al.*(2003) was used. In a succession of 250 ml glass beakers, varying amounts of oil (diesel, castor oil, and lubricating oil) were poured onto 100 ml of seawater to determine the relationships between oil/water selectivity and oil thickness in the water surface. In that order, the measured oil thicknesses were 0.5, 1.3, 1.5, 2.6, and 2.8 cm. Once a steady state was reached, the beakers were filled with 1.0g of water hyacinth biomass. The wet sorbent was taken out after 60 of sorption, drained for two minutes, and weighed. They squeezed the soaked sorbent. Next, using the usual procedure D4007-8I, the liquids that were recovered from the sorbent were centrifuged. The oil capacity of the sorbent was determined using the equation above.

RESULTS

Microbiological Properties of water hyacinth compost samples

The Total Heterotrophic Bacterial Count (THBC) of the water hyacinth compost samples is shown in Table 3.1. The counts range from 1.01×10^8 CFU/g to 1.16×10^8 CFU/g for the stem parts before and after adsorption with crude oil, representing 87.1% drop after adsorption and 1.23×10^8 to 1.67×10^8 CFU/g for the root parts, before and after adsorption with crude oil, representing 73.7% drop after adsorption. The hydrocarbon utilizing bacterial (HUB) counts range from 3.3×10^3CFU/g to 6.51×10^4 CFU/g for the stem parts before and after adsorption with crude oil, representing 5.1% drop in HUB after adsorption and 4.78×10^3CFU/g to 8.62×10^4CFU/g for the root, before and after adsorption with crude oil, representing 5.5% drop in HUB after absorption with crude oil.

Table 3.1: Microbial population on water hyacinth compost (stem and root) before and after crude oil absorption for 42 days

Sample- type	THBC (CFU/g)	HUBC (CFU/g)
Stem	1.16×10^{8}	6.51×10^4
Root	1.67×10^{8}	8.62×10^{4}
	After absorption	
Stem	1.01×10^{8}	3.30×10^{3}
Root	1.23×10^{8}	4.78×10^{3}

Key: THBC-Total heterotrophic bacterial count; HUBC-Hydrocarbon utilizing bacterial count

The isolates identified from biochemical analysis (Table 3.2) include *Acinetobacter* sp., *Klebsiella* sp., *Bacillus*

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sp and *Pseudomonas* sp.

Table 3.2: Biochemical characteristics of bacterial isolates from water hyacinth compost samples

Organism suspected	Catalase	Oxidase	Citrate	Glucose	Lactose	Indole	MR	Vp	Motility	TSI	H2S	Gram stain
Acinetobacter sp.	+	-	-	-	-	-	-	+	-	AB	-	-Rod
Klebsiella sp.	+	-	+	+	-	-	+	-	+	AB	-	+cocci
Bacillus sp.	+	-	-	-	-	-	+	-	+	AB	-	+rod
Acinetobacter sp.	+	-	-	-	-	-	-	+	+	AB	-	-Rod
Pseudomonas sp.	+	-	-	-	-	-	+	-	+	AB	-	-Rod

Proximate quality of water hyacinth compost samples

The results presented in Table 3.3show the proximate composition of water hyacinth samples used in the study. The moisture content of water hyacinth was 42.5 %, others are: % crude protein 3.5±0.40,% ash content 3.5±0.42,% crude fat 0.2± 0.02,% carbohydrate 35.2 ±1.03and % crude fibre 15.1±0.10. Water hyacinth compost is almost hygroscopic and is rich in carbohydrates and crude fibre.

Table3.3: Proximate composition of Water hyacinth

Parameters	Bulk(Stem, Root and Leaves)
Moisture Content (wt%)	42.5± 0.40
Crude Protein (%)	3.5± 0.040
Ash Content (wt %)	3.5± 0.42
Crude Fat (wt%)	0.2± 0.02
Carbohydrate (%)	35.2±1.03
Crude Fibre (%)	15.1± 0.10

Values are mean±S.D of triplicate determinations(n=3)

Themineral composition of water hyacinth compost sample

The values in Table 3.4 are mineral components of the water hyacinth compost samples used in the study; the compost from roots had higher values for almost all the parameters except potassium, sodium and nickel. The compost is rich in minerals.

Table 3.4: Mineral composition of water hyacinth compost from different plant parts used in the study

Parameters	Root	Stem
Nitrogen(mg/kg)	14.83	8.60
Phosphorus(mg/kg)	1.92	1.25
Nitrate (mg/kg)	8.38	1.67
Phosphate(mg/kg)	0.57	0.40
Potassium(mg/kg)	6.51	6.75
Sodium (mg/kg)	17.70	18.48
Magnesium(mg/kg)	3.48	2.02
Iron(mg/kg)	22.73	16.68
Manganese(mg/kg)	0.130	0.030
Nickel(mg/kg)	0.013	0.134
Copper (mg/kg)	0.146	<0.001
Vanadium(mg/kg)	0.021	<0.001
Selenium (mg/kg)	0.003	<0.001

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The Geo-technical properties of water hyacinth compost samples

Table 3.5 contains the values of the geotechnical properties of water hyacinth compost from the stems and roots of the plant. The bulk density of the compost from stems was 0.0139 g/cc, while that from the roots was 0.031 g/cc. The value of the roots is almost three times that of the stems. The particle density of the stems compost was 0.0078g/cc while the particle density of compost from roots was0.021g/cc. Similarly, the roots' particle density is higher than the stems. The pore space volume of the compost from stems was 3.2 ml, while the pore space volume from roots was 2.2 ml. The value for the pore space volume is higher, about one and a half times for the stems than for the roots. The surface area of the compost from stems was 5.4 m^2/g, while the one from roots was 7.788 m^2/g. The surface area value for the roots was almost one and a half times more for the roots than for the stems. The value of the buoyancy of the compost from

The root was 37.9% while the one from the stem was 40.1%; both are floatable; the hydrophobicity of the compost from the root was 15.1% while the one from the stem was 22%; both are hydrophobic.

Table3.5.Geo-technical characteristics of the parts of water hyacinth compost samples

Parameter	Stem	Root
Bulk density(g/cc)	0.0139	0.031
Particle density(g/cc)	0.0078	0.023
Pore space volume (ml)	3.20	2.200
Surface area (m^2/g)	5.4	7.788
Pore volume(cm^3)	17.78	13.75
Buoyancy(%)	40.1	37.9
Hydrophobicity(%)	22	15.1

Results of Crude oil absorption studies using water hyacinth biocompost

The Results of Crude oil absorption studies using water hyacinth biocompost from roots and stems of *Eichornia crassipes* plants are presented in Table 3.6. The values are for experiments conducted in the presence and absence of water. The compost from stems absorbed more crude oil, both in the absence and presence of water. The treatment using the root without water had an absorption capacity value of 4.64 while for the stem the absorption capacity value was 8.22.

Table 3. 6. Oil sorption capacity of water hyacinth compost samples from roots and stems used in the study in the absence and presence of water.

Source of water hyacinth compost sample	Oil sorption capacity value in the absence of Water(-)	Oil sorption capacity value in presence of Water(+)
Root	4.643	4.434
Stem	8.220	5.920

DISCUSSION

The water hyacinth compost (WHC) from the invasive hydrophyte *Eichornia crassipes* has many attributes of a potential sorbent. The stored product exhibited high microbial carrying capacity, up to 167 million CFU per gram. That means applying just 10 grams would provide almost 2 billion cells. These cells are commonly referred to as beneficial microbes. The hydrocarbon-utilizing microbes are among these beneficial microbes. There is a rich population of hydrocarbon-utilizing bacteria absorbed into the WHC (Table 3.1).This microbe-absorbing property makes this product potentially beneficial in the removal of pollutants through bioremediation technology. The bioremediation technology could be through the science of biosorption, where sorptive forces are involved in removing inorganic pollutants, or using the science of biodegradation, where organic, biodegradable pollutants are involved. The WHC showed a strong sorption capacity for crude oil with both the stems and roots derivatives (Table 3.6). This sorption capacity is most applicable for less viscous liquids, such as the Nigerian crude described as the sweet Bonny light. Ashikodi & Abu (2019) reported a high population of hydrocarbon-utilizing bacteria (HUB) in association with the Kenaf (*Hibiscus cannabinus* L.) plant. The bacterial isolates were *Pseudomonas* sp., *Klebsiella* sp., and *Lysini bacillus* sp. These plant-microbe degradative capabilities have been described as phytoremediation in form of phyto degradation (Ashikodi & Abu, 2019). Stems had a higher crude oil sorption capability than water hyacinth roots. This could be because the pollutant's higher viscosity slows down the rate of sorption (Ceylan *et al*., 2014). For sorbents used in water surfaces spill cleanup, sorptive selectivity between oils and water is a crucial feature. The root and stem of water hyacinth have varying oil sorption capabilities when exposed to oil over water baths that contain varying crude oil thicknesses. Because there is little water pickup, the sorbent's selectivity between water and oil may be seen. The stem portion has a greater sorption capacity than the root. Pure oil and systems with an oil/water combination have different oil sorption capabilities. This could be because water is absorbed more readily than oil in the stem and root's pores. Adsorption, absorption, capillary action, or a mix of these may be the process by which sorbents absorb oil (Radetic *et al*.,2008). The specific surface area of the adsorbent and the hydrophobic interaction between the adsorbates serve as the foundation for adsorption. This mechanism is dependent on the sorbent's volume, porosity, and hydrophobicity properties. Non-polar hydrocarbon sorption frequently happens by combining the two processes. As a result, the biomass water hyacinth's capillarity and hydrophobicity both contribute to the overall oil sorption. (Table3.5).These properties would also enhance the applicability of the WHC in the removal of hydrocarbons in an aquatic environment. The mineral composition of the water hyacinth compost samples (Table 3.4) shows that the WHC is rich in minerals. These minerals would affect all the forces of adsorption, absorption and capillary action as observed in this study for the microbes and crude oil, the adsorbates. The relative significance of these mechanisms depends on the properties of the sorbent and sorbate**.** We obtained sorption of up to 8 times the weight of the WHC in this study (Table 3.6).

The moisture content of the water hyacinth compost at 42.5%, carbohydrate at 35.2%, crude protein at 3.5% and crude fibre at 15.1% (Table 3.4) indicates that the water hyacinth compost is hygroscopic and rich in nutrients. The high microbial load of the WHC samples (Table3.1) supports this observation. Because of its high moisture content; water hyacinth may have a limited shelf life. Because of the high moisture content, dehydration would raise the relative concentrations of the other food ingredients and improve the shelf-life/preservation of the water hyacinth meal (Suleiman *et al*.,2020). An example of the adsorption process on the surface of a bioadsorbent based on water hyacinth is shown. Monolayer coverage governs the adsorption process according to the Jovanovic, Langmuir and Halseymodels. According to the Freundlich and Harkin-Jura models, the adsorption process creates monolayer and multilayer coverage. The adsorption process is a cooperative process because of the creation of monolayer and multilayer coverage. Consequently, both chemical and physical interactions take place at the same time during the adsorption process. Weak van der Waals bonds between the adsorbent surface and the adsorbate produce physisorption. The adsorbate physically diffuses across its surface without contact with the adsorbent. Cooperative adsorption is created when chemisorption occurs between the adsorbates to produce the top layer. The results of this study (Table 3.5; 3.6) are in line with the fact that water hyacinth compost has the potential to be used as an adsorbent material for absorbing waste (such as organic or inorganic waste) in water. According to Mohammad et al. (2022), the findings of this study support the idea that water hyacinth may be utilized as an adsorbent substance to absorb trash in water, including both organic and inorganic waste

CONCLUSION

Water hyacinth compost (WHC) samples maintained at ambient laboratory conditions revealed high viable microbial counts of up to 167 million (CFU/g) adsorbed to it. The product is hygroscopic and rich in nutrients and minerals. The geo-technical properties of the WHC show it has a high surface area; it has a bulk density, buoyancy and hydrophobicity that make it floatable in water, making it to be a good absorbent with selective capacities for absorption of crude oil in the presence and in the absence of water. The WHC could absorb up to 8 times its own weight of crude oil. It is, thus, considered a good potential candidate for biotechnological application as an oil absorbent in the first-line response to oil spills in aquatic and terrestrial ecosystems. With the high load of beneficial microbes, including hydrocarbon-utilizing bacteria (HUB), the comp[ost would be a self-cleansing (oil-eating) and biodegradable absorbent. The water hyacinth (*Eichornia crassipes*) plant has been widely studied because it can fulfil various sustainable developmental goals (SDGs) related to clean and safe water, land protection, ecosystem and biodiversity conservation, climate action, increased industrialization and public awareness.

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