

Petroleum Water Contamination Resulting from Change in Land-Use in Paloich, South Sudan: *Azolla pinnata* and *Lemna minor* L. as an Efficient Bio Treatment Tool

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Abstract

The study focused on the change in land-use and phytoremediation and bio-sorption efficacy of Lead and Cadmium from the petroleum-contaminated water by *Lemna minor* L. and *Azolla pinnata*. For the study of the change in land-use in Paloich, satellite imagery of Paloich between 2000 and 2020 was obtained from <http://www.earthexplore.usgs.org/> and analyses. Water samples were collected at different points of Paloich. Experimental set-up consisted of four main samples sets viz: *Lemna minor* L. and petroleum-contaminated water, *Azolla pinnata* and petroleum contaminated water, combination of *Lemna minor* L., *Azolla pinnata* and petroleum-contaminated water then distilled water (control) all containing 3 liters of the water. Samples were left for 28 days. Physico-chemical parameters, heavy metals (Pb and Cd) and bio-sorption capacity were analyzed. The result indicates the changes in the land-use that occurred in the area of study for a given period (2000-2020). Built-up area, mining site and environmental degradation have increased by 8 percent between the periods. The reason behind the increase can be as a result of increase in population. The result also indicates high efficiency in pollutant reduction in the set-up containing the combination of *Lemna minor* L. and *Azolla pinnata* in which the Cd and Pb reduction efficiency recorded was 95% and 97% respectively. While set up containing *Azolla P.* had Pb and Cd reduction efficiency of 83% and 87% respectively. However, the set up containing *Lemna minor* L. has Pb and Cd reduction efficiency of 91% and 85% respectively. A significant absorption of the metals (Pb and Cd) were seen in the samples with the mixture of *Lemna minor* L. and *Azolla pinnata*. As such this study reveals the potentiality of *Lemna minor* L. and *Azolla pinnata* as

some excellent phytoremediation candidates in the treatment of effluents containing Lead and Cadmium; however, we also recommend further studies on the efficacy of such plants in other classes of heavy metals.

Keywords

Remediation, Biotechnology, Macrophytes, Climate Change, Pollution

1. Introduction

One of the ways of counteracting the effect of an increasing population is exploration and production of oil and gas. Most countries, particularly Africa, depend on this sector for economic stability [1]. The benefits of oil exploration and production include easy extraction, power generation, industrial growth, infrastructural development, and many more. The by-products of petroleum products include fertilizers, pesticides, and insecticides, which are important elements for the enhancement of agricultural products [2]. One of the negative effects associated with oil exploration and production is pollution. During oil exploration, opportunities exist for the release of pollutants such as straight chained, branched, cyclic, monocyclic aromatic, polycyclic aromatic hydrocarbons, and inorganic compounds including heavy metals, sulphide, sulphate, nitrate, phosphate, dissolved solids, suspended solids, etc. [3] [4] [5].

About 40% of the country's GDP is dependent on oil exports. The continuous prevention of reports on pollution in Paloich is the reason for the scanty literature showing the extent of oil pollution and how it has impacted negatively on the people of the Paloich community [6]. The increasing number of children born with deformations can be linked to the teratogenic effects resulting from exposure to oil pollution [7] [8] [9]. The incessant increase in the rate of deaths of the inhabitants of the Paloich community due to unknown ills could also be attributed to the result of exposure to oil pollutants. The exposure of the inhabitants of the Paloich community is as a result of poor clean-up of oil pollutants in the field. This pollutant causes soil degradation and pollutes water resources. The presence of toxic chemicals in the environment also contributes to air pollution making breathing impossible for the inhabitants of the Paloich community [10] [11] [12].

These pollutants tend to seep into aquifers and pollute underground water, while some proportion of these pollutants tend to move as runoff into nearby surface water where they cause negative effects on the biota [13] [14] [15]. The release of these pollutants into the environment poses a serious threat to aquatic organisms in nearby surface water and also to the human community in proximity to this surface water. This is because the majority of the population in South Sudan depends on underground or stream water for recreational and farming activities [16] [17] [18]. These pollutants can sometimes sink into the ground, leading to the pollution of underground water, thereby posing serious

toxicity at low level exposure due to the presence of heavy metals which have been associated with chronic and acute toxicological effect on man [19] [20] [21]. Heavy metals have also been shown by many researchers to be detrimental to aquatic organisms, including algae, plants, and other aquatic invertebrate and vertebrate organism [22] [23] [24].

Many physical, chemical and biological methods have been employed to treat this petroleum contaminated water effectively to prevent further degradation of the environment, but most of the technologies are not easily accessible due to cost [25] [26] [27]. It is, therefore, necessary to look for alternative methods to effectively treat petroleum contaminated water, thereby restoring environmental quality. Biosorption and phytoremediation are biological methods that can eradicate the problem associated with petroleum contaminated water, because both processes have been shown by researchers to be effective in the treatment of different wastewater including [28] [29] [30]. The current study aims to determine the effect of the change in land-use in Paloich for petroleum mining activity and to test the role of *Azolla pinnata* and *Lemna minor* L. as an Efficient Biotreatment Tool.

2. Materials and Methods

2.1. Study Area

The Paloich is located at latitude 6.750°N and longitude 30.133°E. The area is characterized by low-lying plains that rise to the highlands in the north. A massive swamp is located in the south and is fed by the White Nile (**Figure 1**). This area has been further characterized as being covered with “Acacia seyal Balanites Savannah, alternating with grass areas. Different types of low and high rainfall savannah cover a substantial part of the area. The climate is characterized by seasonal rainfall and hot temperatures [31]. During the rainy season, which lasts from June to October, the area gets swampy. November to May constitutes the dry season. The climate is hot, with annual mean temperatures between 26° and 28° Celsius. Annual mean rainfall ranges between 600 and 800 mm [32].

Satellite imagery was obtained from www.earthexplore.usgs.org. This includes Landsat (ETM-2000, and OLS-2020). These will be chosen because they provide just the necessary details required and at no cost. Supervised classification was used by a careful training sample was selected using Google earth imageries. The cluster pixels in a data set into classes corresponding to user-defined regions of interest (ROIs) or training classes which are selected as representative areas to be mapped in the output. Maximum Likelihood techniques were used to classify the images. Therefore, six classes were developed. These are built up areas (residential areas, transportation structures, and other erected buildings for domestication), water bodies (rivers, streams, artificial and natural dams, ditches, and any place that holds water during the study), sparse vegetation (farm land, shrubs, grass land, and any area with sparse vegetation), dense vegetation (riparian vegetation, and forested areas), bare surfaces (such as exposed surfaces and dunes),

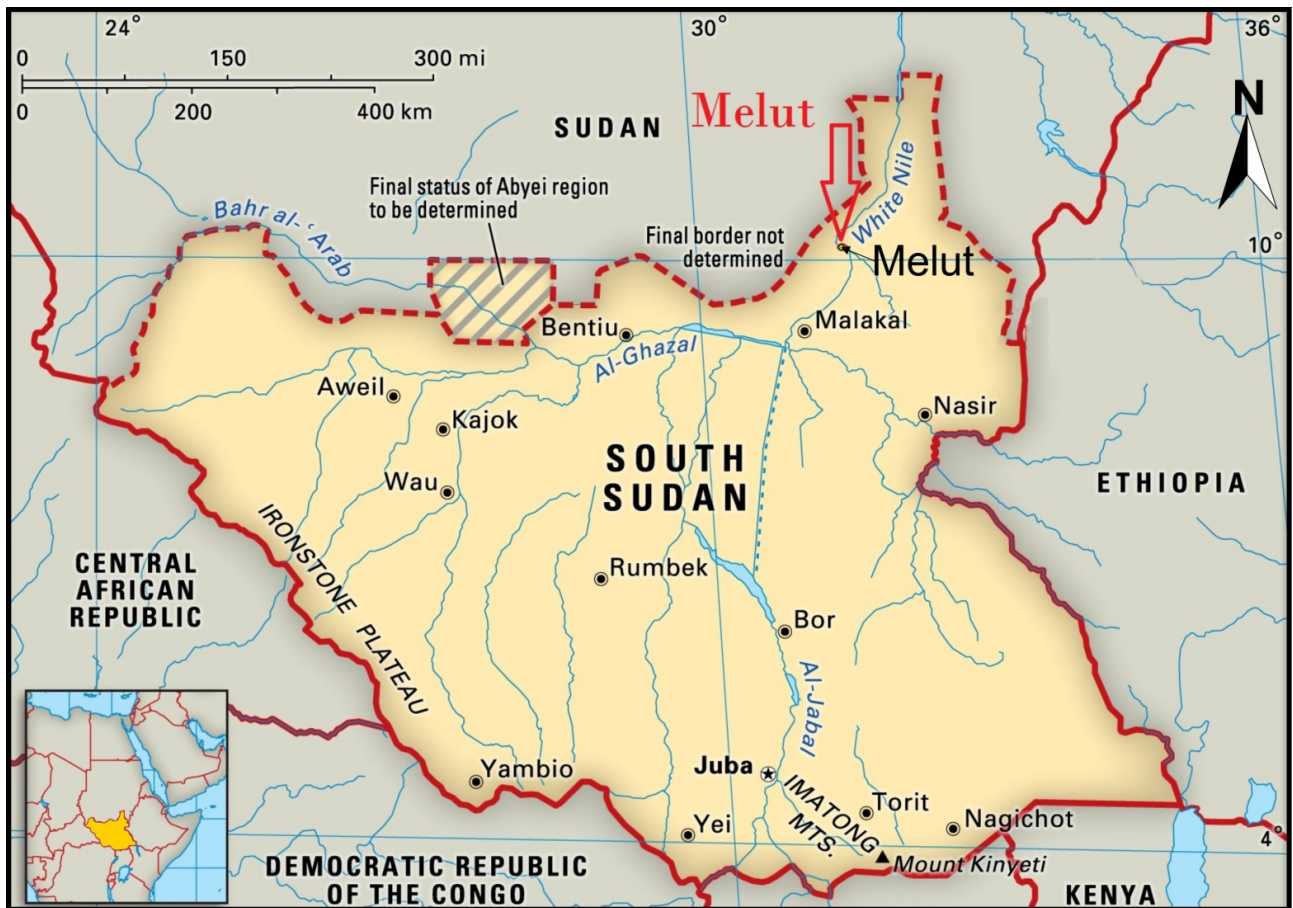


Figure 1. Location map of Study area.

and rocks. Estimating the accuracy of land cover classifications was performed by comparing a classified land-use to a reference map based on Google earth imageries.

2.2. Phytoremediation Setup

The sampled macrophytes were washed thoroughly 3 times using tap water, 2 times using distilled water and kept on a filter paper to remove excess water present in the macrophytes. *Lemna minor* L., *Azolla pinnata* and a combination of *Lemna minor* and *Azolla pinnata* were then transferred into different trough having capacity of 30 liter containing oil polluted water, the control contains only oil polluted water with neither *Lemna minor* nor *Azolla pinnata*. The physico-chemical parameter of the oil polluted water (turbidity, electrical conductivity (EC), nitrate, total dissolved solid (TDS), total suspended solid (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), phosphate, chlorides and hardness) was determined using standard method according to APHA [33]. After 28 days the macrophytes were removed the test macrophytes were removed from the trough and the physico-chemical parameter of the treated water was re-determine according to the previous method employed. *Lemna minor* and *Azolla pinnata* from each of the set-up were removed and separated into leaves, stem and root then oven dried. The dried material was then

grounded and subjected to acid digestion using HNO_3 and HClO_4 . The digested samples were then analyzed for the determination of the concentration of lead (Pb) and cadmium (Cd) in the test macrophytes. The determination of heavy metal concentration was done using Atomic Absorption Spectrophotometer (AAS). The reduction efficiency, bioconcentration factor and biotranslocation factor was thus determined using the formula below:

$$\text{Reduction Efficiency} = \frac{Z - Y}{Z} \times \frac{100}{1} \quad (1)$$

$$\text{Bioconcentration Factor} = \frac{P}{T} \quad (2)$$

$$\text{Biotranslocation Factor} = \frac{E}{P} \quad (3)$$

where Z = Initial concentration, Y = Final concentration, P = metal concentration in root, T = metal concentration in wastewater, and E = metal concentration in shoot.

2.3. Biosorption Setup

Lemna minor L. and *Azolla pinnata* were cut into small pieces and washed 3 times using borehole water and twice using distilled water. The macrophytes were then dried in sunlight for 24 hours. The dry macrophytes were ground using a mechanical blender and sieved using a standard sieve to obtain two sizes of each macrophyte (30 mm and 60 mm). The process materials are stored separately in an airtight container until needed during use. The efficiency of these macrophytes in the biosorption of Pb^{2+} and Cd^{2+} from oil polluted water was tested under conditions such as particle size, contact time and dosage of the particle. The effect of particle size and contact time on the absorption of Pb^{2+} and Cd^{2+} from oil polluted water was determined by placing 2 g each of *Lemna minor* L., *Azolla pinnata* and the combination of *Lemna minor* L. and *Azolla pinnata* in equal proportion separately in a 250 ml conical flask containing the polluted water. The processed materials are of two particle sizes of 30 mm and 60 mm. The mixture was then shaken at 150 rpm using a magnetic shaker for different time intervals of 30, 50, 70, 90, and 120 minutes. The mixture was then filtered and Pb^{2+} and Cd^{2+} were determined using AAS techniques. The effect of absorbent dosage was determined by placing 1 g, 2 g, 3 g, 4 g, and 5 g each of *Lemna minor* L., *Azolla pinnata*, and the combination of *Lemna minor* L. and *Azolla pinnata* in equal proportions separately in a 250 ml conical flask containing the polluted water. The processed materials are of two particle sizes of 30 mm and 60 mm. The mixture was then shaken at 150 rpm using a magnetic shaker for 1 hour. The mixture was then filtered and Pb^{2+} and Cd^{2+} were determined using AAS techniques. The biosorption efficiency was thus determined using the formula below:

$$\text{Biosorption Efficiency} = \frac{C_k - C_l}{C_k} \quad (4)$$

where C_k and C_f are initial and final concentration of Pb^{2+} and Cd^{2+} in the sample before and after shaking [34].

2.4. Effect of Absorbent Dosage

The effect of adsorbent dosage was studied by placing 1 g, 2 g, 3 g, 4 g, and 5 g each of *Azolla Pinnata Lemna minor* L and combination of *Azolla pinnata* and *Lemna minor* L where placed in to a solution 250 ml of solution containing 4.5 mg/l of Pb^{2+} and Cd^{2+} and shaken at 150 rpm using a magnetic stirrer for 1 h at a constant temperature of 25°C. The samples were filtered and the Pb^{2+} and Cd^{2+} concentrations of the solution were determined using AAS technique. Pb^{2+} and Cd^{2+} Percentage removal was calculated using Equation (2) [29].

2.5. Adsorption Equilibrium Study

Adsorption equilibrium study for Ni^{2+} using *Azolla Pinnata Lemna minor* L and combination of *Azolla pinnata* and *Lemna minor* L was performed by placing 1 g, 2 g, 3 g, 4 g, and 5 g of *Azolla Pinnata Lemna minor* L and combination of *Azolla pinnata* and *Lemna minor* L in a solution of 5 mg/l, 10 mg/l, 15 mg/l, 20 mg/l and 25 mg/l of Pb^{2+} and Cd^{2+} and shake at using a magnetic stirrer at 150 rpm for 24 h at a constant temperature of 25°C. The samples were filtered and the Pb^{2+} and Cd^{2+} concentrations of the solution were determined using AAS technique. Langmuir and Freundlich isotherm models were thus determined using Equations (3) and (5) [35].

Langmuir equation is represented below:

$$\frac{1}{q_x} = \frac{1}{b_i q_f} \cdot T_e + \frac{1}{q_f} \quad (5)$$

The equation above was adopted from Kumar *et al.* [29], where T_e is equilibrium Pb^{2+} and Cd^{2+} concentration in solution, q_f is maximum Pb^{2+} and Cd^{2+} absorbed per unit weight of *Azolla Pinnata Lemna minor* L and combination of *Azolla pinnata* and *Lemna minor* L, b_i is affinity adsorbate. The value of q_f and b_i is determined from the slope and intercept. Z_i is the separation factor and is calculated using the formula below:

$$Z_i = \frac{1}{1 + b_i T_g} \quad (6)$$

where T_g is the initial concentration of Pb^{2+} and Cd^{2+} in the solution.

Freundlich Isotherm is represented below:

$$\log(q_x) = \log(K_f) + \frac{1}{r \log T_e} \quad (7)$$

The equation was adopted from Wang *et al.* [36], where K_f is Freundlich constant r is Freundlich coefficient, and K_f and r are determined by plotting a graph of q_x against T [36].

2.6. Adsorption Kinetics

Adsorption kinetics is fundamental in describing the character of an absorbent.

To ascertain the mechanism involved in the adsorption of Pb^{2+} and Cd^{2+} by *Azolla Pinnata* *Lemna minor* L and combination of *Azolla pinnata* and *Lemna minor* L. Pseudo first-order and the pseudo second-order reaction was used [37].

The pseudo first-order is represented below:

$$\log(q_i - q_t) = \log(q_i) - \frac{K_i T}{2.303} \quad (8)$$

The pseudo second order is represented below:

$$\frac{T}{q_t} = \frac{1}{K_2 q_2} + \frac{T}{q_2} \quad (9)$$

2.7. Statistical Analysis

The experimental procedure was triplicate and data obtained was presented as mean \pm deviation as represented using error bar in chart. The mean reduction efficiency of *Lemna minor* L., *Azolla pinnata* and the combination of *Lemna minor* L. and *Azolla pinnata* was compared with the mean reduction obtained from the control using one-way analysis of variance at 5% significant differences.

3. Result and Discussion

3.1. Landuse and Landcover Change

Table 1 indicates the changes in land-use that occurred in the area of study for a given period (2000-2020). Built up areas have increased by 8 percent between the periods. The reason behind the increase could be as a result of an increase in the population. As the population increases, the need for housing/shelter also increases. The water body increased by 2 percent. The activities of the people in the area could be the reason for the increase (**Figure 2**). The present of mining ditches that are created by people during mining activities can help in holding water after rainfall. For that reason, the percentage of water coverage increased in the area. The increment for the sparse vegetation is also significantly high. For the period of the study, it increased by 7 percent. Agricultural land is also part of

Table 1. Area coverage of each land-use classes and change detection between 2000 and 2020.

NAME	LULC 2000		LULC 2020		2000-2020	
	Area (km ²)	%	Area (km ²)	%	Area (km ²)	%
BUILT UP AREA	114	3	374	11	260	8
WATER BODY	39	1	113	3	74	2
SPARSE VEGETATION	789	23	1037	30	248	7
DENSE VEGETATION	1300	38	1511	44	211	6
BARE SURFACE	847	25	259	8	-587	-17
ROCK	358	10	152	4	-206	-6
TOTAL	3446	100	3446	100		

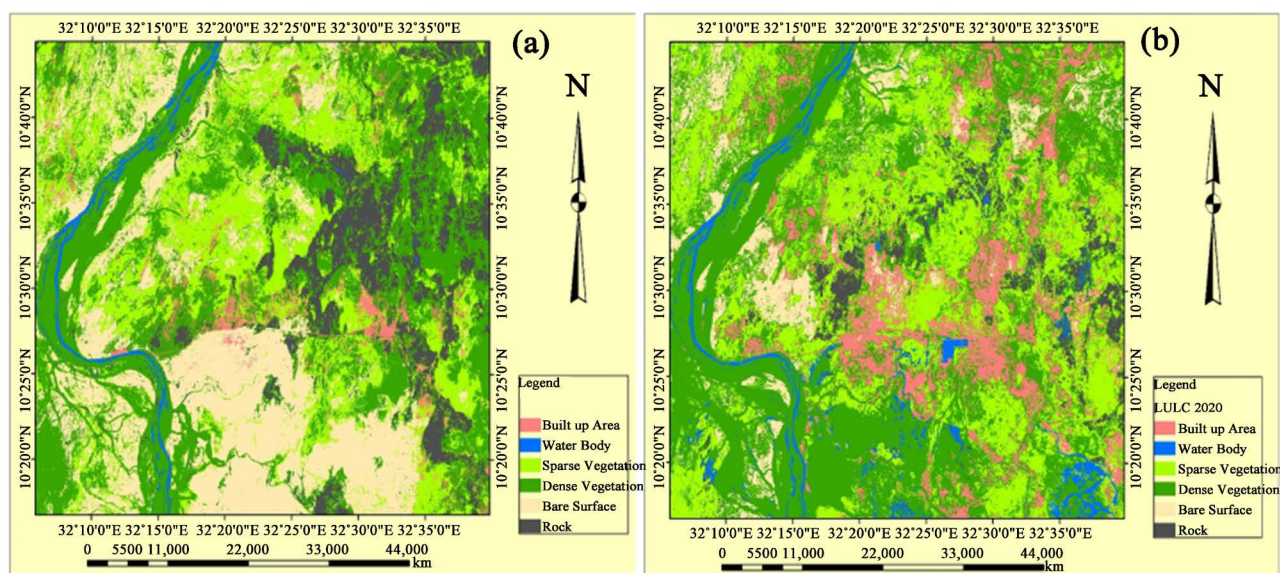


Figure 2. Classified land use land cover of Paloich and neighbouring environs (a) 2000 and (b) 2020.

this sparse vegetation; the increase could be as a result of the increase in agricultural activities by people. Dense vegetation increased by 6 percent. But relating it to the study area, the area is affected by different kinds of conflict which displaces a lot of people from their homes. Many of these areas are empty, which gives the plant the ability to regenerate. The increase in other land-uses, like vegetation, and built-up areas, affect the coverage of bare surface. Since this bare surface is just open land, it gives other land-uses the ability to cover part of this open space. The percentage of rock in the area also significantly dropped to about 6 percent. This decrease could likely be as a result of mining activities in the area. As it is all known, these rocks are formed from the aggregate of different minerals. This gives them the ability to store precious materials like gold, silver, and other precious materials. People break rocks because of this mineral deposit.

3.2. Phytoremediation

The result obtained shows significant differences between turbidity, EC, TDS, TSS, COD, BOD, nitrate, phosphate, chloride and hardness of the petroleum-contaminated water containing *Lemna minor* L. alone, *Azolla pinnata* alone and combination of *Lemna minor* and *Azolla pinnata* if compared with the contaminated water containing neither *Lemna minor* nor *Azolla pinnata* (control). Although, higher pollutant reduction efficiency was recorded in the trough containing a mixture of both *Lemna minor* L. and *Azolla pinnata* (**Figure 3**). The result obtained also shows high Cd^{2+} and Pb^{2+} reduction efficiency from the wastewater with *Azolla pinnata* having 83% and 87% reduction efficiency of Cd^{2+} and Pb^{2+} respectively, *Lemna minor* L having 91% and 85% reduction efficiency for Cd^{2+} and Pb^{2+} respectively while the combination of both plants in a trough shows high pollutant reduction efficiency of 95% and 97% of Cd^{2+} and

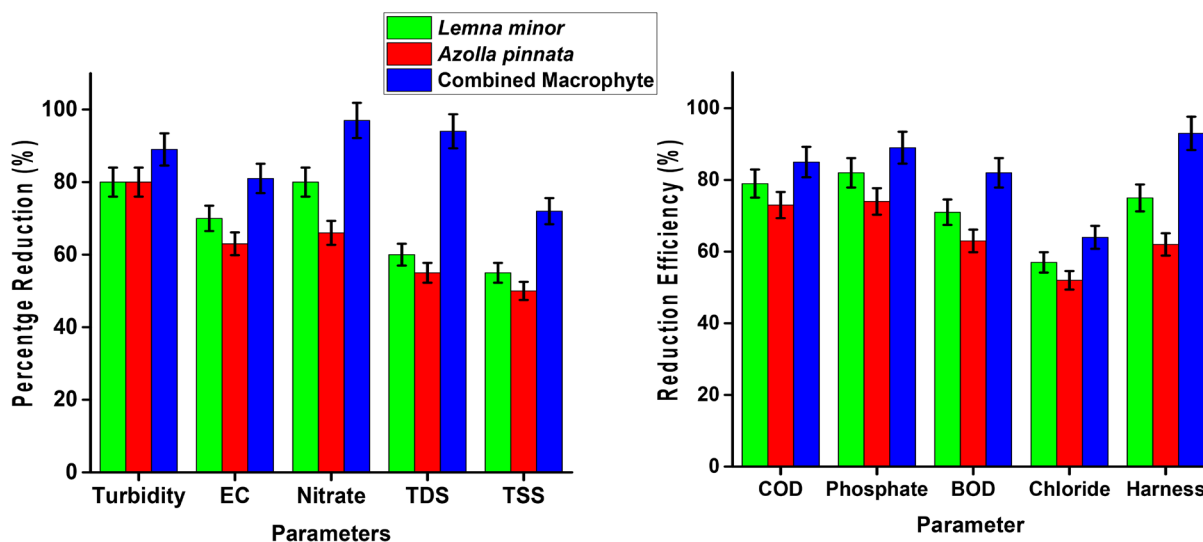


Figure 3. Phytoremediation efficiency of the test plants.

Pb^{2+} respectively. The result obtained shows high bio concentration factor and bio translocation factor of Cd^{2+} and Pb^{2+} of above 1 for the entire test macrophytes category. The reduction in TDS and TSS is responsible for the high EC and turbidity reduction due to the direct correlation between both parameters. This high TDS and TSS reduction efficiency by the entire test macrophytes categories could be attributed to the ability of the root of the macrophytes to retain minute suspended solids or the ability of the test macrophytes to stimulate the growth of microbes which in turn brings about the decomposition of these particles [38]. The high nitrate, phosphate and chloride reduction by the macrophytes could be attributed to the fact that the plants were able to utilize these substances as nutrients for growth and development. Continuous exposure of the plant to the wastewater water would lead to the continuous removal of nitrate and phosphate until 100% efficiency is attain whereas, prolong exposure of the test plants to the wastewater might not necessarily favor continuous chloride removal because chloride is need by the plants in minute quantity [39]. The high BOD and COD reduction efficiency by *Azolla pinnata*, *Lemna minor* L. and combination of *Lemna minor* and *Azolla pinnata* is attributed to the fact that that the test plants stimulate the growth of microorganism which brings about the degradation of organic compounds present in the wastewater [40] [41]. The high biotranslocation factor, bioconcentration factor and reduction efficiency is attributed to the low Cd^{2+} and Pb^{2+} of $0.09 \mu\text{g/l}$ and $0.01 \mu\text{g/l}$ present in the wastewater. This heavy metal concentration is below the maximum concentration of $10 \mu\text{g/l}$ - $15 \mu\text{g/l}$ reported by most researchers.

3.3. Biosorption

The result obtained shows that for *Lemna minor* L. *Azolla pinnata* and Combined material maximum absorption efficiency was obtained at a higher dosage for both Pb and Cd. This result signifies that increasing dosage of the test ma-

crophytes leads to the increasing availability of the more binding site to bind available Pb and Cd. This result shows that higher biomass of *Lemna minor* L. *Azolla pinnata* and Combined material have a higher tendency of removing Pb and Cd than lower biomass of *Lemna minor* L. *Azolla pinnata* and Combined material. The result obtained in **Figure 4** and **Figure 5** shows that the absorption of Pb and Cd by *Lemna minor* L. increases with increasing contact time for both larger and smaller material while for *Azolla pinnata* the absorption of Pb and Cd increases with increasing contact time until 100 min, beyond 100 minute the absorption of Pb and Cd by *Azolla pinnata* decreases with increasing contact time. The absorption efficiency of the combined material tends to increase with increasing contact time but maintain a uniform absorption rate for Pb and Cd beyond 100 minutes. A comparison of the absorption efficiency of Pb^{2+} by larger and smaller sized particles shows that the larger size material and smaller size materials shows higher absorption efficiency by larger sized particle of *Azolla pinnata* than lower sized particle before shaking time of 100 minutes after which smaller sized particle have higher absorption efficiency. The result also shows that a higher absorption efficiency was obtained using small sized *Lemna minor*

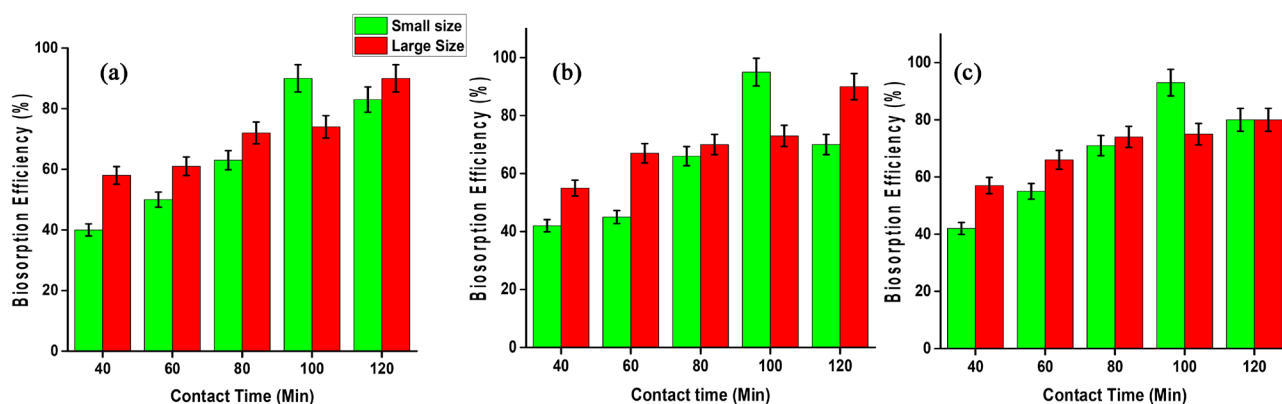


Figure 4. Biosorption Efficiency of Cd by the Test Plants (a) *Lemna minor* L. (b) *Azolla pinnata* (c) Combined material.

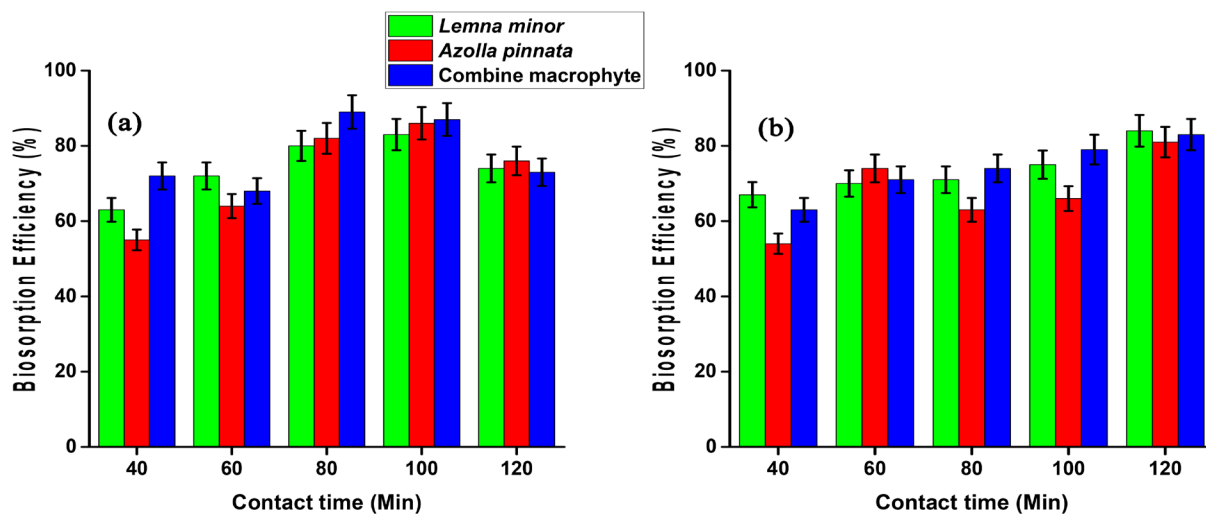


Figure 5. Biosorption Efficiency of Pb by the Test Plants (a) Small Size (b) Large size.

L. if compare to larger sized particles except at shaking time of 50 minutes and beyond 100 minutes. Combined material shows higher absorption efficiency for larger sized particles until shaking time of 70 minutes. Beyond shaking time of 70 minutes higher absorption efficiency was obtained for smaller sized particles. The result display in **Figure 6** shows the increasing biosorption efficiency of Pb and Cd with increasing absorbent dosage and this is as a result of the increasing functional group resulting from the increase in the mass of the absorbent [29].

3.4. Adsorption Isotherms

The Langmuir model obtained gives a nearly perfect fitting for adsorption of Pb and Cd for *Lemna minor* (0.9997 and 0.9967) and *Azolla pinnata* (0.9995 and 0.9974) and the combined material (0.9994 and 0.9938). Although it can be concluded that the adsorption of Pb and Cd onto *Lemna minor* and *Azolla pinnata* correlated well with Langmuir equation with the relationship becoming more linear with R^2 value closer to 1 [42]. The Freundlich model also shows a nearly perfect fitting for absorption of Pb and Cd for *Lemna minor* (0.9998 and 0.9971), *Azolla pinnata* (0.9995 and 0.9953) and combined material (0.9965 and 0.9972) with a high n value of (2.5) *Lemna minor*, (2.9) *Azolla pinnata* and (2.1) combined material which are all within the range of 1 - 10 signifying favorable absorption of Pb and Cd [43] [44].

3.5. Adsorption Kinetics

The biosorption of Pb and Cd by *Lemna minor*, *Azolla Pinnata* and combined material was determined at various initial concentration and time interval for the understanding of biosorption mechanism [35]. The result obtained shows that the Pseudo Second-order kinetic model gives nearly perfect fitting for *Lemna minor* (0.9194) and *Azolla pinnata* (0.9645) and combined material (0.7329).

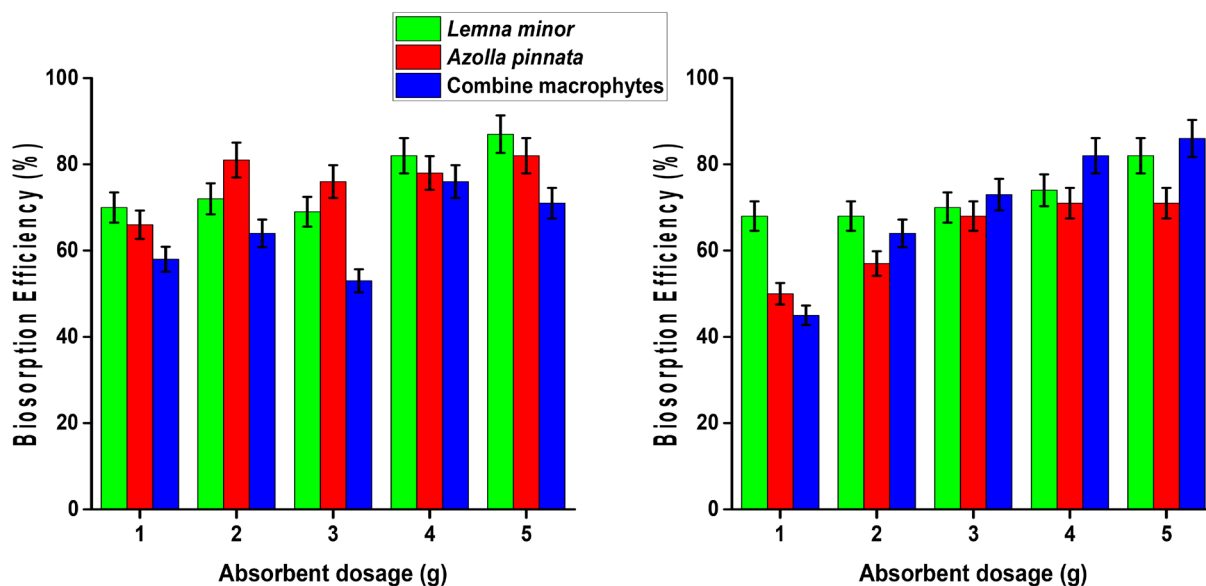


Figure 6. Effect of Absorbent Dosage on the Biosorption of the test plants to (a) Pb (b) Cd.

The correlation coefficient shows that the absorption of Pb and Cd by these macrophytes follows the pseudo second-order kinetic model because poor fittings of (0.7501) *Lemna minor*, (0.5678) *Azolla pinnata* and (0.5404) combine material was obtained for pseudo-first order model. This result obtained signifies that that the absorption of Pb and Cd to the macrophytes is due to the chemical and physical reaction of Pb and Cd and the surface of the macrophytes which leads to the formation of the bond between the valence electron and the negative surface charge of the macrophytes to attain an equilibrium state [45].

4. Conclusion

The petrochemical industry is the world's largest contributor of Pb and Cd particularly in developing countries like South Sudan where water contaminated with Pb and Cd is discharged into nearby water bodies without proper treatment due to low technologic know-how and lack of information. It is thereby paramount for petrochemical industries to utilize the availability of aquatic macrophytes as low-cost absorbents for further treatment of its wastewater before discharge since most industries are unwilling to establish a costly convectional treatment plant. Further investigation is needed on how to increase the efficiency of these macrophytes for the uptake of Pb and Cd. More research needs to be done using more macrophytes such as *Salvinia sp* and *Ludwigia sp*, etc. to discover more novel eco-friendly cheap biosorbent for Pb and Cd removal from petrochemical polluted water.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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