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Phycoremediation of water contaminated with arsenic (As), cadmium (Cd) and lead (Pb) from a mining site in Minna, Nigeria

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ABSTRACT: This study was designed to remediate water contaminated with heavy metals (arsenic, cadmium and lead) using two green macroalgal species, *Spirogyra* and *Cladophora*. The results obtained from this study indicate that both macroalgae can be employed to adsorb and detoxify any of the three heavy metals from aqueous solution. However, it was also discovered from the study that *Cladophora* adsorbed and detoxified more of the cadmium and lead than arsenic as the organism had removal efficiency for cadmium and lead as 88.78% and 94.85% respectively meanwhile for arsenic it was only 23.10%. On the otherhand however, *Spirogyra* adsorbed more of arsenic than cadmium and lead as the organism had a record of 82.76% of arsenic compared to the 28.97% and 47.43%absorption forcadmium and lead respectively. It is therefore concluded based on the results of the present study that reclamation and reuse of water from public or industrial wastewater, or even from water contaminated as a result of precious metal mining is a huge possibility through the application of phycoremediation, using different species of micro and macroalgae.

Keywords: Phycoremediation; Macroalgae; *Spirogyra*; *Cladophora*; Arsenic; Cadmium; Lead.

1. INTRODUCTION

Water, a transparent fluid, is sufficiently abundant on the planet Earth; it covers 71% of the surface of the earth in the form of swamps, lakes, rivers, seas and oceans [1]. But potable water is not always available at the right time or the right place for human or ecosystem use [2]. For water to be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is safe for domestic and other uses, particularly drinking purpose [3]. The quality of our environment is deteriorating on a daily basis in both developing and developed worlds. While it has reached saturation points in the developed cities, and managing it is mounting a huge pressure on their infrastructure, however, the third world nations are being devastated with little or no infrastructure to address the menace of environmental pollution [4]. Heavy metals are a unique class of toxicants since they cannot be destroyed [5]. They are among the major culprits in the pollution of surface, ground, industrial and even most treated wastewaters [6].

Contamination of soil is prominent among the most critical environmental issuesthroughout the world, and it has a hugely harmful impact on people, animals, microorganisms, and plants [7]. The contamination of surface and ground waters by toxic heavy metals is one of the major burning issues, in fact a big problem in many developing countries like Nigeria. This is basically because of the potential hazards they pose to the environment and human beings [8], and of course the expensive nature of the modern technology needed to completely remove these metals from water [9].

Considering the health danger associated with the presence of toxic heavy metals in our water, and considering the high cost of water treatment technology, it becomes pertinent to device a means of improving water quality with regards to removing toxic heavy metals from the water.

When it rains, large concentrations of these heavy metals discharged to the soil via industrial effluents from manufacturing industries and other human activities are washed from the soil into nearby water bodies via run-off [10]. Also, auto-mechanic technicians and various allied artisans constitute a major source of aquatic environment pollution as they usually discharge their untreated wastes into the environment which find their way into the rivers and streams [11]. These contaminate surface waters and by extension sea foods, other aquatic lives and plants/crops that receive water from such contaminated water are endangered too [12]. Also, soil particles excavated during mining procedures are usually washed right inside the river and consequently discharging and contaminating the river with toxic heavy metals. Ingestion of water or food contaminated with these substances, inhalation of contaminated air and or manual handling of contaminate materials are among the major sources of exposure [13]. Subsistence lifestyles can also result in higher risks of exposure because of hunting and gathering activities. This has been exemplified in the case of lead poisoning in Zamfara State of Nigeria in 2009/2010 where poor herdsmen, in an attempt to make ends meet engaged in illegal mining of gold. They brought back home soil that contained extremely high levels of lead [14]. Arsenic, cadmium and lead are among the heavy metals that are extremely toxic to organisms and humans even at a very low concentration. They have been implicated in several organ and system complications particularly in children. Chronic exposure has been implicated in several diseases resulting in organs and system failures [15-17]. The present study was therefore aimed at devising an efficient biosorbent system capable of remediating water sample polluted with arsenic (As), cadmium (Cd) and lead (Pb) obtained from a mining site using two different species of macroalgae.

2. MATERIALS AND METHODS

2.1. Collection of water samples for analysis

Three different plastic containers, four litres each were used for sample collection. The first container was for sample used for microbiology analysis and for algal culture, the second was for the sample used for physicochemical analysis while the third container was for the sample used for the detection of the heavy metals. The first sample was collected from the bank of the river where there was noticeable algal growth while the second and third samples were collected at the very point where the excavated soil was being washed.

The sample for the detection of heavy metal was preserved with concentrated nitric acid to help retard some chemical and or biological changes that inevitably continue after the sample has been removed from the parent source (the river) and samples were refrigerated at 4^{o}C prior to analysis [18].

2.2. Analysis of heavy metals in the water sample from river Kataeregi

The detection and determination of the concentration of the heavy metal in the water sample was done using Atomic Absorption Spectrophotometer, (AAS). Application of AAS is advantageous in that it allows detection of metals in both trace and major concentrations. There are also greater sensitivity and detection limits than in other detection methods. It also allows direct analysis of some types of liquid samples without digestion and there is the advantage of low light interference [19].

2.3. Preparation of growth medium for the growth of algae

Bold Basal Medium (BBM) was used for the growth of the algae. The growth medium is composed of stock solutions and trace metal solution all dissolved in 1 L of distilled water. The stock solution included 25 g of $NaNO_3$, 2.5 g of $CaCl_2$, 7.5 g of $MgSO_4$, 7.5 g of K_2HPO_4, 17.5 g of KH_2PO_4, 2.5 g of NaCl, 50 g of Na_2EDTA, 31 g of KOH, 4.98 g of $FeSO_4$ and 1 ml of concentrated H_2SO_4. The trace metal solutions included 11.42 g of H_3BO_3, 1.44 g of $MnCl_2$, 8.82 g of $ZnSO_4$, 1.57 g of MoO_3, 1.57 g of $CuSO_4$ and 0.49 g of $Co(NO_3)$. The stock solution was dissolved in the trace metal solution according to Culture Collection of Algae and Protozoa (CCAP) Specification [16, 20]. The medium was enhanced with 5 g of well ground chicken dropping [21], sterilized by autoclaving at 121^oC for 15 minutes and stored at 4^oC prior to inoculation.

2.4. Identification of macroalgae

Microscopic identification of the algae was carried out to identify species of algae from the mixed algal culture in the sample container. The algal cells were carefully observed under light microscope for their morphological features. Three different species; *Closterium*, *Cladophora* and *Spirogyra* were identified [22]. After thorough identification of the algae, the three species were separated and grown in separate 500 ml conical flasks containing 250 ml of the enhanced growth medium.

2.5. Screening of algae for heavy metal bioaccumulation

Twelve 100 ml conical flasks containing the enhanced growth medium were set up in three batches; each batch contains three conical flasks labelled Batch 1–3 and one conical flask each designated as control 1–3. The three different species of algae (*Cladophora, Spirogyra and Closterium*) were inoculated in all the conical flasks and monitored for growth. After two weeks, the twelve conical flasks have shown heavy algal growth, 0.5 g each of the compounds of these heavy metals; arsenic chloride ($AsCl_{3)}$, cadmium chloride, ($CdCl_2$) and lead monoxide, (PbO), obtained from a commercial store were weighed, introduced into each of the flasks except for the ones designated as the control, and then monitored for another two weeks to determine the degree of metal tolerance by the algae (Table 1).

2.6. Phycoremediation of heavy metals

The time frame for this study was 90 days. The experimental design consisted of three setups; designated Algae 1 (Ag1), Algae 2 (Ag2) and Control (Ctl). Ag 1 and Ag 2 had nine 100 ml conical flasks each and contained a known concentration of the contaminant in the water sample and the isolates (Algae). The third setup, Ctl consisted another two batches of two 100ml conical flasks each, which were designated, Ctl l and Ctl 2; this group also contained the water sample to be treated but the isolates were not inoculated in them, (control). The study involved a multi metal treatment setup, therefore, each of the eighteen conical flasks used had 0.5196 mg/l (0.0005196 mg/ml) of arsenic, 0.8946 mg/l (0.0008946 mg/ml) of cadmium and 1.2651 mg/l (0.00127 mg/ml) of lead.

Table 1. Studies of metal tolerance.

Batch 1 (*Cladophora*)	**Batch 2 (*Spirogyra*)**	**Batch 3 (*Closterium*)**
Flask 1: Salt of arsenic	Flask 1: Salt of arsenic	Flask 1: Salt of arsenic
Flask 2: Salt of cadmium	Flask 2: Salt of cadmium	Flask 2: Salt of cadmium
Flask 3: Salt of lead	Flask 3: Salt of lead	Flask 3: Salt of lead
Flask 4: Control 1	Flask 4: Control 2	Flask 4: Control 3

The growth medium was inoculated with the identified algae species on day zero and monitored for growth. Because of the slow growth of the algae, the contaminated water containing known concentration of the pollutants was introduced into the growing algae on the 30th day and the rate of algal growth monitored for another 60 days before harvesting.

2.7. Harvesting of algae for analysis

Before digestion to analyze the algal biomass and ascertain the level of bioaccumulation of the heavy metals, the algae were harvested and separated from the growth medium using Whatman filter paper size 41 to obtain the algal biomass. Biomasses obtained from each of the conical flasks were oven-dried at 65^0C for 48 hours. The biomass was then ground into fine particles and kept in a 5 g vial for airtight storage.

2.8. Acid digestion with mixture of H_2SO_4, HNO_3 and H_2O_2 for wet procedures

Digestion of harvested algae became necessary for complete decomposition of the matrices, releasing the analyte embedded therein into solution prior to analysis especially when using AAS, for precise analysis and identification of the elements [23].

Firstly, 0.5 g of the finely ground algal sample was weighed into a boiling tube and 10 ml of sulphuric acid (H_2SO_4) added into the sample in the tube. The solution was heated for about 20 minutes on a hot plate until charred. The solution was allowed to cool for about 5 minutes. Then, precisely 5 ml of hydrogen peroxide (H_2O_2) was added to the solution; it was heated for another 10 minutes until a pale clear yellow solution was obtained. The solution was again allowed to cool for 5 minutes. Then, 5 ml of trioxonitrate (v) acid (HNO_3) was added until reaction was complete. The solution was diluted to a volume of 100 ml, filtered and stored in a corked bottle ready for analysis [15].

3. RESULTS AND DISCUSSION

3.1. Microbiological and physicochemical parameters of the water samples

The Colony Forming Unit (CFU) from the *Salmonella Shigella* Agar (SSA) and the Nutrient Agar (NA) were 1.85×10^7 and 2.01×10^7 respectively as seen in Table 2. These are far above the standard value of WHO, which is 10 and the NAFDAC standard value, which is also 10. This result is not unconnected with the fact that the river is used for bathing (swimming), washing of clothes and other household items. Besides, household wastes including human excreta are consciously dumped into the river by the indigenes of Kataeregi. Based on the results of the microbiological analysis of river Kataeregi, it is therefore not advisable to use water from this river for domestic chores especially cooking or even drinking.

3.2. Heavy metal qualities of the river water

The result of the analysis of the water sample showed that the level of the heavy metals; arsenic and cadmium in river Kataeregi were 0.5196 mg/l and 0.8946 mg/l respectively which were high when compared to the standards set by the Federal Environmental Protection Agency and the World Health Organization, while the concentration of lead; 1.2651 mg/l, was high compared to the standard set by the Federal Environmental Protection Agency (FEPA) but falls within the WHO maximum permissible level (Fig. 1) [24, 25].

Table 2. Microbiological analysis of the water samples.

Parameters (mg/l)	River Kataeregi	WHO standard	Nigeria standard
Coliform (cfu/ml)	1.85 x 10^7	10	10
Total aerobic bacteria	2.01 x 10^7	10	10

Table 3. Heavy metal qualities of the River water.

Heavy metals	Concentrations in mg/l
Arsenic	0.5196
Cadmium	0.8946
Lead	1.2651

3.3. Heavy metal concentrations (mg/l) in the water sample from river Kataeregi in comparison with the standard regulatory values for river and drinking waters.

Table 4 shows the concentration of heavy metal (mg/l) in the water sample from river Kataeregi in comparison with the maximum standard permissible values of these heavy metals for river and drinking waters [2, 6]. It can be seen from Table 4 that the values of the heavy metals from the river Kataeregi were far higher than both the local and international standards for these toxic metals in both river and drinking waters. This implies that usage of this river; especially for drinking portend danger to health. The high amount of these heavy metals may be due to the mining activities in the study area.

Table 4. Heavy metal concentrations (mg/l) in the water sample from river Kataeregi in comparison with the standard regulatory values for river and drinking waters.

Metal	River water		Drinking water		Kataeregi River
	WHO	Nigeria	WHO	Nigeria	
Arsenic	0.02	0.01	0.002	0.001	0.5196
Cadmium	0.01	0.01	0.001	0.003	0.8946
Lead	2.0	1.0	0.01	0.01	1.2651

3.4. Results of the algal screening for heavy metal bioaccumulation

The screening of algae for heavy metals (arsenic, cadmium and lead) bioaccumulation was observed for three weeks. Three different species of macroalgae, isolated from the study area: *Cladophora, Spirogyra* and *Closterium* were screened. After the first two weeks, the specie labelled *Closterium*, started dying and after the third week, there was no more sign of algal growth as the greenish colouration has disappeared and only a turbid brownish colouration was visible in the three conical flasks. It became obvious that the specie *Closterium* may not be able to survive under high concentration of these heavy metals especially in a controlled environment. This leaves *Spirogyra* and *Cladophora* isolates as the most suitable for the treatment of these heavy metals as they continued to proliferate under the same conditions after the *Closterium* had died off.

3.5. Heavy metals concentration in the algal growth medium on day 60 (initial analysis)

The results obtained from the initial analysis indicated that the two macroalgae were adsorbing the heavy metals from the growth medium, though at varying degrees. Results showed a gradual but continuous reduction in the concentration of the heavy metals (As, Cd and Pb) in the growth medium as shown in Figure 1.

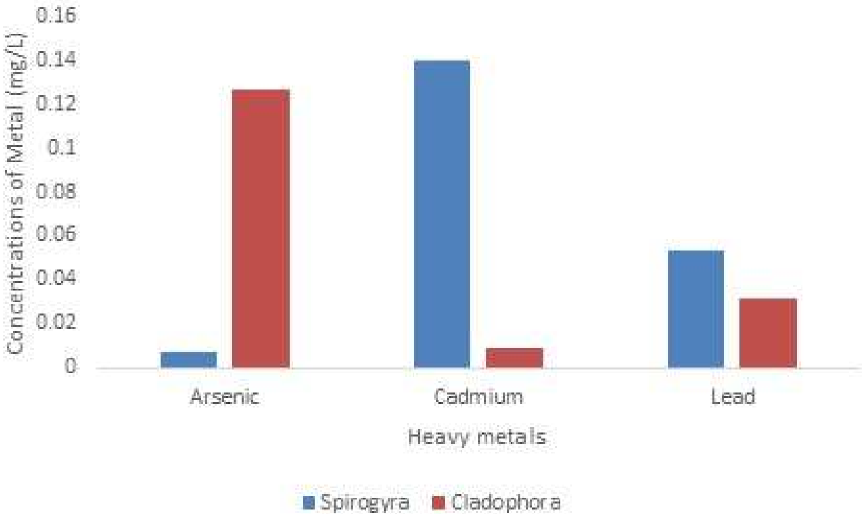

Figure 1. Heavy metals concentration in the algal growth medium after day 60 (initial analysis).

3.6. Heavy metal concentrations of the growth medium and the digested algal biomasses (final analysis)

After harvesting, weighing and digestion of the algal biomass, the growth medium where the algae were grown was analysed to ascertain the concentration of the heavy metals remaining in the growth medium, (Table 5). The biomasses were also taken for analysis to ascertain the concentration of heavy metals adsorbed by the individual alga in each of the conical flasks (Table 6).

Table 5. Concentration of heavy metals in the growth medium at 90 days (final analysis).

		Arsenic (mg/l)	**Cadmium (mg/l)**	**Lead (mg/l)**
Algae	Days	90	90	90
Spirogyra		0.0054 ± 0.001	0.0163 ± 0.002	0.1667 ± 0.019
Cladophora		0.0149 ± 0.002	0.0015 ± 0.001	0.0867 ± 0.010

Table 6. Heavy metals concentration in the algal biomass at 90 days (final analysis).

		Arsenic (mg/l)	**Cadmium (mg/l)**	**Lead (mg/l)**
Algae	Days	90	90	90
Spirogyra		0.3947 ± 0.024	0.2030 ± 0.021	0.4444 ± 0276
Cladophora		0.0588 ± 0.038	0.7055 ± 0.059	0.8889 ± 0.276

3.7. Mechanism of adsorption and sequestration of heavy metals by macroalgae

The green algae have an excellent binding capacity with metals. This feat is attributed to the copious secretion of various types of mucilaginous substances like proteins, or lipids but primarily composed of polysaccharides, on the surface of cell walls of these organisms. These polymeric substances contain functional groups such as aminos, hydroxyls, carboxyls and sulfates, which can act as binding sites for metals [26, 27]. The process of adsorption by algae involves the formation of complexes between a metal ion and functional groups on the surface of the cell wall [28]. Among the secretions include ligands. Ligands are molecules, functional groups or ions that are bound to a central atom of a molecule to form a complex [16]. Particularly, macroalgae species like *Cladophora, Closterium* and *Spirogyra* are capable of secreting a type of ligand called siderophores. Siderophore production by these macroalgae usually leads to severe heavy metal depletion in the environment [30].

The attachment/absorption of heavy metals by extracellular or associated materials such as the polysaccharides and mucilage molecules and other cell wall components like the carboxyl and hydroxyl groups on the surface of the cell wall is a rapid process termed physical adsorption, the metallic ions are transported slowly through the plasma membrane into the cytoplasm (chemisorptions) and then are bound to proteins and other intracellular components where they are finally sequestered [28].

4. DISCUSSION

The results of the present study showed that the two macroalgae species were able to absorb/remove the heavy metals in the water sample. The second (final) analysis of the growth medium on day 90 corroborated the result of the initial analysis of the day 60 as there was significant reduction in the concentration of the contaminants in the growth medium (Table 5).

Another evident of absorption of the contaminants by the algae was observed after the analysis of the digested algal biomasses. The result indicated that significant concentrations of these metals were found in the digested algae as shown in Table 6.

This is in agreement with previous studies by Soma [28],who recorded significant uptake of cadmium, lead and mercury using *Cladophora fasicularis* in an aqueous solution, and Lee and Chang [31], who reported the biosorption capacity of the green algae species *Spirogyra* and *Cladopho*ra to the uptake of lead (Pb^{2+}) and copper (Cu^{2+}) from aqueous solutions. Also in agreement are Nirmal and Cini [32], who revealed that *Spirogyra hyalina* is able to remove Cd, Hg, Pb, As and Co in aqueous solutions.

Although, the two algae were able to absorb the heavy metals, the results also showed that each alga had their preferences among the heavy metals. For instance, arsenic was absorbed more by *Spirogyra* than the *Cladophora.* While the *Spirogyra* absorbed 0.3947 mg/l of the 0.5196 mg/l of arsenic in the growth medium, the *Cladophora* only took up 0.0588 mg/l of the 0.5196 mg/l of arsenic in the growth medium throughout the 90 days duration of the study, suggesting that the Arsenic showed greater affinity for *Spirogyra* than to the *Cladophora*. On the other hand, from the 0.8946 mg/l of cadmium detected in the sample, *Cladophora* absorbed 0.7055 mg/l while *Spirogyra* absorbed 0.2030 mg/l, implying that the cadmiumshowed greater affinity for *Cladophora* than to the *Spirogyra* (Table 6).

Also, from the 1.2651 mg/l of lead detected in the sample, the *Cladophora* was able to take up 0.8889 mg/l while the *Spirogyra* took only 0.4444 mg/l, suggesting that the lead has more affinity for the *Cladophora* than to the *Spirogyra* (Table 6).

These observations are in agreement with the result of another study that was carried out to explore the application of *Cladophora* species biomass as a low-cost sorbent for the removal of copper and lead ions (Cu^{2+} and Pb^{2+}) from aqueous solutions [4]. It showed that the *Cladophora* specie had a remarkable ability to take up Cu^{2+} and Pb^{2+} heavy metal ions. However, the result noted that the *Cladophora* specie has more affinity for Pb^{2+} than Cu^{2+} and as such, higher concentration of the Pb^{2+} was absorbed by the *Cladophora* species from aqueous solutions.

4.1. The affinity of metal ions towards individual macroalga

The affinity shown by metals towards particular specie of microalgae may be due to the dissimilarity in the composition of the cell walls with regards to the polysaccharides and mucilage molecules and other cell wall components like the carboxyl and hydroxyl groups on the surface of the cell wall which avails multiple active sites readily for ion binding, and of course which may vary from species to species. This causes significant disparity in the type and concentration of metal ions that bind to a particular macroalga [22].

Another credible reason for ion affinity to particular algal specie is the presence or otherwise of a gene called phytochelatin synthase in the cytoplasm which directs the synthesis of metal-binding peptides known as phytochelatins. Phytochelatins are glutathione that have very high selective affinity for only a few particular heavy metals [28]. Synthesis of phytochelatin on the cell wall surfaces of algae, as directed by the phytochelatin synthase is activated by the availability of particular heavy metal substrates in the environment [33].

In this study therefore, the affinity/dissimilarity in the type and amount of individual metal removal for both algae as observed, was due to the ability of a particular metal ion to stimulate the active and uninterrupted synthesis of phytochelatins in particular specie of alga. This resulted in the availability of high specific active binding sites on the cell wall of the algal biomass for the very metal ion [34].

5. CONCLUSION

The results obtained from the present study indicated that both species of macroalgae (*Spirogyra* and *Cladophora*) can be used to develop effective absorbents capable of removing these heavy metals (arsenic, cadmium and lead) from aqueous solution, though at different degrees as particular alga seems to have more affinity towards certain heavy metals than to the other. This study discovered that the absorption capacity of *Spirogyra* for arsenic was superior to the absorption capacity of *Cladophora* to arsenic. *Cladophora* absorbed more cadmium and lead when compared to the absorption strength of *Spirogyra* to the two heavy metals. It is therefore concluded based on the result of the present study that reclaiming and reusing of water from public or industrial wastewater, or even from water contaminated as a result of precious metal mining is a huge possibility through the application of phycoremediation; using different species of macroalgae such as *Cladophora* and *Spirogyra.* Thus, these widely distributed and readily available fresh water organisms can be used for the development of efficient biosorbent system for heavy metal removal in aquatic environment.

Authors' Contributions: OPA designed the study, wrote theprotocol and interpreted the data. BUE anchored the field study, gathered the initial data and performedpreliminary data analysis. SAA and MIO managed the literature searches and produced the initialdraft. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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