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PHYCOREMEDIATION OF ZINC BY *SPIROGYRA* AND *RICHTERELLA* SPECIES ISOLATED FROM POND

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Abstract:

This study was carried out to assess the phycoremediation of zinc by *Spirogyra sp.* and *Richterella sp.* isolated from pond. This experiment was carried out for a period of 28 days. For *Richterella sp.*, there was a decrease in concentration of zinc by 50% at the end of 14 day and further reduction in concentration of zinc was 78% at the end of 28 day. Acid digestion of *Richterella sp.* on the 14 day reveals that *Richterella sp.* was able to accumulate up to 0.58mg/100g of zinc in its cell which also decrease at the end of 28 day to 0.28mg/100g. *Spirogyra sp.* was able to reduce the concentration of zinc by 25% and accumulated 0.28mg/100g at the end of 14 day. There was a further reduction in the concentration of zinc at the end of 28 day by 40%. *Spirogyra sp.* was able to accumulate up to 0.25mg/100g of zinc. This study clearly revealed that *spirogyra sp.* was able to remove 40% of zinc while *Richterella sp.* was able to remove 78% of zinc at the end of the experiment. Therefore, both have the ability to remediate zinc but *Richterella sp.* is more effective than *Spirogyra sp.* when used in remediation of Zinc contaminated water.

Keywords: Phycoremediation, Pond, Heavy metal, Zinc, Chromium.

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Introduction

Phycoremediation is the process of employing algae for improving water quality. Algae can fix carbon dioxide through the process of photosynthesis and remove excess nutrients effectively at minimal cost. In addition, photosynthetically produced oxygen can relieve biological oxygen demand (BOD) in the waste water. Micro algae are superior in remediation processes as a wide range of toxic and other wastes can be treated with algae and they are non-pathogenic. The risk of accidental release of pollutants into the atmosphere causing health safety and environmental problems are avoided when algae are employed for remediation. Algae utilize the wastes as nutritional sources and enzymatically degrade the pollutants. The xenobiotics and heavy metals are known to be detoxified/ transformed/or volatilized by algal metabolism. They have the ability to take up various kinds of nutrients like nitrogen and phosphorus. (Muthukumaran et al., 2005). They can utilize various organic compounds containing nitrogen and phosphorus from their carbon sources. Phytoremediation is a novel technique that uses algae to clean up polluted water and soil. It takes advantage of the alga's natural ability to take up, accumulate and degrade the constituents that are present in their growth environment. Algae based waste water treatment systems offer more simple and economical technology as compared to the other environmental protection systems. Photosynthesis can be effectively exploited to generate oxygen from waste water remediation by algae. The choice of algae to be used in wastewater treatment is determined by their robustness against wastewater and by their efficiency to grow in and to take up nutrients from wastewater. By using *Synechocystis salina* almost 60% Cr, 66% Fe, 70% Ni, 77% Hg, 65% Ca2+, 63% Mg2+ and 78% of total hardness were reduced in 15 days of treatment.

Bio-treatment with algae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporating nutrients such as nitrogen and phosphorus causing eutrophication (De la Nou and Basseres, 1989; De la Nou and De Pauw, 1988). Moreover, compared to physical and chemical treatment processes, algae based treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery. Recently, Bhatnagar and Kumari (2013) stated that algae are significantly efficient in treating more than one problem at a time, which is not possible by conventional process of chemical treatment. The phycoremediation shows advantage over other chemical methods as the removal of algal mass from the treated effluents is easy and economic (De la Nou and De Pauw, 1988; Igwe and Abia, 2006; Bhatnagar and Kumari, 2013).
Microalgae remove heavy metals directly from polluted water by two major mechanisms; the first is a metabolism dependent uptake into their cells at low concentrations, the second is biosorption which is a non-active adsorption process (Matagi et al., 1998). Phytoremediation is defined as a process of decontaminating soil and aquatic systems by using plants, fungi or algae to absorb heavy metals. Recently, the use of aquatic plants especially micro and macro algae has received much attention due to their ability to absorb of metals and taking up toxic elements from the environment or rendering them less harmful (Mitra et al., 2012). The algae have many features that make them ideal candidates for the selective removal and concentration of heavy metals, which

Table 1: Composition of Bold Basal Medium

Composition	modified bold basal medium, (Conc. in mg)
$NaNO_3$	0.375
KH_2PO_4	0.263
K_2HPO_4	0.15
$MgSO_4.7H_2O$	0.038
$CaCl_2.2H_20$	0.038
Nacl	0.038
KOH	0.015
$FeSO_4.7H_20$	2.49
H_3BO_3	5.72
$MnCl_2.7H_20$	0.72
MoO_3	0.355
$CuSO_4.5H_20$	0.784
$Co(N0_3)_2.6H_20$	0.245
Na_2EDTA	25
Distilled water	500ml
Urea	1g

include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Cai et al., 1995). The aim of this study was therefore, to remediate zinc contaminant in water using *Spirogyra* and *Richterella* species.

Materials and Methods:

The micro algae, *Spirogyra sp.* and *Richterella sp.* were collected from the pond located at the Federal University of Technology environment, Bosso low cost area and Bosso estate area of Minna, Niger State, Nigeria

Composition of the Culture Media Used For the Algae Cultivation

The algal samples were cultured in proper culture media. The two sp. were cultured in bold basal medium which contains the constituents shown in Table 1.

Experimental Design

Each of the minerals was measured in their various appropriate grams (Table 1). After which it was dissolved in 500ml of distilled water. Two 1000ml conical flask were prepared separately for each algal species. The two algal species were then introduced into each of the prepared media separately. 10mls were measured using measuring cylinder. The growth was monitored for two weeks with colorimeter. After the period of two weeks of growth, the algae were subculture. A fresh media were prepared together with a standard solution of ZnS04 (i.e. 0.78g of ZnSO4 was dissolved in 500ml of distilled water together with the growth minerals). These were done in separate conical flasks for the two species of algae. These (i.e. 500ml that was prepared) were shaked using an incubator shaker for 15mins. It was left to stand for 24hours. Shaking was done to circulate the nutrients in the water. After 24hours, it was then dispensed into three five (5) 100mls conical flask, 2 conical flask for *Spirogyra*, 2 for *Richterella*. The remaining for the control.

Phycoremediation Setup

In the experimental design, the bold basal media prepared in 500ml conical flask which contains both the minerals and ZnSO4 was dispensed in 5 different 100ml conical flask. 10ml of *Spirogyra sp.* was inoculated into the first 2 conical flasks, *Richterella sp.* was inoculated into the next 2 conical flasks. The remaining one was left as control (i.e. it contains no algae, just growth medium and ZnSO4). The experiment was set up in triplicate.

Another control was prepared in 2 separate 100ml conical flask, these contains 10ml of *Spirogyra sp.* and 10ml of *Richterella sp.* respectively, together with the growth medium but no heavy metals (ZnSO4). This was done to determine whether the presence or absence of ZnSO4 will have effect on the algae biomass. After the preparation, the conical flasks were placed under 24 hours fluorescence light bulb and were monitored for a period of 28days.

The analyses were carried out at 14days interval for periods of 28 days after the initial reading has been recorded. One of the flasks was removed for the first analyses at 0day using Atomic Absorption Spectrophometer (AAS) to determine the initial concentration of ZnSO4.

The bioremoval efficiency of the algae was calculated using the formula:

$$\% \text{ Removal} = \frac{C_1 - C_2}{c1} \times 100$$

Where C_1 = initial concentration
C_2 = final concentration.

Results and Discussion

Reduction in zinc concentration by *Spirogyra sp.* and *Richterella sp.*

Preliminary analysis of zinc was 4.65mg/L. Analyses of the media solution containing the algae, growth medium and Zinc was carried out on the 14 and the 28 days.

It was observed that *Spirogyra sp.* was able to reduce the concentration of zinc by 25% at the end of 14 day and further reduction by 40% at the end of 28 day. This clearly revealed that *Spirogyra sp.* has the potential of removing zinc in contaminated water. This could be due to the ability of the algae to utilize zinc as a source of nutrient while *Richterella sp.* was able to reduce the initial concentration of zinc by 50% at the end of 14 days and by 78% at the end of 28 days (Figure 1).

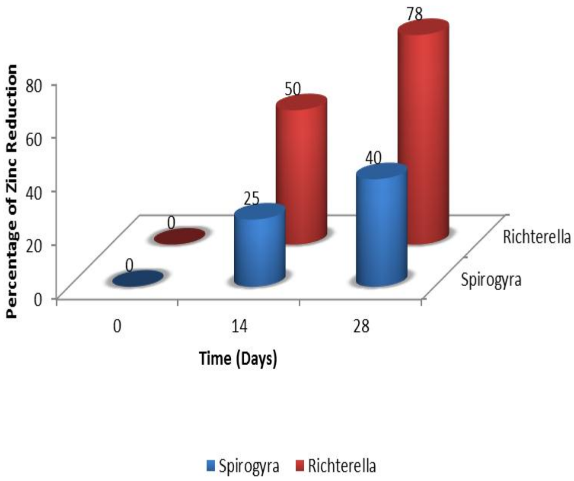

Figure 1: Percentage Reduction in Zinc Concentration by *Spirogyra sp.* and *Richterella sp.*

Figure 1 Show that *Richterella sp.* was effective in zinc remediation by reducing the concentration of the zinc by 50% bioremoval rate at the end of 14 day. At the end of 28 day, the concentration increased to 78% bioremoval rate. The percentage of metal removal increased with time (Figure 1).

Bioaccumulation of zinc by *Spirogyra* and *Richterella* species

For each of the analyses done between the periods of 14 to 28 day, the amount of metal each of the algae species were able to accumulate was determined through acid digestion of the algae and analysis with AAS. On the 14 day, *Spirogyra sp.* was able to accumulate up to 0.28mg/100g of Zn. 0.25mg/100g Zinc accumulated at the end of the 28 day. This shows that *Spirogyra sp. h*as a low affinity for the binding of Zinc. *Richterella sp.* was able to accumulate up to 0.58mg/100g of metal at the end of 14 day. 0.29mg/100g of Zinc accumulated at the end of 28 day. Thus, *Richterella sp.* has a higher uptake of zinc than *spirogyra sp*. The cell divider comprises of mixture of polysaccharides and proteins which offers various dynamic locales equipped for tying metal particles (Rai, 2001).

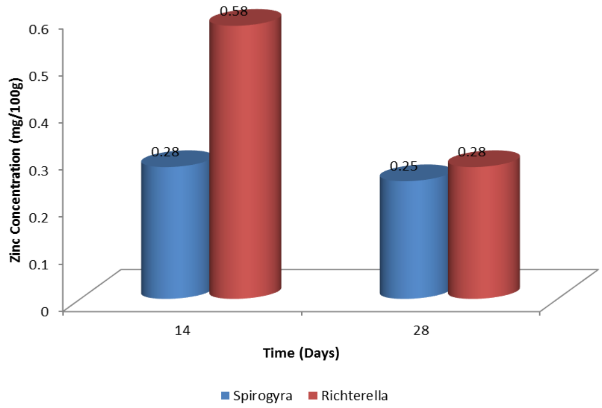

Figure 2: Bioaccumulation of Zinc by *Spirogyra sp.* and *Richterella sp.*

This was similar to the report of Yan and Pan (2002) who reported that the accumulation of metal ions depends on external concentration of metal ions in the solution. If the external concentration of metal ion is higher than the algae biomass, it could lead to toxic effects which could lead to decrease performance of bioaccumulation. This study indicates that both *Spirogyra sp.* and *Richterella sp.* are able to remove zinc from aqueous solution at lower concentrations.

The blue green alga *Phormidium* successfully can hyperaccumulate heavy metals like Cd, Zn, Pb, Ni and Cu (Wang et al., 1995). Liu et al. (2002) reported that *Dunaliella salina*, a green microalgae, have high tendency for zinc accumulation followed by copper and cobalt, the lowest tendency was for cadmium, this may be due to the importance of zinc as hydrogen transferring in photosynthesis. The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups on the surface or inside the porous structure of the biological material. The carboxyl groups of alginate play a major role in the complexation. Different species of algae and the algae of the same species may have different adsorption capacity (Jin-fen et al., 2000).

Effect of Zinc On Biomass Concentration

Phycoremediation of zinc was carried out at biomass concentration of 1.01g for both *Spirogyra sp.* and *Richterella sp.*The absorption rate increases with increasing biomass concentration. In this study, the initial weights of both *Spirogyra sp.* and *Richterella sp.* were measured to determine if the presence of zinc will have effect on the algae biomass. Table 1 shows that there was a significant effect of the metal on the algae biomass when compared with the control.

Table 2: Effect of Zinc on Biomass

Sample	BIOMASS CONCENTRATION (g)		
	0	14	28
A	1.01	1.53	1.65
B	1.01	1.54	1.72
C	1.01	1.41	1.52
D	1.01	1.84	1.96

KEYS:
A= Media+ *Spirogyra*+ zinc
B= Media+ *Richterella*+ zinc
C= Media+ *Spirogyra*
D= Media+ *Richterella*.

This study on metal uptake by these micro algae (*Spirogyra sp.*and *Richterella sp.*) clearly reveal the efficiency of the algae to remove Zinc from a polluted water. In this experiment, it is clearly revealed that both algae has the ability to remove zinc but *Richterella sp.* shows higher efficiency in the uptake of zinc than *Spirogyra sp*. This could be due to the difference in cell wall composition and the intra group differences which cause significant differences in the type and amount of metal ion binding to them.

Conclusion

The accumulation of heavy metals by algae provides an advantage for phycoremediation over other methods which are more costly and not environmental friendly. The results of the present study showed that both *Spirogyra sp.*and *Richterella sp.* are effective for the remediation of zinc but *Richterella sp.* was discovered to be more effective than *Spirogyra sp.* in phycoremediation. However, further study is required before considering this plant Species for phycoremediation.

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