Phycoremediation of Pb, Cd, and of Cu by Spirogyra cummins from wastewater

Azhar Uddin, AM Lall, KP Rao, Suchit A John and Amit Chattree

Abstract

Phycoremediation is the usage of macro-algae or micro-algae for the elimination or biotransformation of pollutants, which include nutrients and xenobiotics from wastewater. Inside the present study, the phycoremediation capacities of live green algae, Spirogyra Cummins changed into evaluated for toxic heavy metals, lead (Pb), cadmium (Cd), and copper (Cu) from wastewater and synthetic solution. Spirogyra Cummins algae proved efficient organic vectors for heavy metal uptake. Phycoremediation studies executed on wastewater effluent found out that ninety-eight percent Pb, ninety-five percent Cd, and ninety-two percent of Cu on ninety minutes of treatments. Experimental consequences located that Spirogyra Cummins has the maximum accumulation of Pb observed via Cd and Cu after ninety minutes of publicity. Cd discovered with the aid of the use of Pb and Cu maximally reduced the general boom performance of the algae measured concentrations for Pb, Cd, and Cu after 90 minutes of exposure.

Keywords: Phycoremediation, heavy metals, Spirogyra Cummins, wastewater, Pb, Cd and Cu

Introduction

Aquatic ecosystems are mainly affected by heavy metals and represent a potential risk to the health of humans. In recent times, the choice of wastewater treatment technique is one of the maximum exciting topics many of the researcher either conventional, bioremediation or preferred mode. Several studies have been reported on the use of algae in bioindication of pesticides (Wong 2000) [32]. Microbes are ideal candidates to decrease the heavy metal ion concentration from ppm to ppb levels (Wang and Chen 2006). Phycoremediation is a bioremediation technique in wastewater remedy that utilises microorganism which includes microalgae. Environmental infection through heavy metals is a severe problem because of their incremental accumulation inside the meals chain (Awofolu 2005) [1].

In contrast to maximum natural wastes and the microbial load in aquatic bodies, steel contaminants are not biodegradable, tending to accumulate in residing organisms, accordingly becoming an everlasting burden on ecosystems (Sivakumar et al. 2014) [15]. Most heavy metals are transition factors with incompletely stuffed d-orbitals. Living organisms require hint quantities (μg L⁻¹) of a few steel ions inclusive of lead, copper, zinc, cobalt, iron and nickel as cofactors for the enzymatic activities. However, heavy metallic ion concentrations at ppm (mg L⁻¹) degree are acknowledged to be toxic to the organisms because of irreversible inhibition of many enzymes by way of the heavy metal ions. The system of accumulation and adsorption of metals with the aid of algae involves adsorption onto the mobile floor (wall, membrane or outside polysaccharides) and binding to cytoplasmic ligands, phytochelatins and metallothioneins, and other intracellular molecules.

For a reason that metal ions in water are generally inside the cationic shape, they are adsorbed onto the cellular floor (Crist et al. 1992; Romera et al. 2007; Singh and Kalamdhad 2012) [2, 12, 14]. Algal cellular partitions are porous and allow the unfastened passage of molecules and ions in aqueous answers. The ingredients of the algal mobile wall provide an array of ligands with different purposeful agencies capable of binding numerous heavy metals. These cells can be used to stay or useless (Zou et al. 2014) [19]. They are commonly rugged organisms with a rapid increase in a single medium, and the algal biomass produced can efficiently be processed into useful biosorbents (Tuzen and San 2010) [18].

However recently it has been stated that stay species reveals better biosorption potential than dead biomass probably because of enzymatic reactions in the course of intracellular uptake.

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Materials and Methods

Algal cultures and water samples

All chemicals used in this study were of analytical reagent grade. The freshwater macroalgae, Spirogyra Cummins algal samples from sample 1 old bridge phul- mandi Naini Allahabad (25° 25'18" N; 81° 51’4” E), sample 2 SHUATS University Campus Forestry Department. The collected samples were subjected to microscopic identification for characterisation of species distribution and selection of source with a high number of Spirogyra species. The cultures were further maintained in Fog’s medium. Slant cultures were prepared from the pure culture for further use. One loopful of algal biomass from best growth obtained above was inoculated in a sterile 15ml test tube with an enriched medium (Bold’s Basal Media).

Wastewater was collected in bulk from the samples A; Yamuna river Address - Sangam yatra mandir Sachcha Baba Nagar Araiil Ghat Naini Allahabad situated (25° 24'14" N; 81° 52'49" E) Sedimentation and filtration through filters paper removed solid particles. After filtration, wastewater was stored at 4 °C in the dark until needed for the experiments.

Ten ml of sample was taken and centrifuged at 6000 rpm for 10 min, and the supernatant was discarded. The heavy metals concentrations in the aqueous environment. BCF is a comparison between heavy metal concentrations on the Spirogyra Cummins with the concentration on the aqueous environment.

BCF =\( \frac{C_{\text{org}}}{C_{\text{medium}}} \)

Where \( C_{\text{org}} \) was heavy metals concentration in Spirogyra Cummin and \( C_{\text{media}} \) was heavy metals concentration in the culture media

Measurement of Chlorophyll

Ten ml of sample was taken and centrifuged at 6000 rpm for 15 min. Supernatants have been discarded and re-suspended in a known volume of methanol, at the same time as pellets extracted with 5 ml of 96% methanol extraction. The tubes were wrapped with aluminum foil and kept in darkish. The samples had been centrifuged again, and the supernatants were used for measuring the optical density at 663 nm and 645 nm towards 96 % methanol as a blank by spectrophotometer. After extraction chlorophyll attention was determined spectrophotometrically and calculated Chlorophyll content material (Chlorophyll a, chlorophyll b total chlorophyll) had been computed using the following equations

Chlorophyll-a (\( \mu g/ml \)) = \{(15.65xA666 – 7.340xA653) x V/ 50 x W} x dilution

Chlorophyll-b (\( \mu g/ml \)) = \{(27.05xA653 – 11.21xA666) x V/ 50 x W} x dilution

Total chlorophyll = chlorophyll-a + chlorophyll-b

Results and Discussion

Microscopic studies of cultures have proven that species infection is found in each the cultures, but the species distribution varies considerably. Within the case of Spirogyra Cummins cultivation, the populace of contaminant species has been low till the 7th day after which they started out growing.

Change in physicochemical parameters

The growth pattern of isolated microalgae in the presence of lead (Pb), cadmium (Cd), and copper (Cu) have revealed that the degree of growth inhibition by Pb n Cd and Cu varied widely between the Spirogyra Cummins. The wastewater contains various toxic contaminants including heavy metals as lead, cadmium, nickel, mercury, arsenic, copper etc. producing a significant poisonous impact on the aquatic
inhibition, after 4 days in solutions contained 2, 5 and 10 mg/l Cr(II) respectively (Nassiri et al. 1997) [8]. Chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium and chromium were reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis (Stobart et al. 1985) [17]. The inactivation of the enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content. The present results showed that lead, copper and chromium toxicity decreased the chlorophyll a content of the two algae under investigation. The highest reduction in chlorophyll content was found in algae exposed to chromium, followed by copper and lead. A large reduction in chlorophyll content due to Cr toxicity can be explained by the destruction of stomata and mesophyll cells, which decreases their efficiency of light utilisation and electron transport rates involving PS I and PS II (Hernández et al. 2004, Munné-Bosch and Alegre 2003) [8,7].

In conclusion, in the present study, the bioaccumulation potential of algal biomass *Spirogyra Cummins* has been assessed for the removal of Pb (II), Cu (II) and Cr (IV) from wastewater and aequous solution. Experiments conducted on wastewater showed a significant decrease in physicochemical parameters and heavy metal content by the algal biomass. The chlorophyll content of both algae was highly suppressed by high reduced by Cd followed by Pd and Cu levels heavy metal removal from wastewaters.

Chl-a as an algal biomass measurement in herbal structures was very famous. Chl-b is used to calculate pigment concentrations. The total Chl-(a + b) is used to degree algal boom (Ramaraj et al. 2013) [11]. Boom device was set up out of doors conditions. Table 2 shows the chlorophyll-a concentration in microalgae determined using the standard method. Biomass measured by total chlorophyll results were average as, 12 μg/mL for Pb, 8.9 μg/mL for Cd, and 6.5 μg/mL, for Cu respectively for *Spirogyra Cummins*. Heavy metals enter algal cells using either active transport or endocytosis through chelating proteins and affect various physiological and biochemical processes of the algae.

The obtained results in this investigation concerning the tolerance of *S. communis* and *C. pyrenoidosa* to the tested heavy metal ions (Pb, Cu and Cr) were in agreement with the results reported by Foster, 1982 and Stokes, 1983 concerning the tolerance and resistance of green algal species to heavy metal ions (Cu, Cd, Pb and Zn). Also, high Cr2+ concentrations reduced cell sizes and caused a decrease in growth rate (Leborans and Novillo 1996) [8]. Nassiri et al., (1997) [8], found no growth inhibition at Cr2+concentrations < 1mg/l, but *Tetraselmis suecica* had 10, 30 and 50% growth inhibition, after 4 days in solutions contained 2, 5 and 10 mg/l Cr2+ respectively (Nassiri et al. 1997) [8]. Chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium and chromium were reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis (Stobart et al. 1985) [17]. The inactivation of the enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content. The present results showed that lead, copper and chromium toxicity decreased the chlorophyll a content of the two algae under investigation. The highest reduction in chlorophyll content was found in algae exposed to chromium, followed by copper and lead. A large reduction in chlorophyll content due to Cr toxicity can be explained by the destruction of stomata and mesophyll cells, which decreases their efficiency of light utilisation and electron transport rates involving PS I and PS II (Hernández et al. 2004, Munné-Bosch and Alegre 2003) [8,7].

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![Fig 1: Percentage of heavy metals removal from media culture of *Spirogyra Cummins* in 60 minutes and 90 minutes.](image)

### Table 1: Change in physicochemical parameters at 14 days interval with *Spirogyra Cummins*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Unit</th>
<th>Initial value</th>
<th>Wastewaters + <em>Spirogyra Cummins</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td></td>
<td>5.8</td>
<td>8.7</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td></td>
<td>Light brown</td>
<td>pale White</td>
</tr>
<tr>
<td>3</td>
<td>Hardness</td>
<td></td>
<td>253</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>Alkalinity</td>
<td>mg/L</td>
<td>187</td>
<td>228</td>
</tr>
<tr>
<td>5</td>
<td>Total Nitrogen</td>
<td>mg/L</td>
<td>200</td>
<td>168</td>
</tr>
<tr>
<td>6</td>
<td>Nitrate (NO₃⁻)</td>
<td>mg/L</td>
<td>3.3</td>
<td>1.86</td>
</tr>
<tr>
<td>7</td>
<td>Phosphate</td>
<td>mg/L</td>
<td>3.77</td>
<td>1.3</td>
</tr>
<tr>
<td>8</td>
<td>Chloride</td>
<td>mg/L</td>
<td>837</td>
<td>456</td>
</tr>
<tr>
<td>9</td>
<td>Ammonical Nitrogen</td>
<td>mg/L</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>Total dissolved solids (TDS)</td>
<td>mg/L</td>
<td>2076</td>
<td>477</td>
</tr>
<tr>
<td>11</td>
<td>Chemical Oxygen Demand (COD)</td>
<td>mg/L</td>
<td>125</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>I. Biological Oxygen Demand (BOD)</td>
<td>mg/l</td>
<td>112</td>
<td>74</td>
</tr>
<tr>
<td>13</td>
<td>II. Test for Dissolved Oxygen (DO)</td>
<td>mg/L</td>
<td>2.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Table 2: Chlorophyll estimation in algae strains exposure to the heavy metal.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spirogyra Cummins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial value</td>
</tr>
<tr>
<td>Chl-a (μg/mL)</td>
<td>8.5</td>
</tr>
<tr>
<td>Chl-b (μg/mL)</td>
<td>3.5</td>
</tr>
<tr>
<td>Total Chl (a + b) (μg/mL)</td>
<td>12</td>
</tr>
</tbody>
</table>

Conclusion
The present findings revealed that live biomass of *Spirogyra Cummins* algae is better Phycoremediation tool for removal of heavy metals under optimised conditions. Further, it showed better reusability potential for removal of lead and cadmium ions from the metal contaminated aquatic system.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Reference
accurate measurement for algal biomass, 2013.


