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

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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, lad (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 % Pb, 95 % Cd, and 92 % 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 90 minutes of publicity. Cd discovered with the aid of the use of Pd and Cu maximally reduced the general boom performance of the algae measured concerning Chl-a (5.8 µg/mL) and Chl b (3.1 µg/mL) content material accompanied through Pd and Cu. The effects confirmed that *Spirogyra Cummins* had been appropriate for Pb and Cd elimination and bioaccumulation of heavy metals from effluent wastewater.

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 Sarı 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

(Doshi, Ray, and Kothari 2009) [3]. The existing look at, therefore, aimed to compare the performance of Spirogyra Cummins sequestering lad (Pb), cadmium (Cd), and copper (Cu) ions from wastewater and aqueous answers. The increase performance of the *Spirogyra Cummins* algal species regarding their Chl a and Chl b content material after heavy metals accumulation was additionally examined. The algae have been distinct from their morphology; one is unicellular while different are multicellular. In literature, no such take a look at has been undertaken to date where an assessment is made between morphologically distinct algae for bioremediation of heavy metals.

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 Arail 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.

Total ten numbers of 25ml test tubes were inoculated with isolated algae, maintained at 24°C ± 1°C and illuminated at 3500–4000 lux light intensity with a light/dark cycle of 16/8-h for ten days. After ten days, inoculated algae from the test tubes were inoculated into 250 ml Erlenmeyer flasks containing Bold's Basal Media for another seven days. After seven days the medium inside the container appear green, these were examined under the microscope. At every 12 days, a new medium was prepared, and the algal cells were inoculated to it to continue the algal cell generation. To avoid bacterial and fungal contamination appropriate amount of antifungal and antibacterial were added to the medium.

Preparation of heavy metal stock solutions

Stock solutions of Pb (II), Cu (II) and Cr (IV) were prepared by dissolving their salts. The trace elements of Pb, Cd, and Cu were added to the culture media. Stock solutions of Pb (II), Cu (II) and Cr (IV) were prepared by dissolving their salts Pb $(NO_3)_2$, 3Cd SO_4. $8H_2O$ and $CuCl_2$. $2H_2O$ in the distilled water. Solutions for adsorption and metal analyses were prepared by appropriate dilution of the freshly prepared stock solution.

Characterization of wastewater parameters

Wastewater was analyzed for various chemical and physical parameters such as pH, Colour, Hardness, Alkalinity, Total Nitrogen, Nitrate (NO3-), Phosphate, Chloride, Ammonical Nitrogen, Total dissolved solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Test for Dissolved Oxygen (DO) estimation by standard prescribed methods in APHA (Eaton *et al.* 2005) [4, 20].

Phycoremediation Experiments

The 1, 3, and 5 mg/l concentrations of Pb^{+2}, Cd^{+2} and $Cu^{+2,}$ were exposed to the *Spirogyra Cummins* culture, respectively. The culture sample of *Spirogyra Cummins* was centrifuged at 9000×g for 10 min, and the supernatant was discarded. The algal cells (biomass) were washed twice with sterile Milli-Q water and re-suspended in sterile Milli-Q water for inoculation into the growth medium. These concentrations were arranged based on preliminary research reviews (Soeprobowati and Hariyati 2014) [16, 29]. The concentrations of Pb, Cd and Cu were measured at the initial time, minutes of 30 and minutes of 90. The heavy metals concentrations in media culture and the *Spirogyra Cummins* were measures with AAS. A reduction of heavy metals (percentage of removal) was calculated as well as *Spirogyra Cummins* population. Bio Concentration Factor (BCF) was calculated to determine the accumulation of heavy metals in the *Spirogyra Cummins*. BCF is a comparison between heavy metal concentrations on the *Spirogyra Cummins* with the concentration on the aqueous environment.

$$BCF = C_{org} / C_{medium}$$

C_{org} was heavy metals concentration in *Spirogyra Cummin*
C_{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 spectrophotometric ally and calculated Chlorophyll content material (Chlorophyll a, chlorophyll b and total chlorophyll) had been computed using the following equations

Chlorophyll-a (μg/ml) = {(15.65xA666 – 7.340xA653) x V/ 50 x W} x dilution
Chlorophyll-b (μg/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 7 th day after which they started out growing.

Change in physicochemical parameters

The growth pattern of isolated microalgae in the presence of lad (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 environment (Oyeku and Eludoyin 2010, Pandey *et al*. 2010, Siddiqui and Sharma 2009) [9, 25, 26, 13, 28]. Data represented in Table 1 and Fig 1. Three indicates the changes in physicochemical parameters before and after treatment with algae *Spirogyra Cummins*. After 90 min there was 98 % Pb, 95 % Cd, and 92 % of Cu on 90 minutes of treatments with algal biomass Fig.1.

Moreover, a significant change was observed in physicochemical parameters of wastewater after 14 days of treatment with algae under investigation. The study suggests that *Spirogyra Cummins* shows promising approach towards the purification process of wastewater at various parameters. Along with bringing the properties such as pH, Colour, Hardness, Alkalinity, Total Nitrogen, Nitrate (NO3-), Phosphate, Chloride, Ammonical Nitrogen, Total dissolved solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Test for Dissolved Oxygen (DO) *etc* showed in Table1. Towards the desirable limit, the algae have been found quite sufficient for the removal of heavy metals also. Among heavy metals, although a significant reduction was observed in all the three metals, the algae are especially useful in the reduction of Pb, followed by Cd and Cu.

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) [6]. 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) [5, 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 aqueous 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.

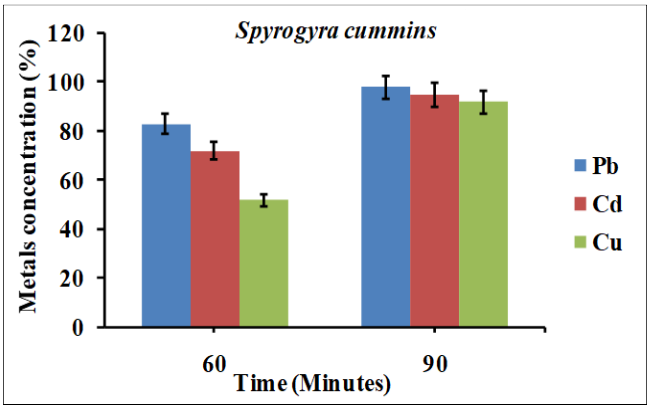

Fig 1: Percentage of heavy metals removal from media culture of *Spirogyra Cummins* in 60 minutes and 90 minutes.

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

SI. No.	Parameter	Unit	Initial value	Wastewaters + *Spirogyra Cummins*
1	pH		5.8	8.7
2	Colour		Light brown	pale White
3	Hardness		253	64
4	Alkalinity	mg/L	187	228
5	Total Nitrogen	mg/L	200	168
6	Nitrate (NO^{3-})	mg/L	3.3	1.86
7	Phosphate	mg/L	3.77	1.3
8	Chloride	mg/L	837	456
9	Ammonical Nitrogen	mg/L	40	19
10	Total dissolved solids (TDS)	mg/L	2076	477
11	Chemical Oxygen Demand (COD)	mg/L	125	72
12	I. Biological Oxygen Demand (BOD)	mg/l	112	74
13	II. Test for Dissolved Oxygen (DO)	mg/L	2.7	3.6

Table 2: Chlorophylls estimation in algae strains exposure to the heavy metal.

Parameter	*Spirogyra Cummins*			
	Initial value	Pb	Cd	Cu
Chl-a (μg/mL)	8.5	5.8	5.2	4.3
Chl-b (μg/mL)	3.5	3.1	2.6	2.2
Total Chl (a + b) (μg/mL)	12	8.9	7.8	6.5

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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