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*Original Research*

# Cd, As, Cu, and Zn Transfer through Dry to Rehydrated Biomass of *Spirulina Platensis* from Wastewater

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## Abstract

Phytoremediation of Cd, As, Cu, and Zn by *Spirulina Platensis* is one of the most cost-effective approaches and environmental friendly technologies used to remediate contaminants from contaminated water. The removal rates of Cd, As, Cu, and Zn in the field experiment were 14.95, 9.45, 35.55, and 73.95 µg/g/d, respectively. The highest concentrations of these metals accumulated in *S. Platensis* after 90 d of the laboratory/field collected samples were 58.9/98.68, 29.86/47.98, 43.28/235.86, and 249.67/390.65 µg/g dry wt., respectively, over the experiment. Only 55% Cd, 35% As, 85% Cu, and 95% of Zn removed from the water were used by *S. Platensis*. The bioconcentration factors were recorded for the metals in field/laboratory: for Cd (BCF=90/536), As (BCF=135/2,155), Cu (BCF=34,200/62,300) and Zn (BCF=32,500/95,300). The data obtained suggest that cyanobacterium *S. Platensis* has promising potential and can be used in a synergistic way to remediate wastewater polluted by Cd, As, Cu, and Zn.

**Keywords:** heavy metals, Cd, As, Cu, Zn, bioccumaulation, *S. Platensis*, phytoremediation, bioconcentration factors (BCFs)

## Introduction

The remediation of heavy metal-contaminated sites is still a big challenge for researchers because of the non-degradability of these metals in the environment. Phytoremediation, a biological technology using plants to remove contaminants from water and soils, has been intensively studied during the past decade due to its cost-effectiveness and environmental harmonies [1, 2]. Metals cause major environmental and human health problems, hence our need for immediate attention for effective and affordable techniques for phytoremediation of metal pollution from water [3, 4]. The contamination of water by heavy metals and metalloids is a major environmental problem. Cd and As, which can co-exist in the environment, present a serious threat to human and animal health because of their capability to enter food chains in large amounts [5, 6]. Cd and As are considered to be toxic in the environment at low levels [7, 8]. Cu and Zn are essential elements for plants and are easily taken up by roots, but are toxic when present in excess concentrations [9]. Considering their adverse effects on the health of humans and other organisms, the appropriate treatment of these metals is of great environmental importance. A variety of treatment methods have been developed for the elimination of these metals from water, including coagulation [10], adsorption [11], ion exchange [12], electrocoagulation [13], and biological process. However, these methods are expensive and require major investments in equipment and facilities. In contrast, phytoremediation is considered a cost-effective and environmentally-friendly technology for the treatment of waters contaminated by heavy metals [14-16].

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Aquatic microalgae have great potential in terms of accumulating toxic metals from contaminated water [17-29], but few studies have investigated the capacity of aquatic plants and other species to accumulate Cd and As from contaminated water [30]. The cyanobacteria *S. Platensis* has recently been reported to accumulate multiple metals from mine tailings and drainage, and to be a hyperaccumulator of Cd, As, and Pb [31]. However, its capacity to directly accumulate metals from water is still well known [32]. *S. Platensis* is an emergent algae with a worldwide distribution, being easily cultivated and controlled, growing naturally in marshes, reservoirs, waterways, and paddy fields, with a broad habitat from northern to southeastern Asia, and Malaysia. The aim of the present study is to assess the ability of *S. Platensis* to bioaccumulate Cd, As, Cu, and Zn from contaminated water under laboratory and natural conditions. The research goal is to assess the broad application of these cyanobacteria in the phytoremediation of metal-contaminated wastewater [32-35].

## Materials and Methods

### *S. Platensis* Cultivation Experiments

The experimental study was carried out over 90 days. *S. Platensis* was grown in a harvesting chamber filled with 1-l of tap water, receiving 24 h of fluorescent light per day. A total wt of about 100 g (fresh weight) of the algae was grown in (1) Milli-Q water (plant control without contaminated water) and (2) metal-contaminated water (Cd, As, Cu, and Zn) collected from a drainage site at the Okhla downstream, New Delhi (India). Water in both chambers was changed every 24 h. Water samples for metal analysis were collected before and 24 h after transplanting *S. Platensis*. Plant samples were collected before transplanting and at 10 days after transplanting. Air and water temperature remained constant (25±1ºC) during the experiment.

In the field experiment, *S. Platensis* algae were collected from the waterlogged areas near Okhla downstream using a Wisconsin plankton net (28 µm mesh) in 250 ml plastic bottles. The material was examined immediately after bringing it to laboratory in living and dry conditions, and photomicrographs were taken with an SZ1450 Nikon microscope. For identification of algal species, the key provided by Prescott was used.

### Bioaccumulation and Analytical Procedures

The cyanobacteria algae samples were separated from water, rinsed with Milli-Q water, and dried in a ventilated oven at 80ºC for 48 hours. The dried samples were ground into a fine powder using a mortar. Plant samples were digested with hydrogen peroxide (0.2 ml), hydrofluoric acid (0.5 ml), and nitric acid (1 ml) for analysis by an Inductively-Coupled Plasma Mass Spectrometer, PerkinElmer Corporation (ICP Optima 3300 RL). The standard reference materials were of Pb, Ni, Zn, and Hg (E-Merck, Germany). The detection limits of Cd, As, Cu, and Zn were 0.001, 0.005, 0.02, and 0.05 mg/ml, respectively. Replicate (n=3) analysis was conducted to assess the precision of the analytical techniques. Triplicate analysis for each metal varied by no more than 5%. Concentrations of metals in water were also determined by ICP-MS. The mean recovery was about 96-98.5% for different metals. The blanks were run in triplicate to check the precision of the method with each set of samples. With concentrations of Cd, As, Cu, and Zn of 2.3±0.3, 12±1 and 340±20 mg/kg dry wt., respectively, was used for quality control. The analysis of the reference material was accurate; indicating a high level of reproducibility (measured concentrations of Cd, As, Cu, and Zn were 1.56±0.30, 2.23±050, 11.9±0.3, and 332±12 mg/kg dry wt., respectively).

### Water and Plant Materials

The concentrations of Cd, As, Cu, and Zn in water collected for the laboratory and field experiment were 43.6±11.86, 5.4±3.9, 2.3±0.65, and 4.50±1.76 µg/L, respectively. Cd and As concentrations exceeded the threshold values recommended for drinking water. The concentrations of Cd, As, Cu, and Zn were 119, 6.08, 0.791, and 2.00 µg/L, respectively. Only the Cd concentration exceeded the threshold set for drinking water. The pH of water in the field experiment was almost neutral (6.79 at 25ºC), and the oxidation-reduction potential was 148 mV. *S. Platensis* used in the laboratory and was collected from the Okhla downstream, near the Okhla industrial area, India.

### Statistical Analysis

Two-way analysis of variance (ANOVA) was done on all the data to confirm the variability of data and validity of results. Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments that showed $p<0.05$ significant and $p<0.01$ strongly significant. Biocencentration factors are the ratio of metal concentration in plant tissues at harvest and initial concentration of metals in external environment (contaminated water).

## Results and Discussion

*S. Platensis* adapted well to contaminated water during the laboratory and field experiment, growing biomass in Fig. 1. The rehydrated samples accumulated metals Cd, As, Cu, and Zn more compared with dry biomass of laboratory and field samples (Figs. 1-8). In the field experiment, however, the plant showed a slight reduction in growth over time due to toxicity of metals. Table 1 showed the concentrations of Cd, As, Cu, and Zn in water before transplanting *S. Platensis* during 1 to 90 d of the laboratory and field experiment. Concentrations of these metals accumulation in *S. Platensis* over the experiment the laboratory and field experiments are presented in Figs. 2-9. However, the relationship between BCF was different for dry and rehydrated samples. Only the relationships between laboratory and

Table 1. Physico-chemical properties of wastewater and metals content of Okhlan downstream.

| Parameters | | Water sample |
|---|---|---|
| pH | | 6.79 |
| BOD | (ppm) | 72.9 |
| COD | (ppm) | 0.45 |
| $Cl^{-1}$ | (ppm) | 0.67 |
| Alkalinity | (ppm) | 0.76 |
| Sulfate | (%) | 2.9 |
| Potassium | (%) | 0.95 |
| Chloride | (%) | 2.65 |
| Carbonate | (%) | 2.09 |
| Magnesium | (%) | 1.86 |
| Metal ions ($\mu gL^{-1}$) | | Water sample |
| Cd | | 43.6±11.86 |
| As | | 5.4±3.9 |
| Cu | | 2.3±0.65 |
| Zn | | 4.50±1.76 |

Values are means of three replicates ± SD

field samples were correlated with ($p<0.01$ for Cd, Cu and Zn) and ($p<0.05$ for As). This means that *S. Platensis* was effective for metals and metalloid accumulation.

## Cadmium (Cd)

Cd concentrations in water showed constant accumulation by *S. Platensis* during the field and laboratory experiments. The accumulation Figs. 2 and 6 and total amount of Cd removed from the contaminated water after 90 d of the experiment was 98.68 µg/g of rehydrated biomass compared with 58.9 µg/g dry biomass. The initial accumulation of Cd in *S. Platensis* was 9.78 µg/g dry wt., which increased to 14.95 µg/g rehydrated dry wt. by the end of the experiment. Only a small amount (55%) of the Cd was removed from the water [9, 20]. Cd accumulation in *S. Platensis*

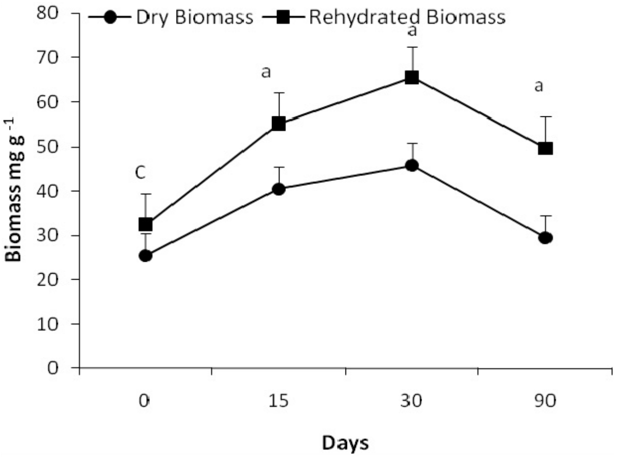


Fig. 1. The effect of wastewater applied on *S. platensis* of biomass (g) at different exposure days. All the values are means of three replicates ± SD, as compared to (C) control.

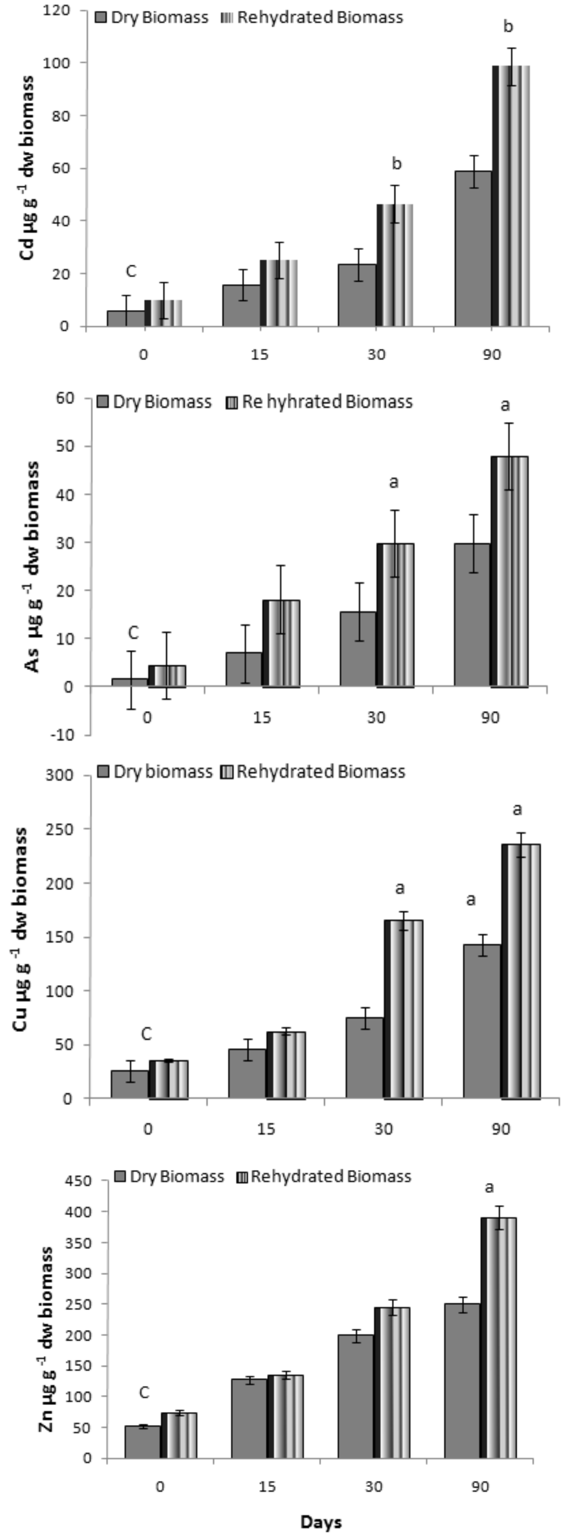


Fig. 2-5. Accumulation of metals (µg/g dw) in *S. platensis* growing wastewater in various days in laboratory. All the values are mean of three replicates ± SD. a=$p<0.01$ for Cd, Cu and Zn, b=$p<0.05$ for As, compared to (C) control.

increased over the course of the field experiment. The highest Cd concentrations in *S. Platensis* were 450- and 300-times higher than the initial concentrations, respectively. Cd concentrations are increased from 9.45/15.8 μg/g of dry biomass, which increase 25.29/25.15 μg/g in rehydrated biomass in laboratory/field samples after 15 d experiment. Background Cd levels in terrestrial vascular plants range from 0.2 to μg/g 50 μg/kg, and Cd levels of 5-10 mg/kg in plant tissue are reported to be phytotoxic [36]. Cd levels of 5-10 mg/kg in plant tissue are reported as phytotoxic [37]. These levels indicate that *S. Platensis* possesses a tolerance to Cd. Moreover, the uptake rates of Cd by *S. Platensis* in the laboratory and field experiments were 4.67/9.78 μg/g dry biomass and 10.58/14.95 μg/g/d, respectively. The bioconcentration factors (BCF: ratio of the total concentration of Cd in the whole algae to that in the growing solution [28]) calculated for *S. Platensis* in the laboratory and field experiments were (BCF=90) and (BCF=536), respectively. The differences in Cd concentrations and BCF values between the two experiments may reflect the shorter duration of the laboratory and field experiments.

Highest Cd concentrations in the roots of *Achillea ageratum*, *Plantago lanceolata*, and *Silene vulgaris* were 338, 1,150, and 249 mg/kg dry wt., respectively, while those in the leaves were 1,367, 569, and 1,163 mg/kg dry wt. High concentrations of Cd in *Dittrichia viscosa* (1136 mg/kg dry wt.) [23]. Although were found Cd concentrations are much higher than those obtained in the present laboratory experiment, the BCF values are similar. In addition, the BCF value obtained in the present field experiment is much higher than those obtained for the other plants. This result indicates that *S. Platensis* has great potential for phytoremediation of Cd [16].

The uptakes of Cd were three aquatic plants growing in ponds contaminated by metal mining. Cd concentrations in the aboveground parts of *Typha latifolia*, *Scirpus sylvaticus*, and *Phragmites australis* were 15, 19, and 15 μg/g dry wt., respectively; those in the underground parts were 1,300, 100, and 320 μg/g dry wt. [12]. The results obtained in the present study indicate that *S. Platensis* is inferior to *T. latifolia*, *S. sylvaticus*, and *P. australis* in terms of accumulating Cd; however, the BCF values obtained for these aquatic plants were approximately (BCF=183), (BCF=17), and (BCF=47), respectively, lower than the BCF values obtained for *S. Platensis* in the present field experiment (BCF=447). This result indicates that *S. Platensis* has greater potential than these aquatic plants in terms of accumulating Cd [2, 16, 20].

## Arsenic (As)

The average accumulation of As shown in Figs. 3 and 7 by *S. Platensis* was in field sample 1.56/9.45 compared with laboratory samples 1.15/5.34 μg/g/d; therefore, total As removed from contaminated water after 30 d was 23.5/46.5 rehydrated biomass compared with 9.45/18.34 μg/g dry laboratory biomass [4, 8]. In the field experiment, As metal accumulations in *S. Platensis* were higher than those in the laboratory experiment. Moreover, As concentrations in *S. Platensis* were higher in rehydrated biomass than those in the dry biomass. The highest concentrations of As in field and laboratory samples of rehydrated/dry biomass after 90 d were 47.98/29.86 and 27.43/15.95 μg/g dry wt., respectively; the BCFs values were (BCF=135) and (BCF=2,155). The As concentrations and BCF values in the field experiment were higher than those in the laboratory experiment.

As concentrations in the aboveground parts of *Equisetum fluviatile*, *T. latifolia*, *S. sylvaticus*, and *P. australis* were 1.5, 5.8, 13, and 11 μg/g dry wt., respectively; those in underground parts were 22, 850, 580, and 270 μg/g dry wt. We monitored the removal of As by water hyacinth and lesser duckweed (*Lemna minor*), for a concentration in water of 0.15 mg As/L [8, 13, 17]. The concentration of As in *S. Platensis* and *L. minor* was 1.8 and 2.5 mg/kg, respectively, after 21 d of the experiment. The author suggested that *S. Platensis* must be harvested every 15 days to avoid the release of As into the water. We assessed the ability of *Lepidium sativum* to uptake As from solutions containing various levels (<0.01-0.8 mg/l) of As [26]. The As concentration in plant shoots ranged from 0.02 (control) to 106 mg/kg dry wt. after 8 days. We conducted some investigation of As accumulation in duckweed (*Spirodela polyrhiza* L.) by cultivating the plant in a culture solution containing 0.02 μM $PO_4^{3-}$ and 4.0 μM arsenate or dimethylarsinic acid [2, 21]. The results revealed that the plant accumulated 0.353 μmol/g dry wt. of As on exposure to 4.0 μM arsenate and 0.02 μM $PO_4^{3-}$, and 0.27 nmol/g dry wt., when exposed to dimethylarsinic acid. The results obtained in the present study indicate that *S. Platensis* is superior to the plants assessed [22, 26, 27] in terms of As uptake.

## Copper (Cu)

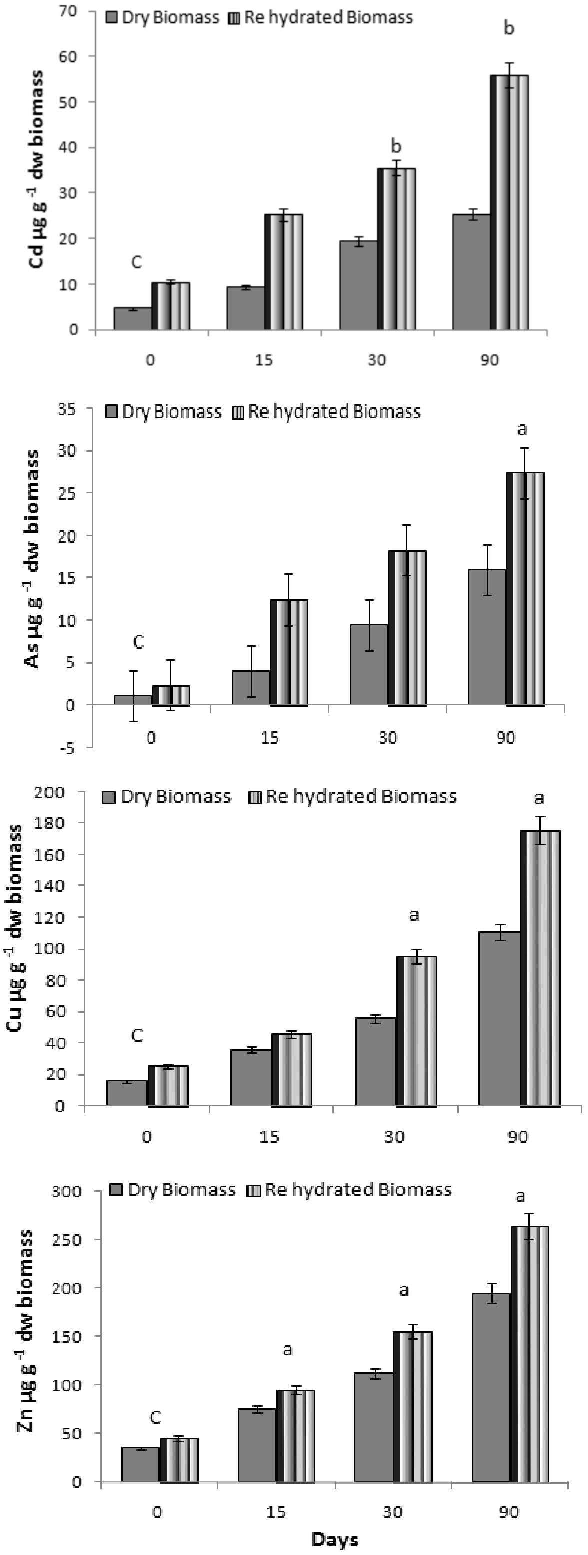
The removal of Cu in *S. Platensis* was 15.78/25.55 μg/g/d dry wt. in laboratory samples, which increased to 110.75/175.45 μg/g dry wt. after 90 days. The accumulation of Cu shown in Figs. 4 and 8 and amount of removed Cu (88%) from water utilized by *S. Platensis* was higher than that of Cd and As; the remainder (12%) may have been precipitated in other compounds [9, 27, 38]. In the field experiment, Cu concentrations in rehydrated sample were higher than those in dry biomass. The highest concentrations of Cu in *S. Platensis* after 90 d were 253.86 in rehydrated and 143.28 μg/g dry wt., respectively; BCF values were 34,200 and 62,300. The significantly higher BCF values of Cu than those of Cd and As may be due to the lower initial concentrations of Cu in water than those of Cd and As. Cu is an essential micronutrient for normal plant metabolism, playing an important role in plant growth [14, 20, 22, 28, 29] and in a large number of metalloenzymes and phytosynthesis-related membrane structure [36].

Aquatic macrophytes are noted for their ability to remove Cu from contaminated water: parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*), and water mint (*Mentha aquatic*). After 21 days, Cu concentrations were 304, 848, and 314 mg/kg dry wt. for parrot feather, creeping primrose, and water mint, respectively, corresponding to average removal rates of 0.16, 0.21, and 0.14 mg/l/day. Water hyacinth assessed the phytoaccumulation of Cu from water containing 10 mg Cu/l. The Cu concentrations in shoots and roots were 130 and 2800 mg/kg dry wt., respectively, after 14 days of the experiment [37, 38]. The removals of Cu were by three aquatic macrophytes (*Pistia stratiotes* L., *Spirodela polyrrhiza* and *S. Platensis*) [30]. The plants were grown in water with Cu concentrations of 1.0, 2.0, and 5.0 mg/l over 15 days. The highest concentrations of Cu accumulated in the three plant species were 0.875, 0.186, and 2.75 µg/g dry wt., respectively. The ability was of *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. to remove Cu (added as $CuSO_4$) from contaminated water. The maximum concentrations were of Cu in *P. pectinatus* and *P. malaianus* after 2 h of hydroponic treatment were 1,130 and 945 mg/kg d wt., respectively [29]. It appears that *S. Platensis* in the present study is good accumulator to the plants assessed by in terms of metal uptake compare with previous studies [2, 9, 24, 25, 30, 31]. The BCF values calculated for the aquatic plants assessed in previous studies were 55-152, 138-1,000, 147, 93-876, and 191-228, respectively, which are much higher than the values obtained for *S. Platensis* in the present study [14, 20, 22, 28, 29].

## Zinc (Zn)

The uptake of Zn shown in Figs. 5 and 9, and *S. Platensis,* was rehydrated/dry biomass samples 264.65/195.55 µg/g dry wt. in laboratory samples, which increased to 390.65/249.67 µg/g dry wt. after 90 d of field samples. The total amount of Zn removed from contaminated water after 90 d of the present rehydrated field experiment was 390.65 µg/g dry wt., in which the initial concentration of Zn in *S. Platensis* was 73.95 µg/g dry wt. over the course of the experiment. The amount of Zn removed from the contaminated water was slightly higher than that accumulated by plants (95%). The concentration of Zn in rehydrated biomass was higher than that in dry biomass in field and laboratory experiments. In the laboratory and field experiments, the uptake rates of Zn in rehydrated/laboratory were 35.65/45.65 and 73.95/52.48 µg/g dry wt/d, respectively; BCF values for *S. Platensis* were 32,500 and 95,300. Significantly higher BCF values of Zn than those of Cd and As may be due to the initial concentrations of Zn, which is low in water compare with Cd and As. The higher concentrations of Zn in comparison with those of Cd, As, and Cu in the present study may reflect the fact that Zn is an essential micronutrient for plant growth [12, 24, 32], and has high mobility and transport; therefore, among heavy metals in plants, the highest content was usually determined for Zn [32, 38-40].

The removal of Zn by three aquatic macrophytes (*P. stratiotes* L., *S. polyrrhiza* and *S. Platensis*) grown for 15 days in water with Zn concentrations of 1.0, 2.0, and 5.0 mg/L [30]. The highest concentrations of Zn accumulated in the plants were 0.98, 1.5, and 6.51 mg/g d wt., respectively. The ability was *P. pectinatus* L. and *P. malaianus* Miq. to remove Zn from contaminated water, reporting maximum concentrations of Zn in the plants of 1,320 and 1,230 mg/kg dry wt., respectively, after 2 h of treatment [24]. The uptake and distribution of Zn after 60 d by 19 wetland plant species growing in water with Zn concentrations of 0.5, 2.0, and 5.0 mg/L. Zn concentrations in whole



Figs. 6-9. Accumulation of metals (µg/g dw) in *S. platensis* growing wastewater in various days in fields. All the values are means of three replicates ± SD. a=$p<0.01$ for Cd, Cu and Zn, b= $p<0.05$ for As, compared to (C) control.

plants showed a wide range from 47.3 (*Phragmites communis* Trin.) to 349 mg/kg d wt in (*Isachne globosa* (Thunb.) Kuntze) [32]. The present results indicate that *S. Platensis* is superior to the other 15 plant species assessed by other researchers [29, 35, 38-40]. This result possibly reflects the fact that Zn concentrations in the water used in the present laboratory and field experiments were much higher than those in previous studies. The BCF values calculated by other researchers for the aquatic plants were 20-54, 196-3,260, 223-240, and 9.4-698, respectively, which are much higher than the values obtained for *S. Platensis* [24, 29, 32, 34, 35, 40]. This result suggests that *S. Platensis* has great potential for accumulating Zn from contaminated water.

## Conclusions

In conclusion, the present study, the aquatic algae *S. Platensis*, which is easily cultivated biomass and controlled, well adapted to contaminated environments, was tested for its ability to accumulate Cd, As, Cu, and Zn from contaminated water in both laboratory and field experiments. The results demonstrate that *S. Platensis* stands out as a good accumulator and phytoremediation of Cd, As, Cu, and Zn. The establishment of a more efficient, natural, and economic cyanobacteria-based system is also required with the aim of making optimal conditions for the successful phytoremediation of metals from contaminated water.

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