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# Duckweed as heavy metal accumulator and pollution indicator in industrial wastewater ponds

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## ABSTRACT

Bioaccumulations of the four heavy metals Cr, Cu, Pb and Zn in *Lemna gibba* (duckweed) as an environmental indicator of contaminated industrial wastewater were detected. Plant pigment content (chlorophyll and carotenoids) were estimated. During the study, heavy metals were ranked according to the preference for bioaccumulation by *L. gibba*, Zn came in the first place followed by Cr, Pb and Cu with bioaccumulation factors 13.9, 6.3, 5.5 and 2.5 respectively. The chlorophyll and carotenoid content in *L. gibba* fronds were altered by the bioaccumulation of heavy metals showing a substantial change in colour from green (lowest degree of bioaccumulation) to pale green (high bioaccumulation) and then degreened (maximum bioaccumulation) fronds. As the bioaccumulation of heavy metals increased in fronds, chlorophyll a content decreased, chlorophyll b content increased and the carotenoids became greater than chlorophyll (a + b) content especially in the pale green fronds. Zinc content in fronds showed greater negative correlations with chlorophyll a, chlorophyll b and total chlorophyll followed by Cu, Pb and Cr. Alternatively, Cr and Cu contents were mostly positively correlated with carotenoid content in *L. gibba* fronds. The accumulation of higher contents of heavy metal content in *L. gibba* than in wastewater samples indicates its phytoremediation potentialities. The visual change in colour of fronds from green to pale green and the degreening accompanied by the increase in heavy metal pollution nominate the species as heavy metal accumulator and pollution indicator.

*Keywords*: Pigments, Chlorophyll, Carotenoids, Degreening, Phytoremediation, Arid regions

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## 1. Introduction

Plant materials exhibit a great potential as biosorbents for the removal of water pollutants. Unlike organic pollutants in water, heavy metals are not degraded through biological processes and their removal is required for water remediation [1,2]. Competing with chemical and traditional treatments, heavy metal ions can be removed by an inexpensive alternative using biological materials such as flowering plants and microorganisms [3,4]. Phytotechnologies have been used for removal of heavy metals from contaminated wastewater [5–7]. The fact that heavy metal content in macrophytes is usually significantly larger than in the wastewater pond led investigators to be interested in their use as biological filters (bioaccumulators) for wastewater and as biomonitors (bioindicators) of heavy metal levels [e.g. 8,9].

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Aquatic plants play an important role in maintaining the purification capability of water and the entire aquatic ecosystem [10]. In the field of ecotoxicology, *Lemna* spp. (duckweed) have been used for the removal of heavy metals from wastewater and constructed wetlands [11,12]. These species present the additional advantage of growing under varied climatic conditions with rapid growth rates [13,14]. Because duckweed is easily raised even in the laboratory, the possible culturing for use as animal feed and for human consumption was also studied [15,16].

*Lemna gibba* (duckweed) is a small-sized freshwater floating macrophyte from the family *Lemnaceae*. The individual plant consists of a leaf-like structure (frond) connected to a fine rootlet [17]. The species inhabits stagnant to gently flowing surface waters, reproduces mainly vegetatively and from seeds only under stress conditions [18]. Plants of *L. gibba* are commonly cultured for wastewater treatment in the Mediterranean climate [19]. The aim of the present work was to investigate the potential use of the visual change in frond colour, due to pigment change, of *L. gibba* occurring in industrial wastewater as an indication of heavy metal pollution.

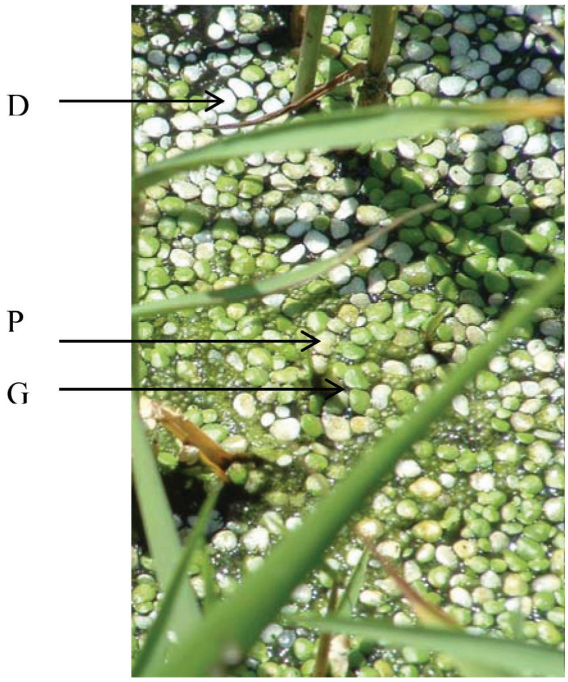

Fig. 1. *Lemna gibba* growing in the study site. G = green plants, P = pale green plants and D = degreened plants.

## 2. Materials and methods

### 2.1. The study site

Plant samples were collected from the industrial wastewater pond of Sadat City, located about 75 km west of Cairo. Plant samples were collected from one population consisting of green, pale green and degreened fronds (Fig. 1). Control *L. gibba* frond sample was collected from a nearby fresh water canal at Menoufia Governorate.

### 2.2. Sample collection

Water samples were collected in pre-acid washed polyethylene bottles of one liter capacity each at 10 cm below the surface water using a silicon/Teflon water pump. Plant samples were collected from the surface of the wastewater pond using a long handed mesh sampler. Water and plant samples were collected (five replications) and preserved in cool ice boxes throughout the field trip and during transportation to the laboratory.

Immediately after returning to the lab, water samples were filtered through 0.45 μm pore diameter membrane filters. The filtrates were stored frozen at –4°C. All plant parts were rinsed three times in de-ionized water, oven dried at 65°C, grounded into powder and stored till further analysis.

### 2.3. Heavy metal analysis

Heavy metal content in water samples was measured in filtrates after a preconcentration–complexation step using (2%) ammonium pyrolodin dithiocarbamate (APDC), solvent extraction into methyl isobutyl keton (MIBK) followed by back extraction in nitric acid [20]. For plant materials, 0.2 g of fronds powder was digested according to the method described by [21]. Determinations of heavy metals in all samples were carried out by Varian inductively coupled plasma atomic emission spectroscopy (ICP–AES). The preference for bioaccumulation of heavy metals by *L. gibba* was estimated using the bioaccumulation factor (BF, ratio of heavy metal content in plant tissue compared to that in water) according to [22].

### 2.4. Pigment analysis

One gram of plant sample was taken from each *L. gibba* sample and soaked separately in 50% acetonitrile (10 ml). After vortex mixing, the samples were filtered in micro filter (45 μm). Pigments were analyzed using a high performance liquid phase chromatography (HPLC) system (Hp 1050) with a UV detector at 220 nm. The separation was accomplished with a ODS, C18 (5 μm 4 × 250 mm) column. The mobile phase consisted of eluent acetonitrile/water (70/30 v/v) to (95/5 v/v) through 5 min. The flow rate was 1 ml /min. The column temperature was 27°C with an injection volume of 10 μl. This analysis was carried out in the Biotechnology Unit of Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

### 2.5. Data analysis

Data were analyzed by one way ANOVA and the Post Hoc tests (LSD and Duncan) were used to determine the least significant differences among the mean values. Pearson correlation coefficient was calculated to test the relationships between the content of heavy metals and pigments in plants.

## 3. Results

### 3.1. *Heavy metals*

Fronds of *L. gibba* grown in the wastewater accumulated Cr, Cu, Pb and Zn from contents of 7.5, 26.5, 20.4 and 121.7 μg/L, respectively (Figs. 2 and 3). Compared to the wastewater, the control water contained significantly lower contents of Cr (2.8 μg/L) and Pb (6.63 μg/L) while only trace amounts of Cu or Zn where detected (Fig. 2). Fronds of *L. gibba* grown in the control water showed the lowest bioaccumulation of heavy metals (Fig. 3). Generally, the bioaccumulation of heavy metals in *L. gibba* fronds obtained from the wastewater increased gradually but significantly from green to pale green then degreened plants. The largest differences in heavy metal bioaccumulation were found between the control and the degreened plants for Cr (6.5 μg/g in control compared to 47.5 μg/g in degreened plants), Cu (13.5 μg/g in control compared to 66 μg/g in degreened plants), Pb (112.5 μg/g as compared to 9 μg/g in the control plants) and Zn (70 μg/g in control compared to 1699.3 μg/g in degreened plants).

In the wastewater *L. gibba* fronds, the bioaccumulation factor (BF) increased with time from green, pale green to degreened fronds, for all study heavy metals (Fig. 4). Maximum BF values were reached in the green, pale green and degreened fronds for Zn with values of 6.75, 9.24 and 13.96 respectively. This was followed by Cr (BF = 1.93, 3.33 and 6.33) and Pb (BF = 1.18, 4.65 and 5.52) in the green, pale green and degreened fronds respectively. Minimum BF values were obtained in all plant samples for Cu attaining its highest value in the degreened plants (BF = 2.49) which was comparatively the lowest among other heavy metals.

### 3.2. *Pigment content*

Comparison of chlorophyll a and b contents in *L. gibba* fronds revealed significantly greater content of chlorophyll b than a in the wastewater samples especially for pale green fronds attaining 0.99 mg/g dry weight for chlorophyll b and 0.42 mg/g dry weight for chlorophyll a (Fig. 5a). Alternatively, chlorophyll b content (1.60) was lower than chlorophyll a (1.93) in the control samples. Fronds of *L. gibba* obtained from the wastewater contained significantly lower chlorophyll (a and b) contents than control fronds. The contents of chlorophyll (a and b) comparatively decreased as the bioaccumulation of heavy metals increased attaining 0.20 and 0.36 mg/g dry weight in the degreened plants.

The carotenoid content increased with the increase in the bioaccumulation of heavy metals in *L. gibba* fronds

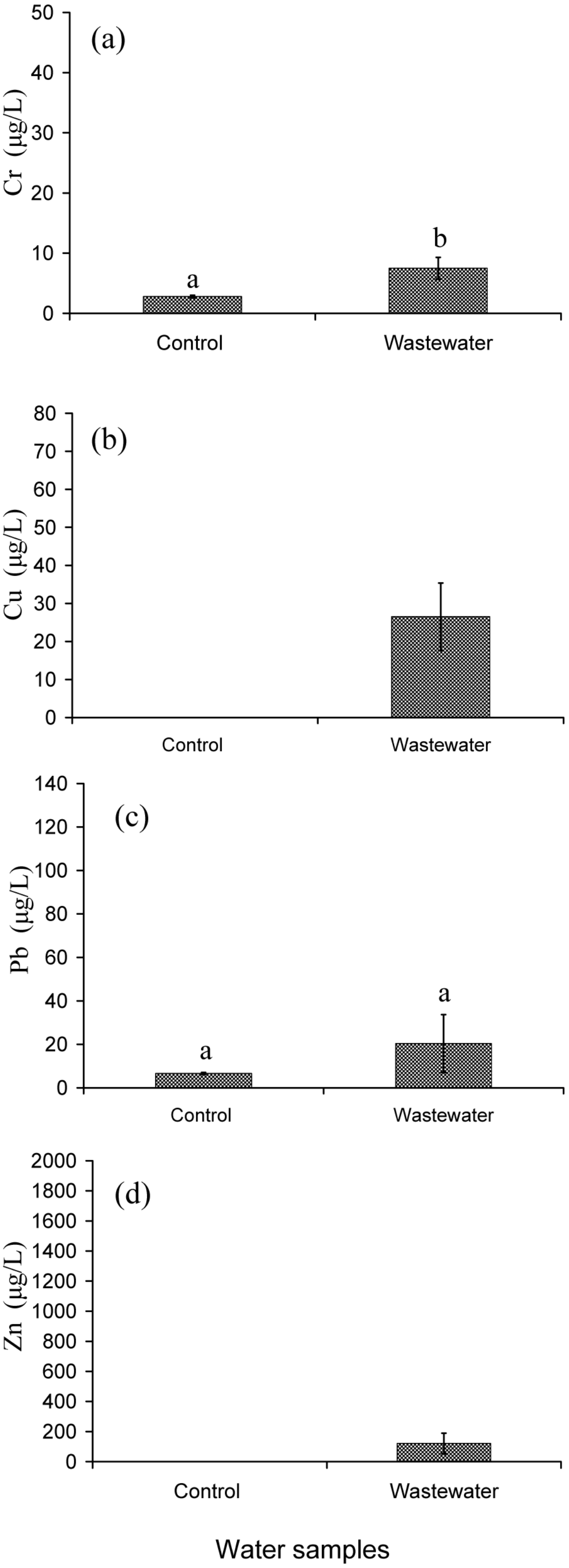

Fig. 2. Heavy metals contents in water samples (μg/L). Error bars represent the standard deviations of the means. Different letters, on top of the bars indicate significantly different values between control and wastewater samples ($P \leq 0.05$).

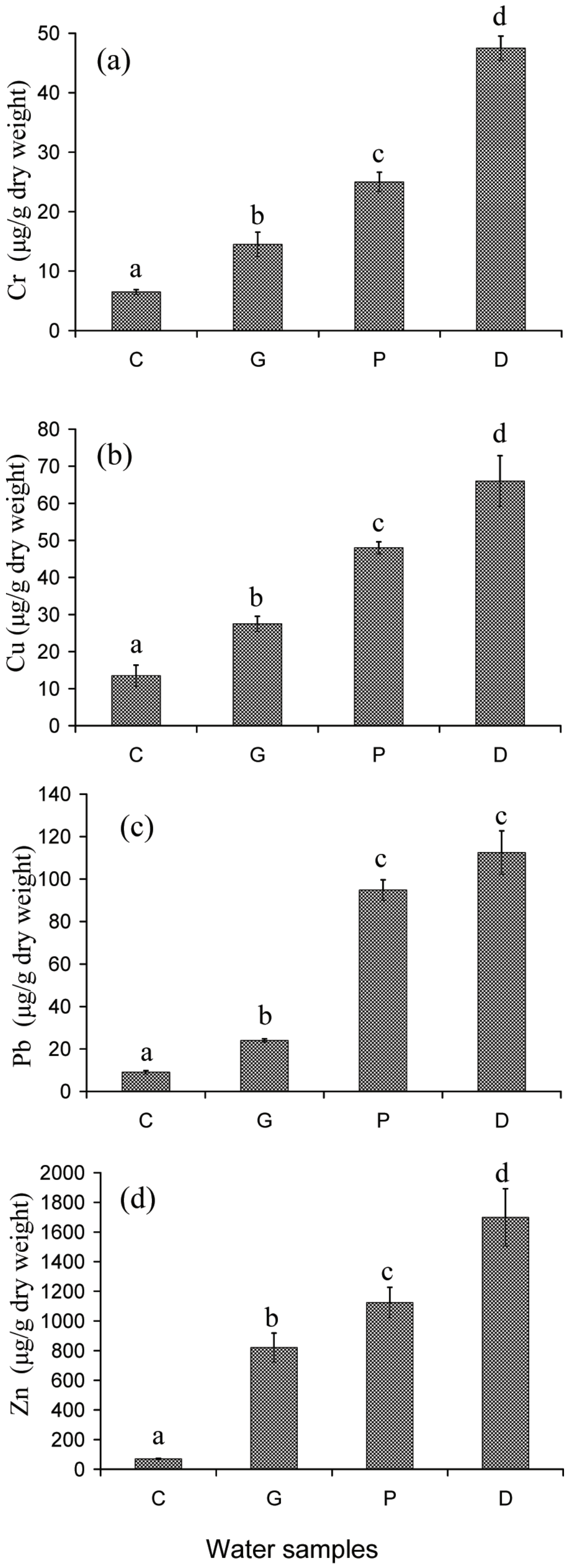

Fig. 3. Heavy metals contents in *Lemna gibba* dry matter (µg/g). C = control plants, see legend of Fig. 1 for G, P and D. Error bars represent the standard deviations of the means. Different letters, on top of the bars indicate significantly different values between plant samples ($P \leq 0.05$).

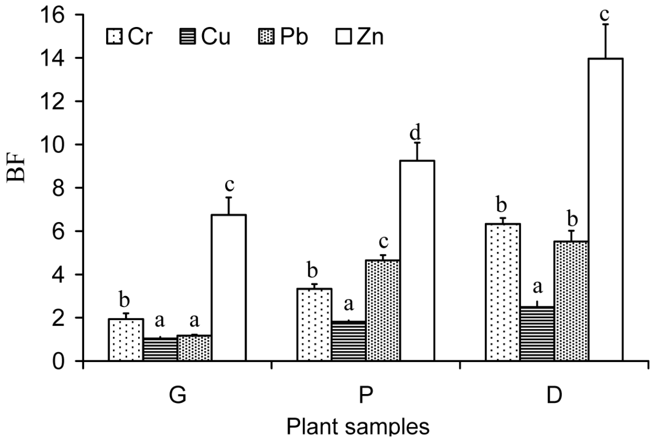

Fig. 4. Bioaccumulation factor (BF) for heavy metals accumulation by *Lemna gibba* fronds raised in wastewater. See legend of Fig. 1 for G, P and D. Error bars represent the standard deviations of the means. Different letters, on top of the bars, in the same plant sample indicate significantly different BF values for the heavy metals ($P \leq 0.05$).

reaching 2.69 mg/g dry weight in the pale green fronds compared to 1.97 mg/g dry weight in the control, whereas degreened fronds attained significantly lower carotenoid content (0.92 mg/g dry weight) than other plant samples (Fig. 5b). Similarly, as the bioaccumulation of heavy metals increased, the carotenoid content in the wastewater fronds became greater than chlorophyll (a + b) content especially in the pale green fronds reaching 2.69 mg/g dry weight content of carotenoids compared to 1.41 mg/g dry weight content of chlorophyll (a + b). The contrary was observed for the control fronds where significantly greater content of total chlorophyll (3.54 mg/g dry weight) was obtained compared to 1.97 mg/ dry weight of carotenoids.

### *3.3. Heavy metal–pigment content relationship*

The correlation between heavy metal contents and pigment contents in *L. gibba* fronds revealed negative relationships with chlorophyll contents and positive relationships with carotenoid content for all study heavy metals (Table 1).

The decrease in total chlorophyll content was mainly associated with the increase in contents of Zn, Cu and Pb reaching correlation coefficients of –0.99, –0.97 and –0.92 respectively. Chlorophyll a degradation was mostly associated with the increase in Zn contents while chlorophyll b degradation was influenced by the increase in contents of Cr, Cu, Zn and Pb.

The degreened stage was excluded from the correlation between heavy metal and carotenoid contents; otherwise weak correlations were obtained (data not shown). Apart from Zn and Pb, Cu and Cr contents where significantly correlated with the increase in carotenoid

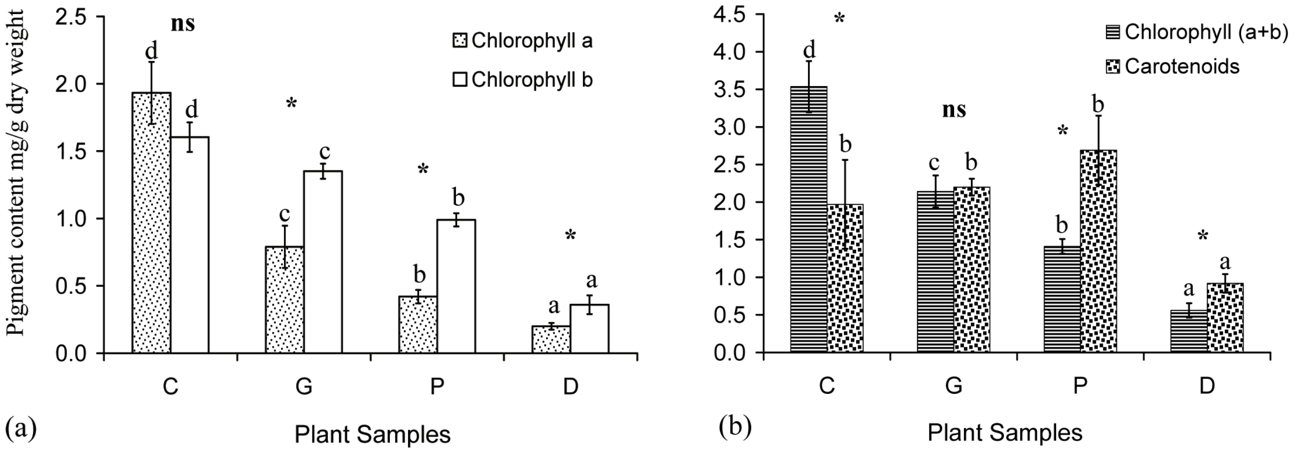

Fig. 5. Pigment content (as mg/g dry weight) of control and wastewater *Lemna gibba* plant samples. C = control plants, see legend of Fig. 1 for G, P and D. Error bars represent the standard deviations of the means. Different letters, on top of the bars, indicate significantly different values between plant samples, for the same pigment ($P \leq 0.05$). An asterisk indicates significant difference in the same plant sample for different pigments, and ns = non significant.

Table 1
Correlation between the contents of heavy metals and pigments in *L. gibba* fronds. Asterisks indicate significance at $P \leq 0.05$

| | Chlorophyll a | Chlorophyll b | Chlorophyll (a+b) | Carotenoids |
|---|---|---|---|---|
| Cr | –0.83 | –0.99* | –0.93 | 0.99* |
| Cu | –0.91 | –0.98* | –0.97* | 0.99* |
| Pb | –0.83 | –0.93* | –0.92* | 0.98 |
| Zn | –0.96* | –0.96* | –0.99* | 0.90 |

content in *L. gibba* fronds attaining correlation coefficients of 0.99 and 0.98 respectively.

## 4. Discussion

Bioaccumulation of heavy metals in *Lemna gibba* increased significantly from green to pale green and degreened fronds. The chlorophyll and carotenoid content in *L. gibba* fronds were altered by the bioaccumulation of heavy metals showing a substantial change in colour from green (lowest degree of bioaccumulation) to pale green (high bioaccumulation) and then degreened (maximum bioaccumulation) fronds. As the bioaccumulation of heavy metals increased, the carotenoid content in the wastewater fronds became greater than chlorophyll (a + b) content especially in the pale green fronds. Decrease in chlorophyll content is considered a sensitive indicator of photoinhibition that may be associated with change in carotenoids according to the study species [23,24].

Macrophytes have been found to be highly effective for recognizing and predicting heavy metal stress in aquatic environments showing toxicity symptoms (i.e. could serve as bioindicators) especially when morphological (visual) alterations were induced [25,26]. Visual symptoms of toxicity in *L. gibba* were mainly manifested by the sharp decline in chlorophyll content in response to increased heavy metals bioaccumulation and the greater chlorophyll b content than chlorophyll a in the wastewater fronds. Photochemical reactions were reported to be inhibited in bioindicator plant species used for remediation of chemical contaminants [27]. Degradation of chlorophyll as a result of heavy metal toxicity has also been reported and a possible explanation is the disorganization of chloroplasts ultrastructure causing decreased chlorophyll content and photosynthetic rate [28–31]. In duckweeds at greater heavy metal contents, reduction in growth, chlorophyll content, disorganized thylakoids and formation of many vesicles in the chloroplast were observed [32,33]. Moreover, alteration of gene expression was also detected in *L. gibba* due to acute copper exposure [34].

Several factors may influence the amount of heavy metals bioaccumulation in macrophytes as the antagonism with other metals [35], or occurrence of organic and chemical pollutants in the wastewater [36]. Similarly, the same species may accumulate specific heavy metals more than the others [37]. By comparing the content of the study heavy metals in the industrial wastewater with the corresponding maximum bioaccumulation, i.e. in the degreened fronds; bioaccumulation factor of about 6.3, 2.5, 5.5 and 13.9 were estimated for Cr, Cu, Pb and Zn respectively. Consequently, if these heavy metals were ranked according to the preference for bioaccumulation by *L. gibba*, Zn would come in the first place followed by Cr, Pb and Cu. Furthermore, Zn content in fronds showed highly negative correlations with chlorophyll a, chlorophyll b and total chlorophyll followed by Cu, Pb and Cr. On the other hand, Cr and Cu contents were mostly positively correlated with carotenoid content in fronds.

Degreening indicated the attainment of maximum bioaccumulation capacity in *L. gibba* where fronds reached a saturation state after which they usually die. The decrease in content of chlorophyll a associated with the increase in content of chlorophyll b and carotenoids are considered as a structural damage occurring in the yellow leaves of the succulent desert species *Zygophyllum* spp. as a visual indication of the senescence syndrome [38]. These changes may be accelerated by the continuous exposure of *L. gibba* to heavy metal contamination. However, degreening in the algal species *Chlorella fusca* (loss of photosynthetic pigments and synthesis of secondary carotenoids) was reported to be temporary occurring under conditions of nitrogen starvation [39]. On the other hand, degreening followed by death of individuals was observed for *L. gibba* as toxicity symptom to heavy metals [40] and for other macrophytes developed on wastewater [41].

In conclusion, *L. gibba* is a potential bioaccumulator and sensitive indicator for heavy metal pollution in industrial wastewater, however, a saturation state is attained after which plant fronds can no longer be useful for bioaccumulation and must be eliminated and replaced with new populations. Large scale experiment is needed for the evaluation of the effective use of the species and to detect the rate at which *L. gibba* loaded with heavy metals should be replaced by new healthy populations. The potential use of *Lemna* as a pollution indicator, and the analysis of the relation of metal bioaccumulation with chlorophyll and carotenoids are valuable environmental tools.

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