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ORIGINAL ARTICLE

Identification of the Mechanism Involved in the Removal Potetial of Textile Pollutants by the Aquatic Plant *Lemna gibba* L.

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ABSTRACT: Some studies asserted that some aquatic plants can remove organic pollutants by degrading them into their biomass. In this work, it was aimed to examine the ability of *L. gibba* L. to remove two dyes, DR-89 and VB-20 and then to elucidate the mechanism of removal. For this purpose, experiments were conducted at 21±1 °C, 12h photoperiod and pH of 6.1± 0.01. For the maximal dye concentration tolerated by the plants (50 mg L^{-1}), the results demonstrated that maximum dye abatement was determined to be about 53% (VB-20) and 23% (DR-89). In order to identify the mechanism of dye absorption, FT-IR, UV-vis and SEM analyses were conducted on the biomass and liquid phases. The results indicated that alcohol, alkene, phenol, and amine functions are involved in dye binding to the biomass surface without demonstrating any phytodegradation phenomenon. Additionally, the SEM analysis confirmed this result showing that the ventral lobe and the thin root of each frond (unlike the dorsal lobe) are colored via a direct interaction with the dye molecules allowing their absorption from the surrounding water. Thus, the invasive plant, *L. gibba* L. could remove organic dyes from contaminated mediums by accumulating them in the biomass without degrading them.

INTRODUCTION

In some countries where the environmental protection legislation is not strict and/or not respected, several industries using synthetic dyes in their manufacturing operations (paper, leather, cosmetic, food, printing and textile), discharge their effluents into the aquatic systems without efficient treatment which led to the subsequent increase in contaminant concentrations in the environment [1, 2]. Among the 10,000 tones/year of total dye consumption in the textile industry approximately 100 tones/year of dyes are released into water streams [3, 4]. These effluents, highly colored and heavily charged with pollutants can persist for long time and led to a variety of environmental and health problems [5-7]. It is thus imperative to develop an efficient method of treatment of polluted effluents in order to cope with this problem and ensure the protection of animal and vegetal biodiversity which constitutes one of the most important topics of the research area nowadays.

Considering treatment processes of contaminated waters, phytoaccumulation process of pollutants has been of great interest to numerous research laboratories as they look for effective and low-cost methods for cleaning the environment. In this process, plants are used as an agent having a natural capacity to absorb and accumulate mineral or organic chemicals present in a gaseous, liquid or solid phase. In the case of an organic pollutant, the

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absorption could be followed by its degradation into the biomass when the phenomenon is usually affected by enzymes and released metabolites by the plant itself [8-10]. Aquatic plants may be an interesting tool to be utilized in such processes since they have great capacity for the accumulation and degradation of chemical pollutants initially present in contaminated waters. As a first step, preliminary studies of contaminant toxic effects should be done in order to determine the tolerance of the macrophytes to these contaminants; this should provide basic information related to their potential use in water depuration [11].

Lemna gibba (*L. gibba*) is a floating weed belonging to the Lemnacea family. This species is found all over the world on the surface of standing or slow-flowing of nutrient-rich fresh and brackish waters and can propagate in anthropogenic wastewaters forming dense green carpets. The plant doubles its biomass in approximately two days under ideal conditions of nutrients availability, light, and temperature [12, 13]. Due to its abundance, simple structure, rapid growth, tolerance to different stresses and easy adaptation to various aquatic conditions, this aquatic species is very suitable for phytoremediation and biomonitoring studies [14].

The use of Lemnacea species for treatment of several mineral pollutants has been well documented [15-20]. However, available research studies for organic pollutant removal remained still limited. In fact, according to the recent review of [16], only 11% of the pollutants treated by these aquatic species concern organic pollutants. Under the present investigation, *L. gibba* was tested for its effectiveness at removing two toxic dyes in simulated contaminated waters at different concentrations. The main goal was to ascertain the mechanism involved in dye removal by the aquatic species using several characterization methods such as FT-IR, SEM and UV-vis Spectroscopy.

MATERIALS AND METHODS

Plant material

The duckweed *L. gibba* was obtained from a natural pond located in Northeastern Algeria (El-Tarf, Algeria). The plants were transported to the laboratory in plastic containers filled with pond water; the best fronds characterized by a green color with the presence of roots were used in phytoremediation assays.

Chemicals

Two textile dyes, Direct Red 89 (DR-89) and Vat Blue 20 (VB-20) provided by the Algerian textile industry located in the city of Constantine were selected as organic pollutants. Their physicochemical data were given in our previous study [21]. The stock solutions were prepared by dissolving 1 g of dye in 1 L of distilled water (pH=6.5, conductivity = 6.0 µS cm^{-1}) and stored in an incubator at 4°C. Just before the experiments, diluted solutions were prepared. All the other chemicals reagents of analytical grade (salts, acids, bases) used as received were purchased from Merck.

Phytoaccumulation assays

The experiments were carried out with healthy *Lemna* fronds which were gently placed in glass beakers of 7 cm high and 5 cm in diameter immersed in a thermostatic bath containing 100 mL of nutrient medium. The test protocols and the composition of the nutrient medium were given in our previous studies [19, 21]. The temperature was kept constant (21 ± 1°C) by using an immersion heater equipped with a bubbler and the pH of the solutions was adjusted to 6.1 ± 0.01 by using HCl or NaOH. Two incandescent lamps (40 W each) provided light for the plants during a photoperiod of 12 h/day. Five nominal initial concentrations of dye pollutant (10, 20, 30, 40 and 50 mg L^{-1} prepared from stock solutions were selected to assess the ability of *L. gibba* in dye accumulation. Twenty and forty *Lemna* fronds were added to solutions enriched with DR-89 and VB-20, respectively. Treatments and controls (without dyes) in triplicate were achieved after a 7-day exposure to pollutants.

Analytical methods

Dye solution

Samples were collected at regular interval (each day) from each beaker for analyzing dye absorbance with a SECOMAM Prim Light V9B S/N 2836 UV/Vis

Spectrophotometer at maximum absorption wavelengths of 495 nm (DR-89) and 580 nm (VB-20) in order to determine dye concentration. Additionally, the different samples were analyzed by a PerkinElmer FT-IR spectrophotometer (IRAffinity-1 model: PerkinElmer) from 4000 to 450 cm^{-1}. The percentage of removal efficiency PR (%) of the target compounds was calculated according to Eq. 1:

$$PR\,(\%) = \frac{\text{Concentration}_{\text{initial}} - \text{Concentration}_{\text{final}}}{\text{Concetration}_{\text{initial}}} \times 100\% \quad (1)$$

Spectroscopic characterization of plant Biomass

The nature of the mechanism involved in dye phytoaccumulation was studied using UV-vis and FT-IR spectroscopy. The content of absorbed dye in plant biomass was extracted with 100% acetone and centrifuged at 2000 rpm for 10 min. The supernatant was then recorded from 400 to 850 nm in UV-visible spectroscope. In parallel, other samples were prepared by mixing *Lemna* biomass with spectroscopy-grade potassium bromide for being analyzed by the PerkinElmer FT-IR spectrophotometer from 4000 to 450 cm^{-1}.

Surface morphology

Surface morphological analysis of *L. gibba* biomass before and after phytoaccumulation experiments were carried out using a Scanning Electron Microscope type AMETEK Materiel Analysis Division at a magnification of 1200x.

Statistical analysis

Three independent assays were performed for each dye concentration. Errors given in the figures represent standard errors of the means. The results were analyzed using a one-way analysis of variance (ANOVA). Comparison between control and treatments was statistically analyzed and the validity of investigation was expressed as a probability value of $p<0.05$.

RESULTS AND DISCUSSION

Dye removal from contaminated water

Preliminary toxicity tests allowed defining dye concentration tolerated by the selected plants. These tests showed that the duckweed species could survive in 50 mg L^{-1} of VB-20 and DR-89 without showing any visible symptom of toxicity (data not shown). This concentration was chosen as the maximal dye concentrations at which the phytoaccumualtion essays were conducted. Thus, the relationship between the initial pollutant concentration and the removal capacity of *L. gibba* was studied in the interval 10-50 mg L^{-1}. After 7 days of treatment, significant ($p < 0.05$) decrease in dye concentration was observed for all the tested concentrations compared with the corresponding controls (without dyes). Figure 1 shows that under the conditions of the present work, the removal of the textile dyes by *L. gibba* was most efficient, mainly in the case of VB-20, when dye concentration in water was low. This fact is due to availability of sufficient active sites for absorption of dye molecules. On the other hand, the same figure shows that DR-89 dye abatement from the contaminated solution was slightly low compared to VB-20. It seems that L. *gibba* showed quite different efficiency toward the two dyes (VB-20 > DR-89) that has to be related to their different chemical structures. The red dye (DR-89) has higher molecular weight which made it less attractable by absorption. The results expressed as percentage of removal efficiency (PR) revealed a satisfactory percentage dye efficiency of 74 and 30% for 10 mg L^{-1} of VB-20 and DR-89, respectively (Table 1). Several studies asserted that duckweed species were effective in bioremoving dyes present in mono-solute solution or in mixture [22-24]. The biological removal efficiency fluctuates from one study to another. Tôrôk *et al*. [25] found that *L. minor* L. was effective in eliminating 98% Malachite Green and 96% Crystal Violet at a concentration of 40 mg L^{-1}. However, Yaseen and Scholz [6] demonstrated that for dye mixture containing 2 mg L^{-1} RB 198 and 8 mg L^{-1} BR 46, removals were around 53%; this percentage decreased to 41% for the contaminated solution containing 5 mg L^{-1} of each pollutant. These

results confirm some relationships between plant phytoremediation efficiency and initial dye concentration.

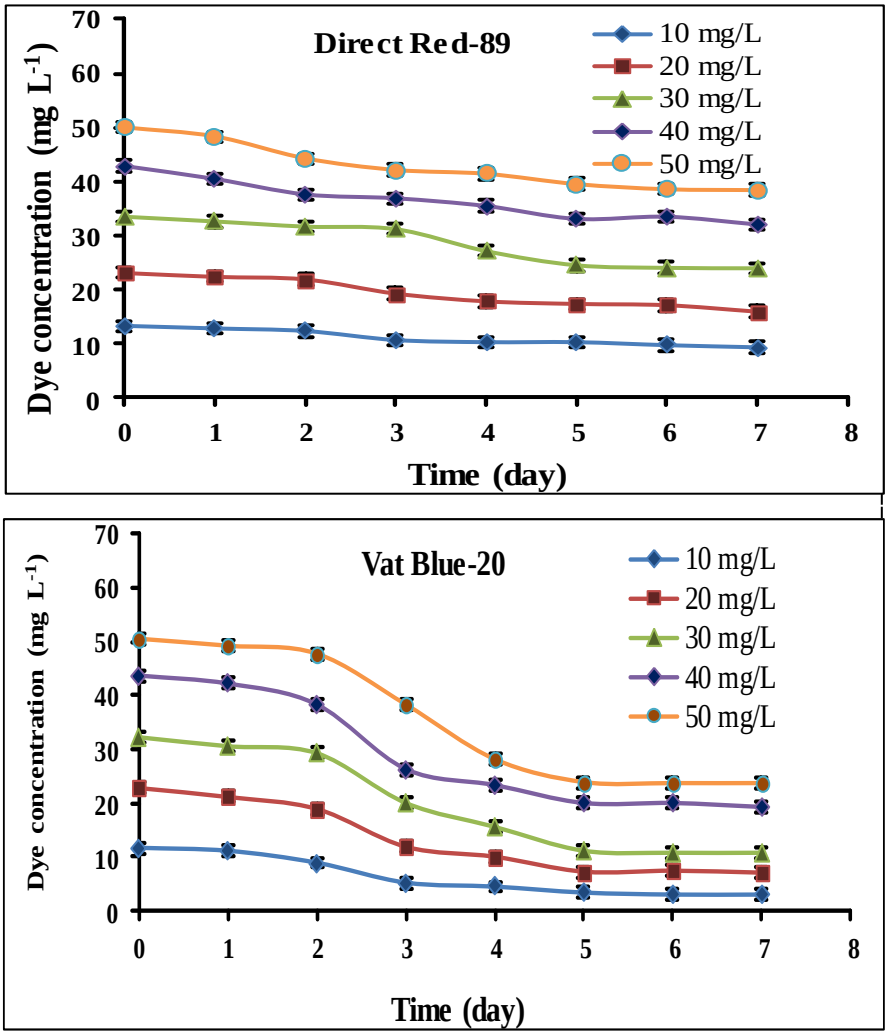

Figure 1. Removal of VB-20 and DR-89 by *L. gibba* at different initial concentrations (V=100 mL, pH=6.1±0.01, T=21±1°C, Time=7 days). Vertical bars indicate standard deviation, n=3

Table 1. Dye removal efficiency for different initial dye concentrations

Initial dye concentration (mg L^{-1})	Removal efficiency (%)	
	VB-20	DR-89
10	74.60	29.67
20	69.11	30.93
30	66.67	28.44
40	55.93	25.19
50	53.11	23.17

Removal mechanism

Some research works demonstrated that aquatic plants can remove textile dyes from contaminated mediums with phytodegradation mechanism. For example, *Lemna* species remove dyes and transform them into different intermediate compounds [17, 26-28]. Other researchers highlighted furthermore the synergistic effect between plants and microorganisms for the transformation of these xenobiotics into less harmful products [29]. Other studies however investigated the removal potential of hazardous dyes by some aquatic plants without elucidating the

phenomenon mechanism. In the present work, in order to examine the mechanism of dye removal by *L. gibba*, the content of plant biomass was extracted in acetone before and after dye exposure and the different spectral sweeps using UV-vis and FT-IR analysis were registered. As shown in Figure 2, the UV-vis spectra (A) representing dyes diluted in acetone exhibit a shift from 580 to 560 nm and from 495 to 480 nm in λ_{max} of VB-20 and DR-89, respectively. This is due to the dissolution of dye compound in acetone rather than in water. The two spectra (B) remained essentially unchanged from the UV-vis spectra (A) since the peaks of the two samples did not disappear suggesting that *L. gibba* accumulated the two pollutants in its biomass without phytodegradation [25]. The two peaks at 430 nm and 663 nm for both dyes correspond to the presence of chlorophyll; those peaks appeared in control spectra (C) (plants not exposed to pollutants).

Similarly, [30] proved that UV/Vis spectra analysis illustrated the decrease of a dye pollutant absorbance throughout 24 h of the test period and showed significant percentage of solution discoloration.

We conclude as a first result that the dye removal from water could be effected without significant structural change in dye molecules. To confirm this, FTIR analysis of both VB-20 and DR-89 was performed in the liquid phase before and after 7 days of treatment with *L. gibba* L. The results (not shown) indicate that the phytoaccumulation of the pollutants took place without modification of the molecular structure of the two dyes. Indeed, no change in the peaks of the spectra was recorded. Similar results have previously been reported for the phytoaccumulation of several dye pollutants. The fungi *A. niger* accumulated Blue Acid 161 and removed it from a contaminated medium without significant structural change in dye molecules [31]. *L. minor* also showed the potential in remove methylene blue dye with accumulation to be the prominent mechanism [32]. According to [33], transmission electron microscopic imaging of *T. angustifolia* (cattails) revealed the accumulation of Reactive Red 141 in tissues after 28 days of exposure.

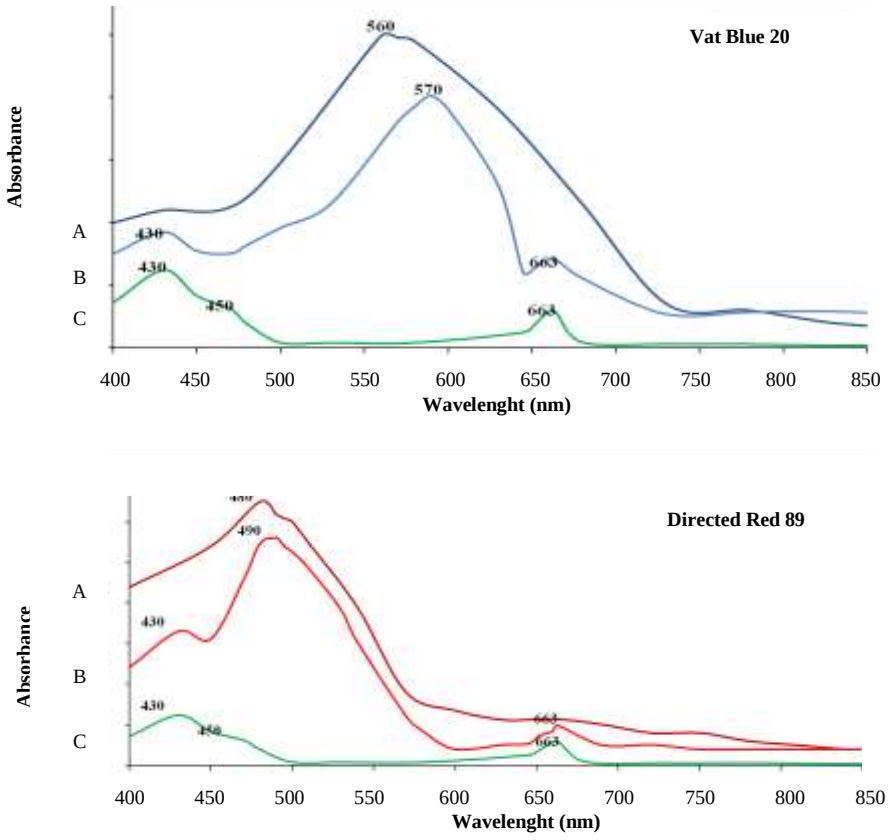

Figure 2. UV-vis spectra of (A) VB-20 and DR-89 diluted in acetone, (B) Phytoaccumulated VB-20 and DR-89 content extracted with acetone from *Lemna* biomass and (C) Photosynthetic pigments extracted with acetone from controls.

Further analysis was carried out by FT-IR spectroscopy on *Lemna* biomass to get information about the nature of dye binding mechanism. Figure 3 shows the spectra of *Lemna* biomass before and after dye exposure. In the case of DR-89, Figure 3a shows peaks at 1012, 1641 and 1408 cm^{-1} which are assigned to the stretching of C-OH of alcohol, C = C of alkene and the deformation of phenol functions. Those peaks shifted at high and low frequencies to 1016, 1658, 1406 cm^{-1} respectively after being exposed to dye pollutant for 7 days. In Figure 3b, the peaks assigned to the primary alcohol shifted at high frequencies to 1018 and 1045 cm^{-1} as well as the peaks obtained at 3369 and 3394 cm^{-1} which are assigned to the primary amine functions that are shifted to low frequencies at 3361, 3392 cm^{-1}. This suggests that alcohol, alkene, phenol, and amine are responsible for the binding of both dyes to the plant biomass. Similar works indicated that functional groups contained in dye molecules are bound to the aquatic plant surface. Reema et al. [32] showed, after FT-IR analysis, that C=O carbonyl, amine group N-H and P-O present in Methylene Blue dye are bound to L. minor surface.

Based on these results and according to previous works, it can be concluded that the removal of the two dyes can be explained by a the phytoaccumulation phenomenon that takes place in two steps: the first step is the passive stage (rapid elimination) when the dye adsorbed on the plant surface by electrostatic attraction and the second stage (step of gradual elimination) when the dye accumulated in plant biomass and transferred inside the cells [19, 31]. Finally, it can be concluded from this experimental study that the possible mechanism involved in removal of dyes

from contaminated water using *L. gibba* consisted of two-stage process: the biosorption of dye molecules leading to the binding of solutes to the plant biomass and then the phytoaccumulation of the molecules by the actively growing plants.

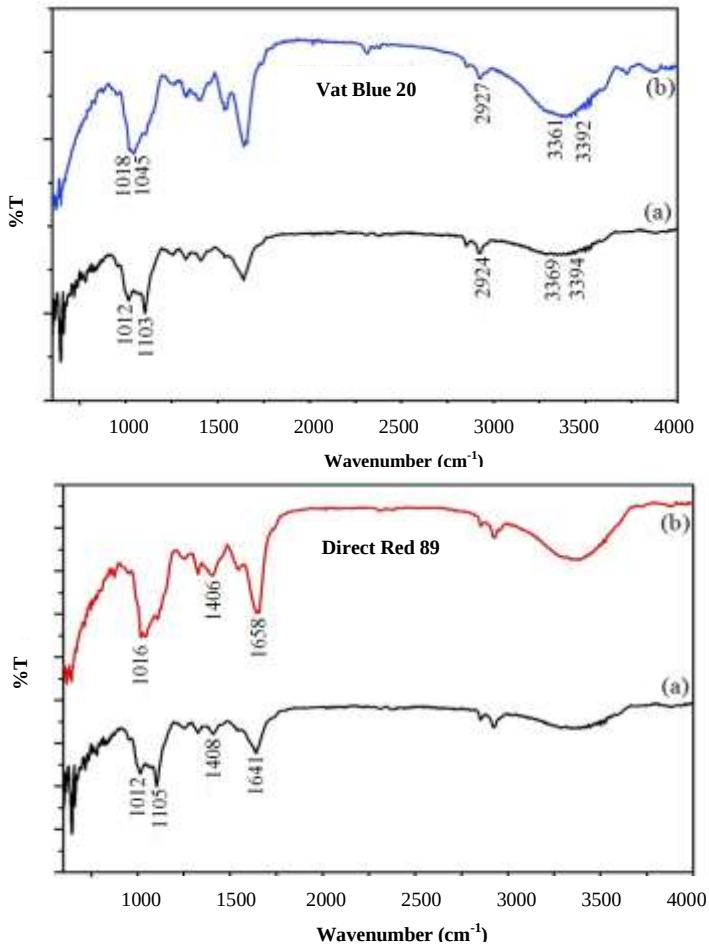

Figure 3. FT-IR spectra of *Lemna* biomass (a) after and (b) before 7 days of treatment with *L. gibba*.

Morphology and SEM imaging of plant biomass

The duckweed *L. gibba* possesses fronds formed of dorso-ventrally lobes. The aerial dorsal lobe contains chlorophyll pigments and the ventral lobe is colorless and partially submerged providing the buoyancy for the plant [34]. Morphological appearance of the fronds is depicted in Figure 4a. To identify the sites where dye molecules accumulated in plant tissue, the visual morphology of *Lemna* biomass was observed by electronic microscopy. From Figure 4a showing duckweed fronds before and after dye phytoaccumualtion, it can be observed that the dorsal lobe was not colored by the two dyes, because the cuticle surface makes it impermeable and repellent to the aqueous dye solution; however, the ventral lobe and the thin roots were colored in red (DR-89) and in blue (VB-20) because the cuticle is not present in the ventral lobe and root tissue avoiding contact with the dye molecules in water. The SEM micrograph provides a visual characterization of the plant tissues at a higher resolution. The morphology of the native plant (Figure 4b) is of rough appearance with a porous surface and pores of various shapes and diameters; these properties are considered as necessary for the phytoaccumulation capacity of the two dyes [32]. The morphology of the plant after phytoaccumulation of the two dyes was still rough with small pore enlargement due to the accumulated dye molecules on the plant tissue (Figure 4c, d). It is suggested that the dye-induced pores result in the increased uptake of water and organic compounds by the fronds. However, further study will be needed to determine the extent of this phenomenon on a wide range of chemicals that induce this effect. Also, more work in the future is needed to elucidate the knowledge of the accumulative and degradative pathway in several plants which could help understand and enhance the role of aquatic macrophytes as a vital based phytoremediation agent for water pollution.

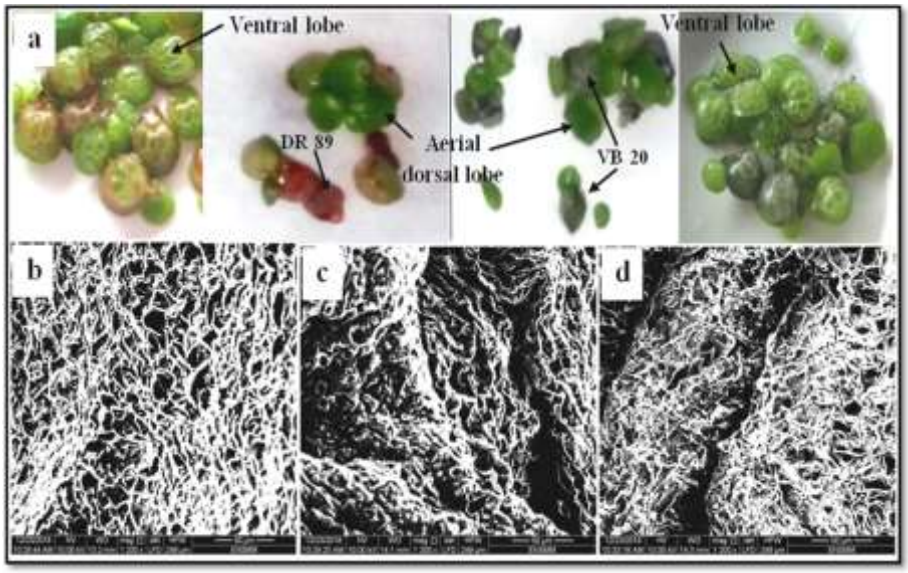

Figure 4. (a) Visual analysis of *L. gibba* before treatment, (b) SEM micrograph of *Lemna* biomass before dye phytoaccumulation. (c) SEM micrograph of *L. gibba* after phytoaccumulation of VB-20, (d) SEM micrograph of *L. gibba* after phytoaccumulation of DR-89.

CONCLUSIONS

The results obtained in this study showed that *L. gibba* could be an interesting species for the phytoremediation of the two dyes VB-20 and DR-89. It was found that the biological process was influenced by initial dye concentration. The phytoremediation mechanism was determined by UV-vis and FT-IR analysis, which explained that the dyes were removed in two steps: the first step is the adsorption on the biomass surface and the second one is phytoaccumulation without any phytodegradation. Additionally, FT-IR analysis showed that the biomass functions namely alcohol, alkene, phenol and amine are implied in dye retention. The

Morphological change was also observed in the SEM micrographs due to the dye coverage into *L. gibba* surface.

The present results indicated that the aquatic species *L. gibba* may be a good candidate for the removal of organic pollutants contaminating aqueous media. Eventually, the present process could be coupled with other biological species (aquatic plants, microorganisms, activated sludge) for further increasing the effectiveness of the biological treatment.

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Conflict of interests

The authors declare no conflict of interest

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