Acta Chim. Slov. **2015,** *62,* 452–461 **DOI:** 10.17344/acsi.2014.1109

Scientific paper

Biological Removal of Triphenylmethane Dyes From Aqueous Solution by *Lemna minor*

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Received: 09-10-2014

Abstract

The aim of this study is to investigate and develop a phytoremediation method for the removal of two *triphenylmethane dyes* (crystal violet and malachite green) using an aquatic plant, *Lemna minor*. The effects of operational parameters, such as aquatic plant quantity, initial dye concentration, initial pH of the solutions and temperature of the medium were studied in order to determine the optimum phytoremediation conditions. The plant's photosynthetic pigments were determined quantitatively in order to detect its response to abiotic stress. During the phytoremediation experiments the parallel sub-processes (phytoextraction, phytodegradation) were observed and analysed. The mechanisms of phytoremediation were studied using Fourier transformation infrared spectroscopy, ultraviolet-visible spectroscopy, thin layer chromatography and Energy-dispersive X-ray spectroscopy. Results show that the plant tolerated high concentrations (300 mg/L) of dyes. It was able to remove the dyes from the environment and to accumulate them in its cells for up to a significant percentage (crystal violet was removed by about 80% and malachite green by 90%).

Keywords: *Lemna minor*, phytoremediation, aquatic plant, triphenylmethane dyes, photosynthetic pigments

1. Introduction

Various industries such as textile, leather, paper, cosmetics, medicine, and food factories use dyeing for nylon, wool, silk, plastics or biological stains. More than 10.000 different dyes and pigments are known to be used in industries, and 0.7 million tons of synthetic dyes are produced annually worldwide, as reported by Saratale,[1] and approximately 280.000 tons of textile dyes are discharged every year.[2]

The wastewaters from these industries are very difficult to treat effectively due to their contents of organic pollutants, dye intermediates and organic solvents, which contaminate water bodies and environment. Several methods were proposed for removing organic pollutants from aqueous solutions, such as physical, chemical or biological remediation treatments. Many recent studies focus on developing new alternative strategies of wastewater treatments, from which the biological remediation is one of the most cost-effective.

Phytoremediation is an eco-friendly process for the removal of different organic and inorganic contaminants[3] using different types of plants which can act as biofilters and bioaccumulators for hazardous pollutants. Phytoremediation studies represent a useful tool for monitoring and carrying out the process of decontamination of water ecosystems, offering more information on adsorption, uptake, translocation, accumulation, and tolerance mechanism of the pollutants, as well as their damage control.[4]

In the past many plants were tested in remediation processes, including aquatic and terrestrial plants. The duckweed species from the botanical family of *Lemnaceae* have grabbed the attention of researchers in recent years.[5,6] The free-floating aquatic plant, *Lemna minor*, is considered to be an ideal test system for water remediation research[7–9] because of its physiological properties (small size, big surface) and high multiplication rate.[10,11] *Lemna minor* is considered one of the fastest growing plants, with a productivity of 10–30 tons of dry mass/ha·year, confirmed by Bich,[12] having a good capacity to adapt to different conditions, like a wide range of pH (4.5–8.3), temperature (6–33 °C), and different kinds of wastewaters.[13, 14]

Recent studies have reported that *Lemna minor* has great potentials in the phytoremediation of inorganic pollutants.[9,15] It is a good accumulator of heavy metals like As, Pb, but it can also tolerate and bioaccumulate Zn, Cd, Cu, and Cr in high concentrations.[16–18]

Malachite green (MG) and crystal violet (CV) are triphenylmethane dyes, having antibacterial, antifungal and antihelmintic properties in small quantities.[19] On the other hand, in higher concentrations they cause several health problems, having carcinogenic and mutagenic effects on living cells.[20–22]

The present study examines and develops a phytoremediation process to remove CV and MG triphenylmethane dyes from aqueous solutions using an aquatic plant, *Lemna minor.* It is the first time that *Lemna minor* is used in the removal of these two triphenylmethane dyes from aqueous solutions. In order to optimise the aquatic plant's phytoremediation capacity, the effects of the operational parameters such as biomass quantities, initial concentration, initial pH, and temperature were studied. The plant's efficiency in removing the two dyes was determined, the results being compared and discussed in details. It was attempted to detect the plant's responses to abiotic stress by measuring the quantity changes of photosynthetic pigments. To analyse the phytoremediation process mechanism we used Fourier transformation infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-Vis), Energy-dispersive X-ray spectroscopy (EDX) and thin layer chromatography (TLC). The phytoremediation mechanism and the parallel sub-processes were studied by spectrophotometric and chromatographic methods.

2. Experimental

2. 1. Plant Material and Growing Conditions

The free-floating aquatic plant, *Lemna minor* was chosen to be used in the phytoremediation studies because of its unique physical and biological properties and its high tolerance for abiotic stresses. The plants were grown in the greenhouse of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania, with the addition of Complex III fertilizer (0.5%). Following the 30 day growing period, they were used for the phytoremediation experiments.

2. 2. Methods for Dye Determinations

Two cationic triphenylmethane dyes, CV and MG were selected as organic pollutants for the phytoremediation experiments. The triphenylmethane dyes were purchased from Penta (Czech Republic). The dyes concentrations were determined spectrophotometrically (CV λ_{max} = 590 nm and MG λ_{max} = 618 nm) using a double beam UV-visible spectrophotometer (UV-Vis: GBC Cintra 202). The stock solutions of CV and MG were obtained by dissolving 1 g of dye in 1 L distilled water. The working solutions were prepared by diluting the stock solutions with a Hoagland nutrient solution.

2. 3. Process Characterisation

The percentage of removal efficiency of the *Lemna minor* was calculated by equation (1) below, where E (%) is the dye removal efficiency, C_i is the initial dye concentration and C_f is the final dye concentration measured from the aqueous solutions in mg/L.

$$E(\%) = \frac{C_i - C_f}{C_i} \cdot 100 \quad (1)$$

The plants phytoremediation capacity was calculated by the formula (2), where Q_{max} is the plant's uptake capacity (mg/g), C_i is the initial concentration (mg/L), C_f is the final concentration (mg/L) measured from the aqueous solutions, V is the volume of the solution (L), and m is the plant quantity (g).

$$Q_{max} = (C_i - C_f) \cdot \frac{V}{m} \quad (2)$$

2. 4. Experimental Set-up and Procedure

The phytoremediation experiments were carried out in controlled conditions (at room temperature 23 ± 2 °C, illuminated with a lamp with the 14/10 h light/dark photoperiod), in 250 ml Beaker glass containing 200 ml synthetic wastewater and the aquatic plants along with the macro- and micronutrients in batch mode. The Hoagland nutrient solution contains 1.25 mM KNO_3, 1.25 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.25 mM KH_2PO_4, 11.6 μM H_3BO_3, 4.5 μM $MnCl_2 \cdot 4H_2O$, 10 μM Fe(III)EDTA, 0.19 μM $ZnSO_4 \cdot 7H_2O$, 0.12 μM $Na_2MoO_4 \cdot 2H_2O$, and 0.08 μM $CuSO_4 \cdot 5H_2O$ (the chemicals were purchased from Merck Germany), as suggested by Csog et al.[23]

The aquatic plants were left for an acclimatisation period for 3 days in the Hoagland nutrient solution, then the plant's dye removal efficiency was analysed in mono-

dyes aqueous solutions, for a 12–14 days period until equilibrium was reached. Samples were withdrawn from the aqueous solution every day for monitoring the plant's removal efficiency. The absorbance of the supernatants was measured using UV-spectrophotometer at the characteristic wavelength.

To determine the *Lemna minor* phytoremediation potential (the uptake of CV and MG dyes from aqueous solutions) the following parameters were studied: the effect of plants quantity (m_{plants} = 1–5 g (fresh weight); C_i = 100 mg/L, V = 250 mL, T = 23 ± 2 °C, until 12 days); the effect of initial concentrations (C_i = 40 – 300 mg/L; m_{plants} = 3g (fresh weight), V = 250 mL, T = 23 ± 2 °C, until 14 days); the effect of initial pH (initial pH between 2–9 for CV, 2–6 for MG; m_{plants} = 3g (fresh weight), C_i = 290 mg/L, V = 250 mL, T = 23 ± 2 °C, until 12 days), the effect of temperature (T_1 = 5 °C, T_2 = 23 °C, T_3 = 40 °C and T_4 = 50 °C only in the case of MG; m_{plants} = 3g, C_i = 100 mg/L, V = 250 mL, for a phytoremediation period of 10 days).

The initial pH was adjusted using 0.1 M HCl and 0.1 M KOH solutions, in order to study the effect of this parameter. The working pH was monitored daily with a pH meter (Hanna Instruments pH 212 Microprocessor pH Meter) during the experiments.

The effect of temperatures experiments were analysed using a Thermomix UM/ Frigomix S B. Braun Biotech International water bath.

2. 5. Study of the Phytoremediation Mechanism

After the phytoremediation experiments (0–14 days) the remained synthetic water and plants were further analysed. The phytoaccumulated dye's content was analysed from 1 g fresh plant, frozen with liquid nitrogen and homogenized with 5 mL ethanol. The samples were shaken and centrifuged at 10000 rpm for 10 min. The supernatants' absorbance was determined spectrophotometrically. Fourier transformation infrared spectroscopy (FTIR) was used to further characterise the treated biomass after the phytoremediation experiments.

2. 6. Photosynthetic Pigments Determinations

The content of photosynthetic pigments (chlorophyll *a, b* and carotenoids) was determined to investigate the biochemical responses of the live biomass on the abiotic stress. The photosynthetic pigments were isolated with extractions using organic solvents from the stress exposed biomass (1 g of fresh plant sample was extracted with 5 ml of ethanol and centrifuged at 5000 rpm for 10 min). The chlorophyll *a*, *b* and total carotenoid x + c (xanthophylls and carotenes) pigments quantitative determination was evaluated spectrophotometrically, at the maximum absorbance of 664 (A_{664}), 648 (A_{648}) and 470 (A_{470}) nm respectively. The content of these photosynthetic pigments was calculated using the specific absorbance coefficients and equations (1–7) suggested by Lichtenthaler, detailed by Lichtenthaler[24] and Buschman,[25] the results are given in mg/g fresh weight.

The basis for spectrophotometric quantification of pigments is the Lambert-Beer law. The concentrations for chlorophyll *a* (C_a) and chlorophyll *b* (C_b) are given by different equations, where α is the specific absorbance coefficient in L/g cm and A is absorbance, Z is the four extinction coefficients of chlorophyll *a* and *b*.

$$C_a = \left[\frac{\alpha_{(b)\max b\cdot} \cdot A_{\max a}}{Z}\right] - \left[\frac{\alpha_{(b)\max a} \cdot A_{\max b}}{Z}\right] \quad (1)$$

$$C_b = \left[\frac{\alpha_{(a)\max a\cdot} \cdot A_{\max a}}{Z}\right] - \left[\frac{\alpha_{(a)\max b} \cdot A_{\max a}}{Z}\right] \quad (2)$$

$$Z = \left(\alpha_{(a)\max a} \cdot \alpha_{(b)\max b}\right) - \left(\alpha_{(a)\max b} \cdot \alpha_{(b)\max a}\right) \quad (3)$$

The carotenoids is determined as sum of specific absorbance for chlorophyll *a*, and *b* and total carotenoids x + c. The concentration of carotenoids $C_{(x+c)}$ is given by the following equation:

$$C_{(x+c)} = \frac{A_{(a)470} - (\alpha_{(a)A_{470}} \cdot C_a) - \left(\alpha_{(b)470} \cdot C_b\right)}{\alpha_{(x+c)A_{470}}} \quad (4)$$

The photosynthetic pigments from the plants can be extracted using different solvents and their concentrations can be calculated using the specific absorbance coefficients for the respective solvents. The following equation was given for the determination of photosynthetic pigments concentrations extracted with ethanol:

$$C_a = 13.36 \cdot A_{664} - 5.19 \cdot A_{648} \quad (5)$$

$$C_b = 27.43 \cdot A_{648} - 8.12 \cdot A_{664} \quad (6)$$

$$C_{(x+c)} = \frac{(1000 \cdot A_{470} - 2.13C_a - 97.64C_b)}{209} \quad (7)$$

2. 7. Energy-dispersive X-ray Spectroscopy (EDX) Analysis

To determine the elemental composition of the aquatic plants, *Lemna minor* samples were washed with distilled water and dried. Then the dried samples were analysed with a Scanning Jeol JEM 5510LV (Japan) coupled with Oxford Instruments EDX Analysis System Inca 300 (UK).

2. 8. Characterisation of the Parallel Phytodergaradation Process

During the phytoremediation experiments, the phytodegradation of dye molecules in the rhizosphere was observed. Thin layer chromatography was performed for the detection of degradation products, using a mobile phase: methanol, ethyl-acetate, n-butanol, water and acetic acid in 1:2:3:1:0.2 (v/v). The degradation compounds from the mono-dye solutions after phytoremediation were extracted with ethyl acetate.

2. 9. Statistical Analyses

Values shown in the figures and tables represent average values ± standard deviation of three replicates (n = 3). Statistical analysis was performed using t-Test in Microsoft Excel package. The lowest values at the effect of parameters (1g plant quantity, 40 m/L initial concentration, 5 temperature, and pH 2) was used as corresponding control to determine the significant differences between the treatments. The values were considered statistically significant at $P < 0.05$.

3. Results and Discussion

3. 1. Characterization of the Phytoremediation Process

In order to evaluate the phytoremediation efficiency of the *Lemna minor*, we studied the effect of operational parameters. The first parameter studied was the effect of plant quantity on the efficiency of phytoremediation. Different plant quantities have an impact on the phytoremediation process mechanism, affecting directly the plant's surface binding capacity and its uptake capacity. The results of the effect of biomass quantity are presented in the Fig. 1.

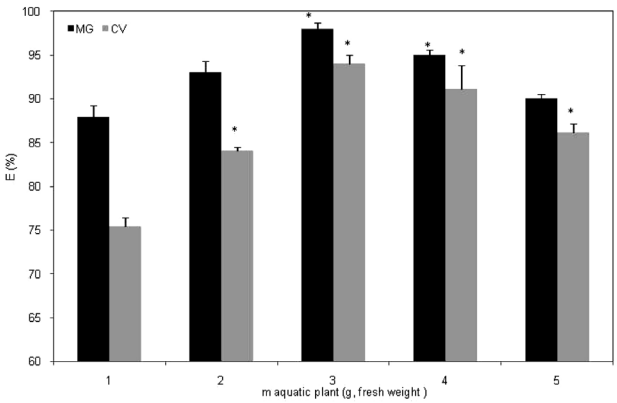

Fig. 1. The effect of *Lemna minor* plant quantity on the removal efficiency of the CV and MG dyes; m_{plants} = 1–5 g (in fresh weight), V = 250 mL, initial pH = 3.5 (MG)/3.8 (CV), C_i = 100 mg/L, T = 23 ± 2 °C, until 12 days; values mean ± standard deviations (n = 3); *significant difference at $P < 0.05$.

Increasing the quantity of the plant has a beneficial effect on the removal efficiency. The larger amount of biomass provides more surface area for the phytoextraction processes. Our results agree well with the findings of Khataee,[26] who used *Lemna minor* to phytoremediate Acid Blue 92 in aqueous solutions. As Fig. 1 makes clear, in our experiments the optimal weight of *Lemna minor* is 3 g with the mentioned parameters, when exposed to CV (E = 96%) and to MG (E = 98%) dyes. It was observed that a higher quantity of plant material decreased the efficiency of the process (the phytoremediation efficiency of 5 g plant is 90% for MG, and 86% for CV). These results indicate that under these experimental conditions eutrophication could occur in the aqueous medium, and this affects the plant's uptake capacity by decreasing surface binding capacity.

The *Lemna minor* removal efficiency for CV and MG dyes were analysed at five different initial concentrations. During the phytoremediation experiments the process equilibrium was reached after 14 days. The effect of the initial concentrations on removal efficiency is presented in Fig. 2.

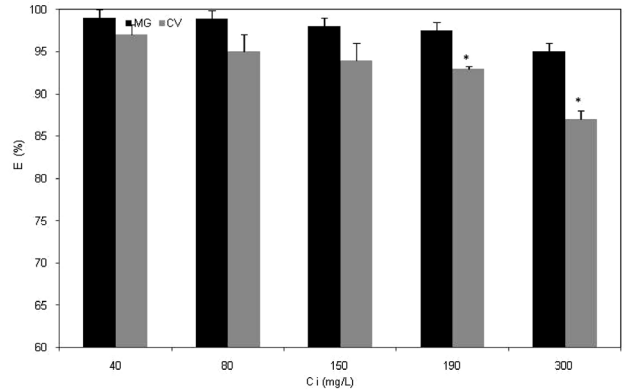

Fig. 2. The effect of the initial dye concentrations on the *Lemna minor* dye removal efficiency; C_i = 40–300 mg/L, m_{plants} = 3 g (in fresh weight), V = 250 mL, T = 23 ± 2 °C, until 14 days; values mean ± standard deviations (n = 3); *significant difference at $P < 0.05$.

According to the obtained results, dye removal efficiency of the *Lemna minor* was highest at lower concentrations (at 40 mg/L initial concentration of dye, E = 96% in the case of CV and E = 98% for the MG). It was observed that the plants were able to tolerate higher concentrations of dyes (190, 300 mg/L), however, removal efficiency decreased. The calculated phytoremediation capacity in removing CV and MG dyes are presented in Table 1.

The pH is an important factor in the phytoremediation process, affecting the plants directly. The used CV and MG dyes have a cationic structure in the form of chloride and oxalate salts. The CV and MG dyes diluted in Hoagland nutrient solutions have an acidic pH (3.5 and 4).

Table 1. The calculated *Lemna minor* phytoremediation capacity (Q_{max}) after the removal experiments with CV and MG dyes; C_i = 40–300 mg/L, m_{plants} = 3g (in fresh weight), V = 250 mL, T = 23 ± 2 °C, until 14 days; values mean ± standard deviations (n = 3).

The initial concentration of dyes (C_i = mg/L)	The calculated phytoremediation capacity in case of CV dye (Q_{max} = mg/g)	The calculated phytoremediation capacity in case of MG dye (Q_{max} = mg/g)
40	2.64 ± 0.23	2.91 ± 0.42
80	4.85 ± 1.06	5.29 ± 0.24
150	8.09 ± 0.39	9.9 ± 0.28
190	11.64 ± 0.25	12.72 ± 0.33
300	17.85 ± 0.98	18.06 ± 1.08

The experimental aqueous solutions' initial pH were set in the pH range between 2 and 9 in case of CV, without precipitation, and, in case of MG, between 2 and 6. At higher pH the MG solution precipitates. The oxalate is known as chelating agent forming complexes with metal ions[27] from the Hoagland nutrient solutions. The experimental results concerning the effect of the initial pH on the efficiency of removing the two dyes are shown in Table 2.

Table 2. The effect of the initial pH on CV and MG dye removal efficiency with *Lemna minor* aquatic plants; initial pH = 2–9 for CV, 2–6 for MG; in conditions of m_{plants} = 3g (in fresh weight), C_i = 290 mg/L, T = 23 ± 2 °C, until 12 days; values mean ± standard deviations (n = 3); *significant difference at $P < 0.05$.

The initial dye concentration (C_{dye} = mg/L)	The initial pH of the dye solutions	The final pH of the dye solutions	Dye removal efficiency (E = %)
	CV		
100	2.1	2.2	20.02 ± 2.35
	3.1	7.1	68.3* ± 2.03
	5.1	7.48	76.4* ± 2.53
	7	7.53	78.2* ± 1.67
	9	7.58	74.4* ± 2.06
	MG		
100	2.01	2.08	10.4 ± 3.4
	3.05	5.96	82.1* ± 2.09
	3.4	7.39	96* ± 1.36
	4.02	7.6	98.5* ± 0.8
	5.01	7.81	98.4* ± 0.65

It was observed that the maximum amount of CV dye uptake was achieved at pH 7 and, in the case of MG, at pH 4. It was also noticed that during the phytoremediation process, the final pH of solution became stable generally at the pH of ~7, except for the initial pH of 2. This indicates clearly that the aquatic plants try to maintain the pH around 7 to avoid the negative effects of the induced abiotic stress. At a lower pH, the H^+ ions compete effectively with the dye's cations, showing a decrease in dye decolourisation efficiency. At very acidic pH ($pH_{initial}$ = 2), the *Lemna minor* removal efficiency was very low (E = 20% in the case of the CV and E = 10% in the case of the MG) so that the pH value of the solutions remained acidic (pH_{final} = 2). The dye removal efficiency of the *Lemna minor* is inhibited at extreme conditions. The *Lemna minor* could tolerate a wide range of pH between 4.5 and 8.3. Reema[28] has found that the *Lemna minor* activity in the removal of Methylene blue is highest in the pH range of 6–7.5. These observations are in accordance with our results.

The temperature could have a major effect during the phytoremediation process on the plants' biochemical processes, such as enzyme activity, translocation of nutrients and photosynthesis of plants.[29] *Lemna minor* is one of the most adaptive aquatic plants. Its optimal growth temperature is between 6 and 33 °C. Lower or higher temperatures can be considered stress factors for the plant and can influence phytoremediation efficiency.

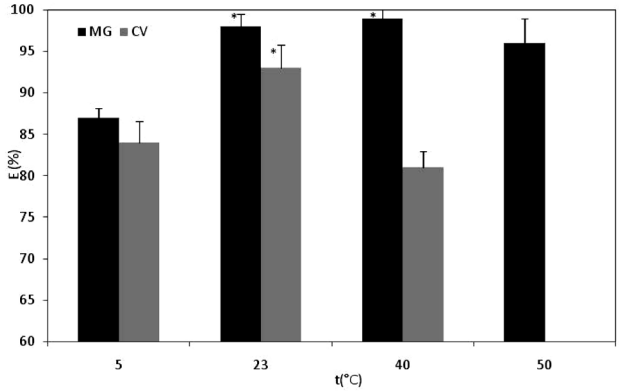

Fig. 3. The effect of temperature on the dye removal efficiency of the *Lemna minor* in the phytoremediation process; T_1 = 5 °C, T_2 = 23 °C, T_3 = 40 °C and T_4 = 50 °C only in the case of MG, in conditions of m_{plants} = 3g, C_i = 100 mg/L, V = 250 mL; values mean ± standard deviations (n = 3); *significant difference at $P < 0.05$.

Our results concerning the effect of temperatures on the phytoremediation process are presented in Fig. 3.

It was observed that during the experiments the plant showed no critical thermal deactivation in its removal capacity. The comparative results of the two dyes show that the decolourisation efficiency was more substantial in case of the MG. It can be concluded that the optimal temperature for removing MG by *Lemna minor* is 40 °C (E = 98%), which suggests that the process is an endothermic one. At higher temperatures the physiological functions of the plant decrease obstructing the phytoextraction capacity, while the phytorsorption capacities of the plant increase. In case of CV, the optimum phytoremediation temperature is room temperature (E = 92%). This means that temperature is a limiting factor in the CV dye uptake and CV phytoextraction is an exothermic process.

3. 2. Study of the Phytoremediation Mechanism

It was noticed that phytoremediation methods can be explained by the parallel sub-processes, such as phytoextraction/phytoaccumulation in the plants and phytodegradation in the aqueous solutions.

The phytoaccumulated dyes' nature and the plant's binding mechanism were studied using different spectrophotometric determinations (UV-Vis and FTIR analysis).

The content of the aquatic plant's pigments was extracted before and after the phytoremediation experiments and the characteristic spectra were registered using UV-Vis spectroscopy. The results are presented in Fig. 4.

The results demonstrate that the plant's cells contain the accumulated dyes in an intact form without degradation. The wavelength bands of the dyes are similar to the control dyes' wavelength bands, except that the peak maximums are shifted from λ_{max} 586 to 571 nm by CV and from λ_{max} 617 to 620 nm in case of MG.

The FTIR analysis of plant samples provides information about *Lemna minor* structural functional groups and about the nature of the binding of dyes. The FTIR spectra of the control dyes (A), control plants (without dye treatment, B) and after the phytoremediation experiments (C) were recorded and were compared to determine the vibrational frequency changes for the functional groups in the plants after the dye treatments. The FTIR comparative spectra results are shown in Fig. 5 for CV and MG dyes.[30]

The comparative spectra shows that in the region of 1700 to 500 cm^{-1} the spectra of the treated plants contain a large number of organic components attributed to the plant and specific fingerprint peaks of the dyes.

After the phytoremediation experiments with CV

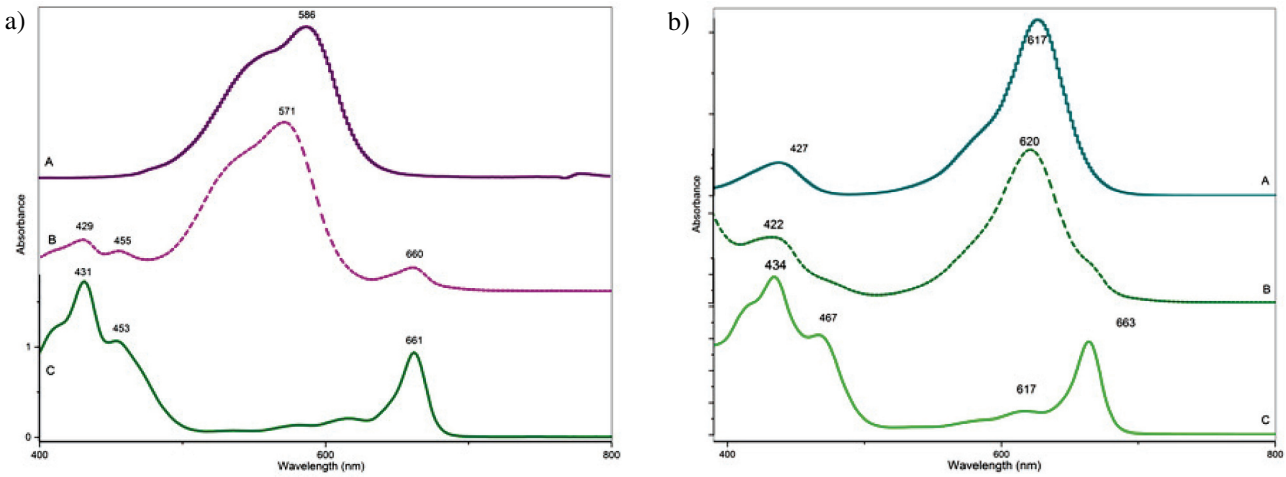

Fig. 4. UV-Vis spectra of the CV and MG diluted in EtOH (A), the phytoaccumulated CV and MG content extracted with EtOH from *Lemna minor* (B), and the photosynthetic pigments extracted with EtOH from control *Lemna minor* (C) [The a) for the CV and b) for the MG dye].

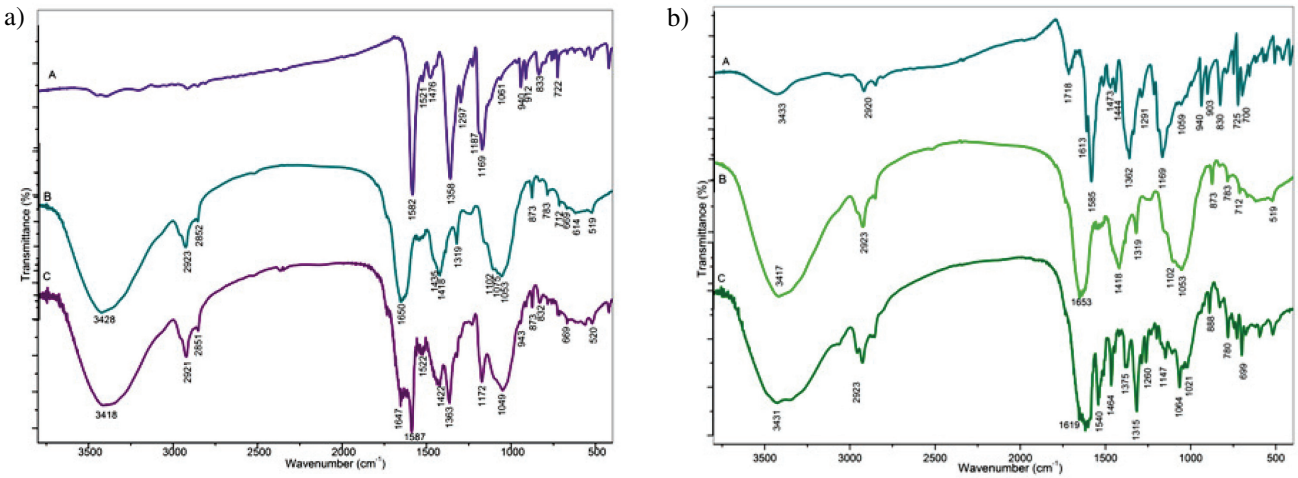

Fig. 5. a) FTIR spectra of the CV dye (A), *Lemna minor* control plant (B) and *Lemna minor* after the phytoremediation of CV; b) FTIR spectra of the MG dye (A), *Lemna minor* control plant (B) and *Lemna minor* after the phytoremediation of MG

dye, in the *Lemna minor,* FTIR spectra (C) reveal the appearance of new peaks at 1587, 1363 and 1172 cm^{-1}. The same case was noticed after the MG treatments, where the exposed *Lemna minor* plant spectra shows new peaks at 1464, 1375, 1315, 1147 cm^{-1}. The new peaks are substituted for the mono- and para-disubstituted benzene rings, the benzene rings' C=C stretching vibrations and the asymmetric stretches of Ar-NR_2 peaks from the dyes which clearly indicates the CV and MG uptake by the aquatic plants.

Changes in the peaks of absorbance intensity were also observed in case of the treated aquatic plants spectra: at the peak of 1650 cm^{-1} in case of the *Lemna minor* treated with CV, and at 1100 cm^{-1} in the case of the *Lemna minor* treated with MG. The bands < 800 cm^{-1}, which represent the fingerprint zone corresponding to the phosphate and sulphur functional groups, exhibit minor changes after the phytoremediation process. These results fit well the findings of Ayed,[31] who studied the triphenylmethane dyes' decolourisation on *Staphylococcus epidermidis.*

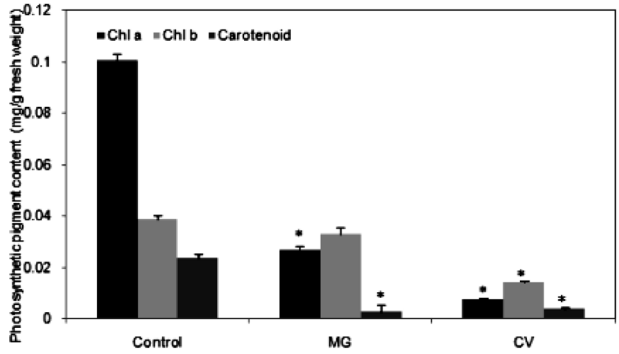

Fig. 6. The *Lemna minor* aquatic plant's photosynthetic pigment from the control plants and after the phytoremediation experiments with triphenylmethane dyes (CV and MG); C_i = 100 mg/L, m_{plant} = 3 g (in fresh weight), V = 250 mL, pH = 3.5 (MG)/3.8 (CV), T = 23 ± 2 °C, until 12 days); values mean ± standard deviations (n = 3); * significant difference at $P < 0.05$.

3. 3. Plant Responses to Abiotic Stress

It was previously reported in the literature that the dyes' toxic effect on the biosynthesis of the plants can be monitored by determining the changes of several biochemical markers.[32] The content of the plants' photosynthetic pigments (chlorophyll *a, b* and carotenoid) was analysed quantitatively in order to measure the responses of the *Lemna minor* to the abiotic stress induced by CV and MG dyes. The results are shown in the Fig. 6.

We can conclude that during the phytoremediation experiments with CV and MG dyes, the chlorophyll (*a, b*) and carotenoid pigment concentrations have decreased in *Lemna minor* significantly, in both cases. The observed photosynthetic content was more affected after phytoremediation experiments involving CV (a decrease of more than 70%), which is in accordance with the removed dye quantity, and can be explained by the fact that the *Lemna minor* is more sensitive to the exposure of CV dye.

3. 4. EDX Analysis

The elemental composition of the *Lemna minor* after the phytoremediation experiments with triphenylmethane dyes were determined with EDX spectra analysis in order to confirm the phytotoxicity symptoms of the dyes on the aquatic plants. The results of EDX spectra are presented in Table 4.

It can be noted that the *Lemna minor* aquatic plant contains generally macro- (C, N, P, and O) and microele-

Table 4. EDX analysis of the *Lemna minor* control and the aquatic plant after the phytoremediation experiments with triphenylmethane dyes; C_i = 100 mg/L, V = 250 mL, initial pH = 3.5 (MG)/3.8 (CV), T = 23 ± 2 °C, until 12 days; values mean ± standard deviations (n = 3), *significant difference at $P < 0.05$.

Nr.	Elements	Wt (%) Content of the control plant	Wt (%) Content of the plant after the phytoextraction of MG	Wt (%) Content of the plant after the phytoextraction of CV
1	C	48.46 ± 2.61	54.61 ± 4.26	58.06 ± 3.83
2	N	8.29 ± 1.62	7.85 ± 0.80	6.88 ± 2.89
3	O	27.98 ± 4.69	33.96 ± 5.8	22.41 ± 5.59
4	Na	0.33 ± 0.26	0.20 ± 0.04	0.13 ± 0.14
5	Mg	1.66 ± 0.2	0.15* ± 0.06	0.18* ± 0.07
6	Al	0.32 ± 0.17	0.48 ± 0.35	3.18 ± 4.03
7	Si	0.04 ± 0.01	0.06 ± 0.04	0.20 ± 0.11
8	P	2.09 ± 0.23	0.31* ± 0.15	1.33 ± 1.21
9	S	1.38 ± 0.35	0.19 ± 0.15	0.54 ± 0.35
10	Cl	0.55 ± 0.15	0.00*	0.08* ± 0.04
11	K	6.90 ± 1.8	0.10* ± 0.12	0.34* ± 0.24
12	Ca	1.77 ± 0.76	1.78 ± 1.59	6.85 ± 6.2
13	Mn	0.07 ± 0.04	0.02 ± 0.01	0.10 ± 0.07
14	Fe	0.16 ± 0.03	0.28 ± 0.27	0.71 ± 0.57

ments (such as Mg, Ca, Mn, Fe). The elemental changes in concentration were determined from the untreated and treated plant spectra. As known from the literature, the most important macro-elements in the plants play an important role in the formation of carbohydrates, lignin, cellulose, proteins, and nucleotides.[33,34]

According to our study, after the phytoremediation experiments with CV and MG dyes, the aquatic plant suffers some changes in elemental content, such as Mg, Na, S, Cl, and K. The observed Mg reduction can be correlated with the decrease of chlorophyll concentration. Our results agree with the findings of Wang[35] and Zhao[36] on *Brassica napus* and *Phytolacca americana* plant species. They also mention that Mg, Mn, Zn and Fe are involved in many essential biological processes and have an important role in the biosynthesis and stability of the chlorophyll.

Our results indicate that the decrease of concentrations in the macro- and microelements can be interpreted as phytotoxicity symptoms induced by the triphenylmethane dyes. These findings also point to the ionic homeostasis, which is deleterious to plants by inhibiting plant growth, causing oxidative damage and chlorosis in the leaves.

3. 5. Parallel Phytodegradation Processes

During the phytoremediation process the dye molecules can be degraded in the rhizosphere. TLC analysis was used to analyse the degraded products remained in the mono-dye solutions. The results are shown in Fig. 7.

Compared to the control dye new spots can be observed, which indicate the dyes' degradation.

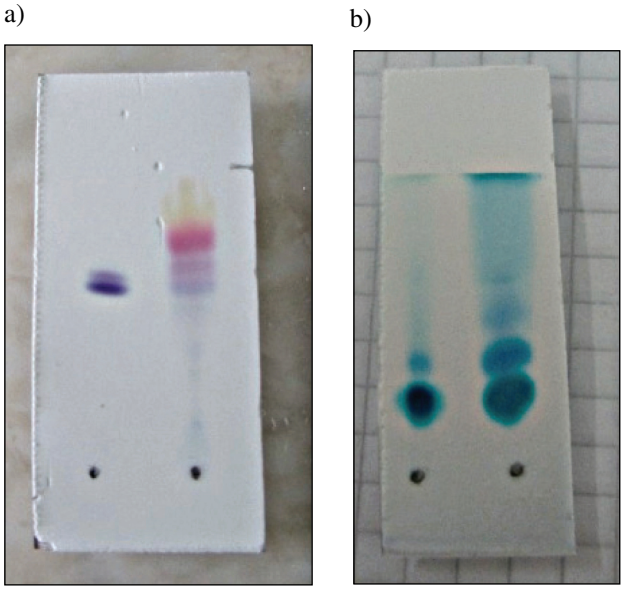

Fig. 7. TLC analysis of control dyes (CV and MG) and the degraded metabolites after the phytoremediation experiments with CV (a) and MG (b) dyes (C_i = 100 mg/L, m_{plants} = 3 g, V = 250 mL, initial pH = 3.5 (MG)/3.8 (CV), T = 23 ± 2 °C, until 12 days).

4. Conclusions

The present study showed that *Lemna minor* plant promises good results in the phytoremediation process of two triphenylmethane dyes. The optimised experimental parameters that allow the highest phytoremediation efficiency were determined. It was found that the biological processes and the plant's removal efficiency are influenced by plant quantity, initial concentration, initial pH and temperature. The equilibrium was reached after 14 days with a maximum dye uptake at 3 g plant. The study of the influence of pH on dye removal efficiency led to interesting results. It was concluded that *Lemna minor* has a buffer effect on the pH of the dye solutions. During the phytoextraction process equilibrium was reached at the neutral pH. Temperature is one of the major environmental factors which effect the plant's physiological and biochemical changes and also the plant's phytoremediation capacity. In our work, the highest efficiency was reached at room temperature in the case of CV uptake and at 40 °C in removing MG.

The phytoaccumualtion mechanism and the phytotoxicity symptoms of the plant were determined by the UV-Vis, FTIR, EDX methods and through a total photosynthetic pigment quantity analysis. The phytotoxicity symptoms were supported by EDX analysis. Changes in the Mg amount of *Lemna minor* samples correlated very well with the chlorophyll determinations.

The phytoremediation process mechanism of the two triphenylmethane dyes was studied by determining the parallel sub-processes: phytoextraction/phytoaccumulation and phytodegradation.

5. Acknowledgement

This paper is a result of a doctoral research made possible by the financial support of the Sectoral Operational Programme for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project POSDRU/159/1.5/S/132400 –"Young successful researchers – professional development in an international and interdisciplinary environment"

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Povzetek

Namen raziskav je bil razviti fitoremedijacijsko metodo za odstranjevanje dveh trifenilmetanskih barvil (kristalno vijolična in malahitno zelena) z vodno rastlino *Lemna minor*. Za določitev optimalnih fitoremedijacijskih pogojev so bili proučevani učinki količine rastline, začetne koncentracije barvila in pH-ja ter temperature medija. Za ugotavljanje odgovora rastline na abiotski stres so bila kvantitativno določana rastlinska fotosintetska barvila. Vzporedno s temi eksperimenti sta bila opazovana in analizirana tudi fitoekstrakcija in fitodegradacija. Mehanizem fitoremedijacije je bil proučevan s pomočjo FTIR spektroskopije, UV spektroskopije, tankoplastne kromatografije in X-žarkovne spektroskopije. Rezultati kažejo visoko koncentracijsko toleranco barvil (300 mg/L). Rastlina je bila zmožna odstraniti barvila iz okolja in jih akumulirati v svoji celični zgradbi in sicer okrog 80% kristalno vijolične in 90 % malahitno zelene.