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RESEARCH ARTICLE

Phytoremediation processes of domestic and textile effluents: evaluation of the efficacy and toxicological effects in *Lemna minor* and *Daphnia magna*

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Abstract

Phytoremediation has been proposed as a potential biotechnological strategy to remediate effluents before their release into the environment. The use of common aquatic plant species, such as macrophytes (e.g., *Lemna* spp.) as a cleanup solution has been proposed decades ago. However, the effectiveness of such processes must be assessed by analyzing the toxicity of resulting effluents, for the monitoring of wastewater quality. To attain this purpose, this work intended to quantify the efficacy of a *Lemna*-based wastewater phytoremediation process, by analyzing toxicological effects of domestic and textile effluents. The toxic effects were measured in *Lemna minor* (same organisms used in the phytoremediation process, by quantifying toxicological endpoints such as root length, pigment content, and catalase activity) and by quantifying individual parameters of *Daphnia magna* (immobilization, reproduction, and behavior analysis). Phytoremediation process resulted in a decrease of chemical oxygen demand in both effluents and in an increase in root length of exposed plants. Moreover, textile effluent decreased pigments content and increased catalase activity, while domestic effluent increased the anthocyanin content of exposed plants. *D. magna* acute tests allowed calculating a EC_{50} and Toxic Units interval of 53.82–66.89%/1.85–1.49, respectively, to raw textile effluent; however, it was not possible to calculate these parameters for raw and treated domestic effluent (RDE and TDE). Therefore, in general, the acute toxicity of effluent toward *D. magna* was null for RDE, and mild for the treated textile effluent (TTE), probably due to the effect of phytoremediation. Exposure to textile effluents (raw and treated) increased the total number of neonates of *D. magna* and, in general, both textile effluents decreased *D. magna* distance swim. Moreover, although both effluents were capable of causing morphological and physiological/biochemical alterations in *L. minor* plants, organisms of this species were able to survive in the presence of both effluents and to remediate them.

Keywords Macrophyte · Biomarkers · Pigment analysis · Catalase activity · Behavior · Crustaceans

Abbreviations

AA	Anthocyanins
Car	Carotenoids
CAT	Catalase
Chlor a	Chlorophyll *a*
Chlor b	Chlorophyll *b*
COD	Chemical oxygen demand
PC	Phytoremediation control
PE	Pre-treatment
RDE	Raw domestic effluent
RTE	Raw textile effluent
TD	Total distance moved
TDE	Treated domestic effluent
TT	Total swimming time
TTE	Treated textile effluent

Introduction

Nowadays, water resources are becoming increasingly scarce and many of them are polluted by anthropogenic sources, such as household wastes and different water-dependent industrial

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activities. In this regard, it is known that wastewaters from domestic origin contain pathogens, suspended solids, nutrients (nitrogen and phosphorus), and other chemical pollutants, such as heavy metals, detergents, pharmaceuticals, and pesticides, whose toxicity has been widely discussed (Al-Jlil 2009; Rajasulochana and Preethy 2016). On the other hand, wastewaters produced by the textile industry contain large amount of dyes and other chemicals, some of which are carcinogenic non-biodegradable agents that may pose a major threat to health and to the environment (Ghaly et al. 2014 and references therein; Kharat 2015). Despite their composition and complexity, both domestic and textile wastewaters have been recognized as one of the major factors contributing for worldwide aquatic pollution, fundamentally because they contain chemicals with toxicological concern; in addition, these substances may block the sunlight, thereby impairing photosynthesis and increasing the biological oxygen demand in receiving waters, compromising the fundamental reoxygenation process (Kharat 2015; Seow et al. 2016). Therefore, it is of high importance to dispose of these liquid residues in a proper manner with the goal of providing adequate environmental and human health protection. In this sense, the treatment of wastewater before such effluents enter receiving natural water bodies is a critical point (Gogate and Pandit 2004; Seow et al. 2016).

In addition, one of the most relevant problems related to wastewater treatment is the incomplete reduction of the load of all the contaminants present in wastewaters, to attain specific limits that are established by governmental laws (Pawęska and Bawiec 2017). This drawback represents a potential risk to aquatic organisms exposed via the environment, living in receiving water bodies (Hernando et al. 2005). In this sense, phytoremediation has been proposed as an effective, low-cost, preferential cleanup option for moderately contaminated sites (Türker et al. 2014). Plants can directly participate in detoxification processes, through contaminant incorporation and subsequent metabolization, or immobilization within the plant, or indirectly, through the promotion or support of rhizospheric microorganisms that effectively carry out the detoxification process, and one of these processes is the phytofiltration (Ibañez et al. 2018). Particularly, phytofiltration involves the use of aquatic plants, either floating, submerged, or emergent, to remove pollutants from solution, mainly through their root system although in some cases, fronds are also involved directly in the removal process (Olguín and Sánchez-Galván 2012; Paisio et al. 2017). This strategy is of special relevance for the treatment of liquid industrial residues due to their action as “nutrient pumps” (Herath and Vithanage 2015). Taking into account this role of phytoremediation, it is possible to say that the use of aquatic macrophytes (e.g., *Lemna* spp.) for the complete treatment of wastewater derived from specific treatment plants can be important, particularly during the final tertiary treatment phase, allowing completely purified wastewaters to be released into the environment (Chaudhary and Sharma 2014; Saha et al. 2015). These plants that may be used on phytoremediation processes can be ultimately deposited in landfills, but may also be incinerated; incineration has been proposed as a way of reducing the plant volume and also to generate energy by means of heat (Souza and Silva 2019). In addition, these plants may also be used in the ceramic industry, incorporating the biomass used in the treatment of ceramic blocks, which corresponds to an efficient method of transformation of the obtained products (Lima et al. 2015).

Another topic to consider is that in order to assess the efficiency of an applied wastewater treatment strategy, adequate evaluation tools must be used. Regarding this aim, traditionally the quality of effluents is based on the control of chemical, biochemical, and physical parameters and even more in the detection of specific pollutants (Hernando et al. 2005). However, it is not sufficient to assess the environmental risk because they are not real measurements of the toxicity effects on the aquatic ecosystem (Chang et al. 2009; Rizzo 2011). Thus, with the aim of avoiding this drawback, toxicity tests have been used to evaluate whether effluent detoxification takes place (Klamerth et al. 2010; Lyu et al. 2018). However, it is necessary to take into account that toxicity and chemical measures are complementary analytical tools for monitoring wastewaters quality, which can contribute with reliable indices of the toxic impact of effluents in the aquatic environment. A very popular bioassay used internationally for toxicity screening of chemical compounds, and also for the monitoring of effluents, is undoubtedly the toxicity test with freshwater Daphnids, particularly with *Daphnia magna* (Persoone et al. 2009).

Therefore, this work evaluated the efficiency of phytoremediation of domestic and textile effluents, collected from wastewater treatment plants from north of Portugal using an aquatic macrophyte, *Lemna minor*, as an alternative treatment procedure. Then, the toxicity of the treated effluents was analyzed by applying *D. magna* tests. Additionally, key physiological and biochemical characteristic (catalase activity) of the plants were evaluated in order to evaluate sub-individual responses that may compromise additional biological functions of particular ecological importance.

Materials and methods

This work was divided in three steps: (1) phytoremediation process of effluents (textile and domestic) with *L. minor*; (2) quantification of toxicological endpoints in *L. minor* individuals used in the phytoremediation process; (3) toxicity characterization of effluents and phytoremediation process efficiency using *D. magna* ecotoxicological assays.

Collection and characterization of effluents

Samples of effluents of both domestic and textile origins were collected at effluent treatment plants in the north of Portugal (Guimarães and Vieira do Minho). Both effluents were subjected to a complete (preliminary and secondary) treatment process, after which treated effluents, complying will all regulatory requirements, are released into freshwater streams. Samples were collected directly from the pumping stations of both plants, properly accommodated in 30-L plastic jars, and transferred to the laboratory where they were evaluated in terms of some characteristics, such as chemical oxygen demand (COD) and pH. After the determination of the following measurements, samples were accommodated at 4 °C until further processing.

Chemical oxygen demand (COD) determination

The organic matter content was determined by measuring COD. For this purpose, a HACH commercial kit (2125915 COD HR) was used, following the method 410.4 of US EPA (EPA 1993). This method is based on the ability of the oxidizable organic compounds to reduce the dichromate ion to chromium ion, which was quantified according to its absorbance at 610 nm. An HANNA multiparametric spectrophotometer (model HI 83214) was used to determine absorbance values, which correspond directly to COD concentrations (mg/L).

Phytoremediation process

The effluents were subjected to phytoremediation by *L. minor* in order to provide additional treatment (tertiary), to optimize the decontamination initially generated in the corresponding treatment plants. *L. minor* was cultivated according to the procedures described in Alkimin et al. (2019) and the phytoremediation process was conducted in glass flasks ($n = 8$), with 1 L of effluent, in which *L. minor* were inoculated so as to cover 70% of the surface. The process was conducted during 5 days in controlled conditions (23 ± 1 °C; $16h^L:8^D$ light intensity 5500 lx) and the same conditions were adopted for both effluents. For comparison of results, a similar exposure was conducted with *L. minor* cultured in modified Steinberg medium (phytoremediation control—PC) (OECD 2006). At the end of the exposure period, *L. minor* individuals were removed, and specific endpoints were analyzed to assess the effects of both effluents on *L. minor* individuals, namely those that were used during the phytoremediation process. For the determination of pigment levels and catalase activity (as described below), the plants were stored in Eppendorf® microtubes at − 80 °C until the analysis. The resulting effluents were used in ecotoxicity tests with *D. magna* to assess their ecotoxicological profile, after being subjected to the phytoremediation process.

Lemna minor analyses

Morphological parameter

Root length was used as morphological parameter. This parameter was measured in at least 3 plants per replicate ($n = 8$), and for all conditions (PC, and RTE and RDE after phytoremediation process). In addition, this same parameter was also analyzed in non-exposed plants from the stock culture (pre-treatment—PE).

Pigment content determination

Chlorophyll *a* (Chl a) and *b* (Chl b), carotenoids (Car), and anthocyanins were extracted using approximately 25 mg of *L. minor* tissue. Biomass was macerated in 1.5 mL of acetone:water (9:1 *v*/*v*) for Chlor a and b and Car extraction, and in 1.5 mL of methanol 1%:HCl:water (90:1:1) for anthocyanin (AA) extraction (Sims and Gamon 2002). After this procedure, samples were centrifuged (Eppendorf 5810 Refrigerated Centrifuge) during 5 min at 4 °C and 15,000×*g*, and absorbance readings (470, 529, 537, 647, 650, and 663 nm) were performed in 96-well plates, in microplate reader Thermo Scientific, model Multiskan GO, version 1.00.40, with SkanIt Software 3.2. The calculation was performed according the following Eqs. 1, 2, 3 (Sims and Gamon 2002), and 4 (Wellburn and Lichtenthaler 1984). The results were expressed in milligrams per gram of fresh weight (mg/g FW).

$$\text{Chlor a} = 0.01373 \times A_{663} - 0.000897 \times A_{537} - 0.003046 \times A_{647} \quad (1)$$

$$\text{Chlor b} = 0.02405 \times A_{647} - 0.004305 \times A_{537} - 0.005507 \times A_{663} \quad (2)$$

$$\text{AA} = A_{529} - (0.288 A_{650}) \quad (3)$$

$$\text{Car} = 1000 \times A_{470} - 2.27 \times \text{Chl a} - 81.4 \times \text{Chl b}/227 \quad (4)$$

Catalase determination

Catalase (CAT) was chosen as biochemical marker since this enzyme is an important option to prevent the occurrence of oxidative damage (through reactive oxygen species accumulation) that can compromise the plant physiology, by causing severe oxidative damage, thus inhibiting growth and grain yield (Caverzan et al. 2016). CAT activity was determined in a 96-well microplate. Spectrophotometric readings were performed in the previously described microplate reader. CAT activity was assayed by the procedure described by Aebi

(1984). This activity was quantified based on the degradation rate of the substrate H_2O_2, monitored at 240 nm for 5 min. The results were expressed by considering that 1 U of CAT activity equals the number of moles of H_2O_2 degraded per minute per milligram of protein. Protein quantification was performed at 595 nm using the Bradford method (Bradford, 1976), adapted to microplate with bovine γ-globulin as standard, in order to express enzymatic activities per milligram of protein on the analyzed samples.

Ecotoxicological tests—*D. magna* maintenance and exposure

To analyze the efficiency of phytoremediation of *L. minor*, ecotoxicological tests were performed using the macroinvertebrate *D. magna* (clone K6) that is routinely kept at the Center of Environmental and Marine Studies at University of Aveiro, Aveiro, Portugal and cultivated according Daniel et al. (2018) and ASTM (2014). Tests were carried out using the raw effluents and effluents resulting from phytoremediation treatment.

Daphnia magna acute test

Independent experiments were used to assess the acute toxicity of the effluents to *D. magna*. The tests were performed in agreement with a standard protocol (OECD 2004), under the same laboratory conditions described above. The acute assays were conducted in glass flasks containing 50 mL of test solution and 50 mL of clean ASTM medium in the control. A total of 25 animals (< 24 h old) were divided into five groups of five animals per treatment concentration. For acute tests, a range of dilutions between 1% and 100% (being 100% pure effluent and 1% the highest dilution) of both effluents were used to calculate EC_{50}; these tests were performed in triplicate. After EC_{50} calculation, the obtained values also were transformed in acute toxic units ($TUa = 100/EC_{50}$) following the specifications: < 1, low toxic; > 1 to < 4, toxic; > 4, very toxic (Verma 2008).

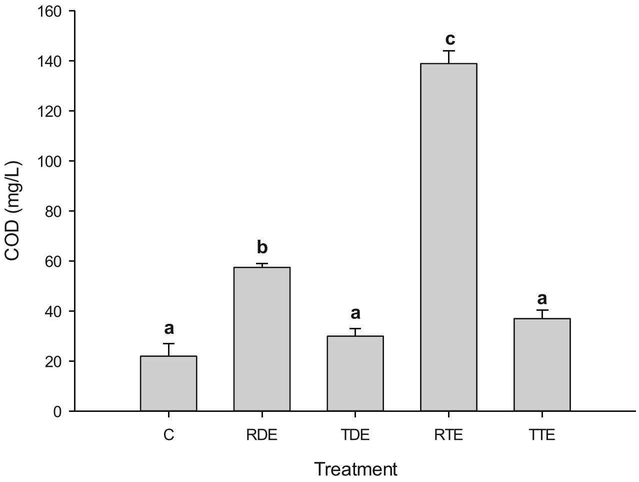

Fig. 1 Chemical oxygen demand (COD) of domestic and textile effluents before and after the phytoremediation process. C: control RDE: raw domestic effluent, TDE: treated domestic effluent, RTE: raw textile effluent; TTE: treated textile effluent. Different letters represent significant differences between treatments ($p < 0.05$). For each parameter, mean and standard error are shown

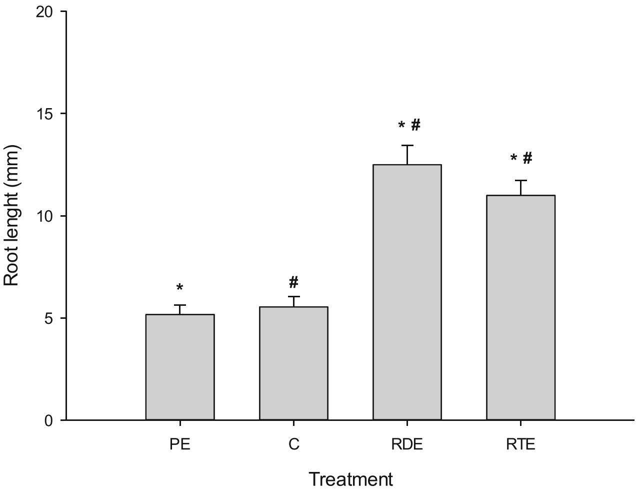

Fig. 2 Root length of *L. minor* plants exposed to domestic and textile effluents and to uncontaminated control (Steinberg's solution), during 5 days. PE: pre-treatment, C: control, RDE: raw domestic effluent, RTE: raw textile effluent. Equal symbols represent significant differences between treatments ($p < 0.05$). For each parameter, mean and standard error are shown

Subchronic reproduction test

Subchronic reproduction test was conducted with *D. magna*, based on the OECD guideline 211 (OECD 2012); the exposure period was modified according to the studies of Ribeiro et al. (2011) with *D. magna*, and of Lameira (2008) and Vacchi et al. (2016) with *Daphnia similis*. The test was ended when the exposed daphnids were in their fourth brood, approximately 17 days of exposure. This method measured the subchronic toxicity in terms of reproduction, using ten neonates (<24 h old) for each effluent concentration. Exposure media were totally renewed every other day. This test used sublethal dilutions of each effluent, calculated from data obtained in acute tests: 25%, 50%, and 100% of raw (RDE) and treated (TDE) domestic effluent; 5%, 10%, and 15% of raw textile effluent (RTE); and 25%, 50%, and 100% of treated textile effluent (TTE).

Swimming behavior test

The behavior assay was performed with 16 animals, exposed to sublethal concentrations of both effluents, with and without phytoremediation treatment (same as described above) plus

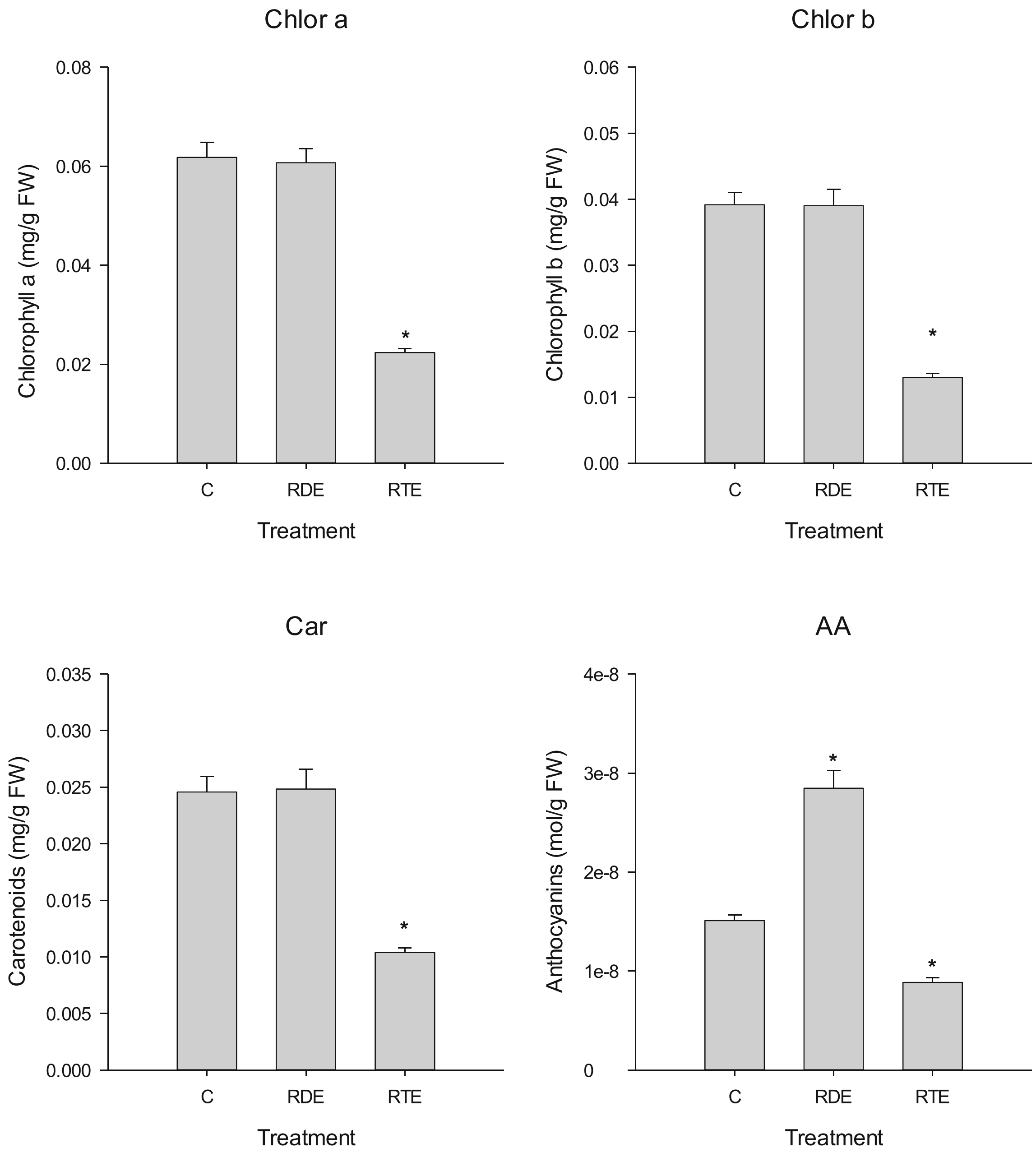

Fig. 3 Pigments of *L. minor* plants exposed to domestic and textile effluents and to uncontaminated control (Steinberg's solution), during 5 days. C: control, RDE: raw domestic effluent, TE: raw textile effluent. "*" represents significant differences between treatments ($p < 0.05$). For each parameter, mean and standard error are shown

control (clean ASTM), distributed in six-well plates (one organism per well) with 10 mL of solution per well. To develop this test, animals < 24 h old were used and exposure was of 96 h duration, with behavioral analysis being made at 48 and 96 h after the onset of exposure in order to evaluate the effluents' effects through the period of exposure. Medium was renewed every other day. Immediately before each behavioral analysis, the animals were transferred to 24-well plates with 2 mL of the respective contaminated solution. Each plate was composed by 16 treated (exposed) animals, plus 4 control animals and 4 animals subjected to the phytoremediation control (PC). The behavior analysis started after 10 min of adaptation to new conditions. Movement of the animals was tracked using the Zebrabox (Viewpoint, Lyon, France) tracking system using a 25-frame-per-second infrared camera over a period of 20 min. Movement was stimulated by alternating light and dark periods during two cycles with $5^{L}:5^{D}$ min ($300^{L}:300^{D}$ s) each cycle (Dionísio et al. 2020). Typically, *D. magna* individuals are less active during dark periods and move more during light phases. Data outputs were obtained at each 5 min and the following parameters were calculated: total distance moved (TD) and total swimming time (TT).

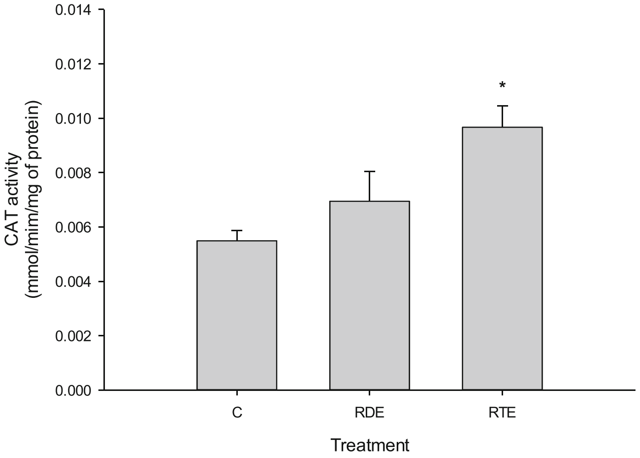

Fig. 4 Catalase activity of *L. minor* plants exposed to domestic and textile effluents and to uncontaminated control (Steinberg's solution), during 5 days. C: control, RDE: raw domestic effluent, RTE: raw textile effluent. *Significant differences between control group ($p < 0.05$). For each parameter, mean and standard error are shown

Statistical analysis

To calculate the EC_{50} value, we used the software Probit in IBM SPSS Software version 25, and a one-way analysis of variance (ANOVA, with appropriate post hoc test) was conducted for the other analysis. The type of ANOVA (parametric or non-parametric) and post hoc test (Dunnett's, Dunn's, or Tukey test) was chosen depending on whether normality and homogeneity of data were demonstrated by analysis of the residuals with the Shapiro–Wilks test. Test statistics (ANOVA) and analysis of normality were conducted using the software SigmaPlot version 12.5 and a significance level of 0.05 was adopted.

Results

Physical–chemical parameters and *L. minor* toxicity

Chemical oxygen demand (COD—Fig. 1) and pH were the physical–chemical parameters analyzed; COD values in the controls were close to 20 mg/L, while raw effluents of both types (domestic and textile) had higher levels of this parameter. On the other hand, COD in effluents after the phytoremediation process returned to basal values, close to control. The other parameter was pH, for control treatment this value was 5.53 (± 0.1); for RDE, TDE, RTE, and TTE, the values were 7.79 (± 0.1), 7.55 (± 0.15), 7.96 (± 0.12), and 7.77 (± 0.17), respectively; and in general, control had lower pH values than treated and non-treated effluents. Despite not having being quantified, our observations lead us to conclude that the here-proposed treatment was responsible for a significant reduction of the effluent's color intensity, that was not quantified.

Root length (Fig. 2) was the vegetal morphological parameter analyzed; exposure to both effluents caused a significant increase ($p < 0.05$) of this parameter in exposed plants, when compared with pre-treatment (PE) plants and subjected to control.

Pigments contents (depicted in Fig. 3), such as Chlor a and Chlor b levels, were not affected in *L. minor* exposed to RDE. On the other hand, when plants were exposed to RTE, the contents of these pigments significantly decreased ($p < 0.05$). Similar results were obtained for the pigment carotenoid. However, AA levels showed different patterns: in RDE-exposed plants, this pigment was significantly increased ($p < 0.05$), while in RTE-treated plants, exposure led to a decrease in their content ($p < 0.05$).

The oxidative stress responses on *L. minor* plants used in the phytoremediation process were measured by quantification of CAT activity (Fig. 4). In plants exposed to the TE, the activity of this enzyme was significantly increased ($p < 0.05$).

Ecotoxicological tests—*D. magna*

The tests with *D. magna* started with the determination of the effective concentration value. This determination was impossible for RDE since the effluent was not capable of causing effect (immobilization) even before treatment. Consequently, and even when submitted to the treatment by *L. minor*, this effluent was not capable of causing immobilization to juveniles of *D. magna*. However, it was possible to calculate an EC_{50} value for the RTE, which was found to be between 53.82% and 66.89% of effluent, and with a TUa calculated between 1.85 and 1.49, allowing to classify the effluent as "toxic." This effluent, after being treated by *L. minor*, was not causative of any mortality. Respect to control, it did not exert acute toxicity on *D. magna*.

The number of neonates from the first and the fourth broods (Table 1) were not affected by effluent exposure ($p > 0.05$); however, the total neonate number suffered an increase when exposed to RTE 10% and 15%, and to TTE 50% and 100% ($p < 0.05$), showing a lower toxicity of TTE respect to RTE. The age of release of the first brood was only significantly changed after the exposure to a TDE 25% ($p < 0.05$).

After 48 h (Fig. 5) of exposure to RDE, *D. magna* individuals showed some differences in their swimming behavior: TT was the most affected parameter, with significant differences ($p < 0.05$) being reported in two light and dark cycles. On the other hand, TD was only significantly different ($p < 0.05$) in comparison with the control treatment, in organisms exposed to the concentration of 25%, in the first light/dark cycle, with a significant decrease ($p < 0.05$) in the swimming distance. After 96 h of exposure (Fig. 6), the TT followed the same pattern, with significant differences ($p < 0.05$) being registered for all cycles; TD was affected in the first light cycle, while in

Table 1 Results obtained in the *D. magna* subchronic reproduction assay exposed to raw and treated domestic and textile effluents

	Number of neonates						Days	
	1st brood		4th brood[a]		Total neonates		Age (1st brood)	
	Average	SE	Average	SE	Average	SE	Average	SE
Control	9.89	± 0.90	24.11	± 0.71	66.22	± 1.83	7.89	± 0.10
RDE 25%	13.29	± 0.94	17.86	± 1.20	64.86	± 3.22	7.86	± 0.13
RDE 50%	11.33	± 0.86	17.78	± 2.57	69.22	± 4.80	7.78	± 0.14
RDE 100%	11.56	± 1.33	25.11	± 2.55	80.44	± 2.50	7.67	± 0.16
TDE 25%	12.13	± 0.74	25.13	± 2.55	77.00	± 7.39	7.00*	± 0.00
TDE 50%	12.33	± 0.47	28.11	± 2.05	78.22	± 3.53	7.78	± 0.14
TDE 100%	11.71	± 1.06	33.71	± 1.71	83.00	± 1.65	8.00	± 3.01
RTE 5%	9.78	± 0.83	31.56	± 0.70	79.33	± 2.06	8.11	± 0.10
RTE 10%	14.00	± 2.82	21.33	± 2.78	85.11*	± 2.31	8.22	± 0.14
RTE 15%	14.67	± 2.65	25.67	± 1.57	96.22*	± 4.43	8.33	± 0.16
TTE 25%	10.60	± 1.76	16.20	± 3.81	65.80	± 4.89	7.60	± 0.22
TTE 50%	10.00	± 1.87	24.38	± 3.33	84.38*	± 5.17	7.63	± 0.17
TTE 100%	10.56	± 0.77	33.33	± 2.52	99.67*	± 2.25	8.00	± 0.16

RDE raw domestic effluent, *TDE* treated domestic effluent, *RTE* raw textile effluent, *TTE* treated textile effluent, *SE* standard error

*Significant differences between treatment and control group ($p < 0.05$)

[a] Approximately after 17 days of exposure

the second dark cycle, the swimming distance was significantly impaired.

Exposure for 48 h to TDE (Fig. 7) was not generally causative of significant changes ($p > 0.05$) in terms of light/dark cycles; however, animals exposed to PC during the first light cycle had their TT decreased, and their TD increased after the second light cycle ($p < 0.05$). However, 96 h (Fig. 8) of exposure to this effluent caused a decrease ($p < 0.05$) in TT on both light cycles and also following the second dark cycle. TD was significantly impaired ($p < 0.05$) after light cycles in animals exposed to PC; TD was also impacted ($p < 0.05$) after the dark cycle in daphnids exposed to PC and TDE.

In general, 48 h of exposure to RTE (Fig. 9) caused a decrease ($p < 0.05$) in TD in all cycles, being significant in dark cycles. TT was not so affected, showing significant reductions in PC-exposed animals in first light cycle and in organisms exposed to 15% ($p < 0.05$). After 96 h (Fig. 10), TT was only significantly inhibited in PC-exposed organisms ($p < 0.05$) in the first light cycle; TD was not compromised ($p > 0.05$).

In general, after 48 h of exposure to TTE (Fig. 11), daphnids did not suffer behavior alterations in terms of TT ($p > 0.05$), with the exception of PC-exposed organisms in light cycles, a parameter that was significantly decreased ($p < 0.05$); light cycles too provoked a significant decrease ($p < 0.05$) in TD-exposed daphnids. On the other hand, after 96 h of exposure (Fig. 12), TT was significantly affected ($p < 0.05$), with a decrease in this parameter in the first light cycle and in both dark cycles when animals were exposed to PC. In addition, TTE exposure increased swimming distance ($p < 0.05$) in animals exposed to 25% in the first light cycle. The same parameter was affected by PC and 100% exposition ($p < 0.05$) decreasing TD in both dark cycles and just in the second dark cycle, respectively.

Discussion

L. minor phytoremediation-based process

Results from the phytoremediation process using *L. minor* showed to be efficient, by decreasing the COD in both effluents. COD is one of the most widely used parameters indicating organic pollution applied to both wastewater and surface water, and it is defined as the amount of oxygen required to oxidize the organic matter present in wastewater (Arrojo 2006). The higher the COD, the higher the amount of organic pollution in the water sample. Thus, COD is considered one of the most important quality control parameters of an effluent in wastewater treatment facilities (Wu et al. 2011). In other words, *L. minor* was capable of significantly decreasing the amount of organic compounds present in both effluents. This decrease was extremely important since the post-treatment effluents presented COD values of the same order of magnitude of those presented by the control treatment

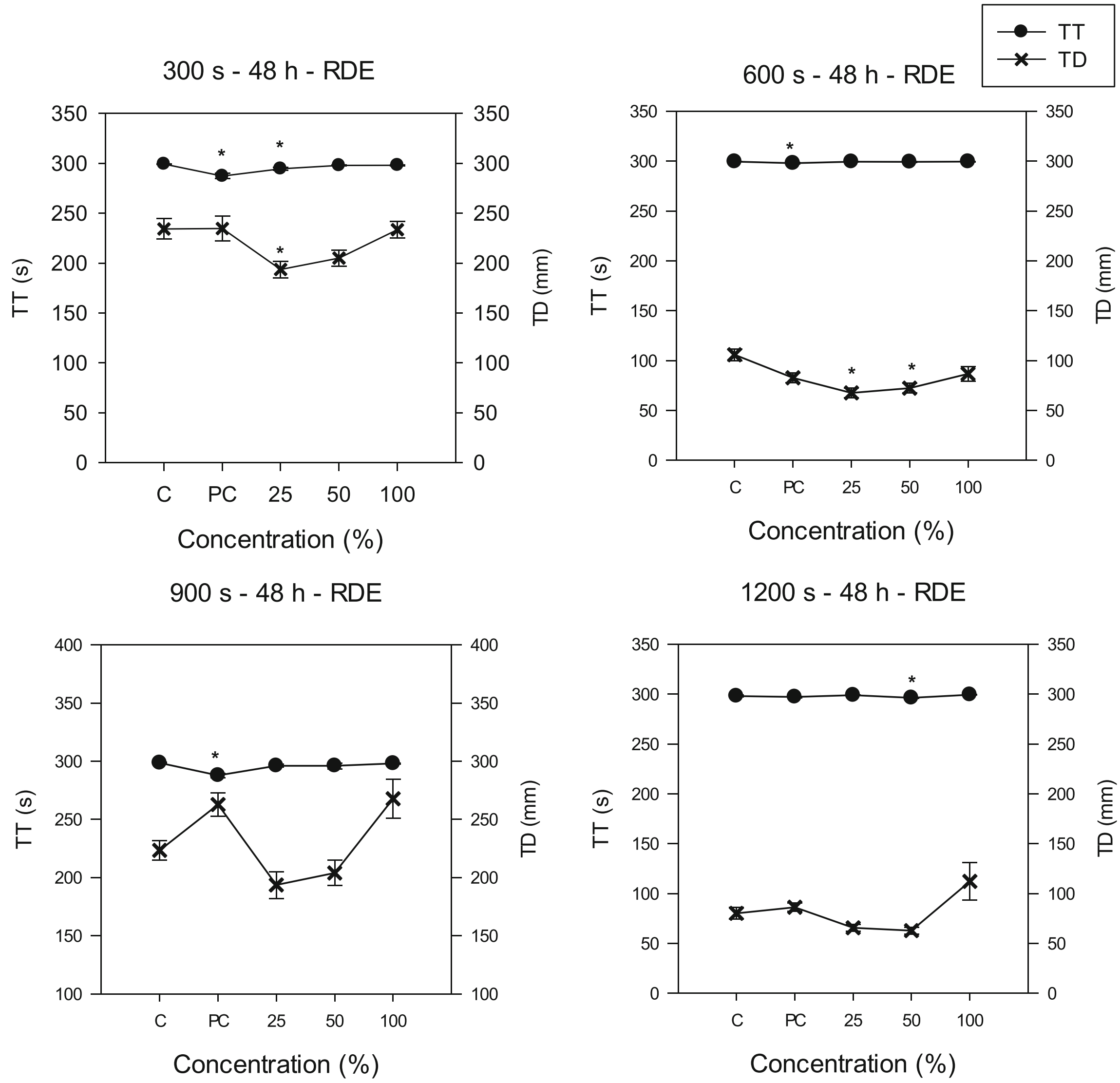

Fig. 5 Effects of raw domestic effluent (RDE—48 h of exposure) on *D. magna* locomotion in dark and light period. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. "*" significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

(Steinberg's media). Moreover, both effluents prior to the phytoremediation process also exhibited low COD values and therefore they would not require additional treatment. From this point of view, it is important to highlight that the treatment by *L. minor* reduced these values even further, which is a positive aspect of phytoremediation since these treated effluents would produce a lesser impact on the environment after being discharged, in relation to their untreated form. Organic wastes mineralize after being discharged into the receiving water bodies and the resulting nutritive elements stimulate plant production, leading to eutrophication (Kanu and Achi 2011). In this situation, the biomass increases considerably and surpasses the assimilation limit by herbivores. The excessive production of organic matter leads to the buildup of "sludge" and the mineralization process consumes all dissolved oxygen from the water column, which is responsible for fish mortality (Kanu and Achi 2011). Thus, *L. minor* could be considered an efficient phytoremediator plant for DE and TE treatment, being potentially used to perform the tertiary treatment of these effluents in order to ensure their final purification, as it was demonstrated by the COD reduction achieved in this work.

On the other hand, it is important to note that the mentioned reduction in COD values by the treatment by *L. minor* was higher for RTE, reaching removal efficiencies around 73%, while for RDE this reduction was of 48%. These variations can be due to the nature of the organic compounds contained in each effluent, which is a decisive factor for their degradability. Thus, more easily degradable organic compounds could be present in higher

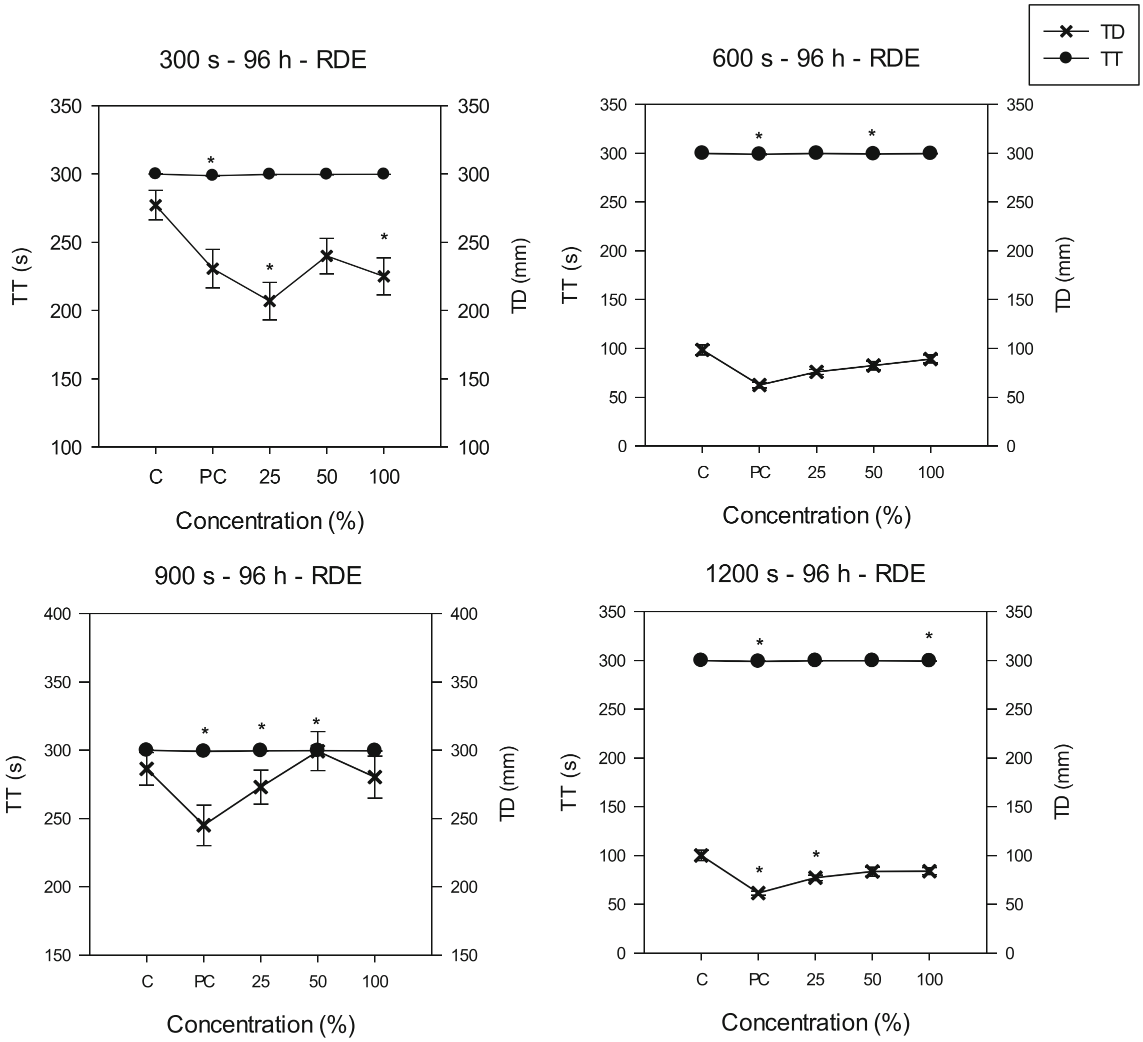

Fig. 6 Effects of raw domestic effluent (RDE—96 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. "*" significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

amounts in textile compared to domestic effluents. Moreover, it is important to mention that in textile samples, a coloration was detected, which was reduced during the here-tested phytoremediation process. In this regard, it is not negligible the data showing that *L. minor* was able to discolor them since it is known that dyes are complex and environmentally troublesome contaminants. The toxic effects of dyes towards the aquatic environment result from their long persistence in the environment, accumulation in sediments and in biota, decomposition of pollutants into carcinogenic or mutagenic compounds, and also low aerobic biodegradability (Samchetshabam et al. 2017). The here-obtained results are in line with previously published data. Chaudhary and Sharma (2014) emphasized that duckweeds are functionally simple, yet easy to maintain, and they can provide a highly effective tertiary treatment, with a performance that is equal or even superior to conventional wastewater treatment systems now recommended for large-scale operations. This efficiency was again demonstrated by Sivakumar (2014) who found a decrease in COD and in color of industrial effluents suggesting the possibility of using *L. minor* for the phytoremediation of various parameters in selected textile industry effluents and in any type of textile industry effluents. In addition, Patel and Kanungo (2010) found a high nutrient removal rate by *L. minor*–based phytoremediation of DEs. These works confirm the possibility of a wide use and improved efficiency of *L. minor* in effluent phytoremediation processes, which are in agreement with our study. Finally, the here measured pH values were decreased after the phytoremediation process, and both effluents became more neutral than alkaline. This pH reduction might be due to microbial action under anaerobic conditions. During microbial respiration, organic matter decomposes and releases CO_2 which may be responsible for decreasing the pH

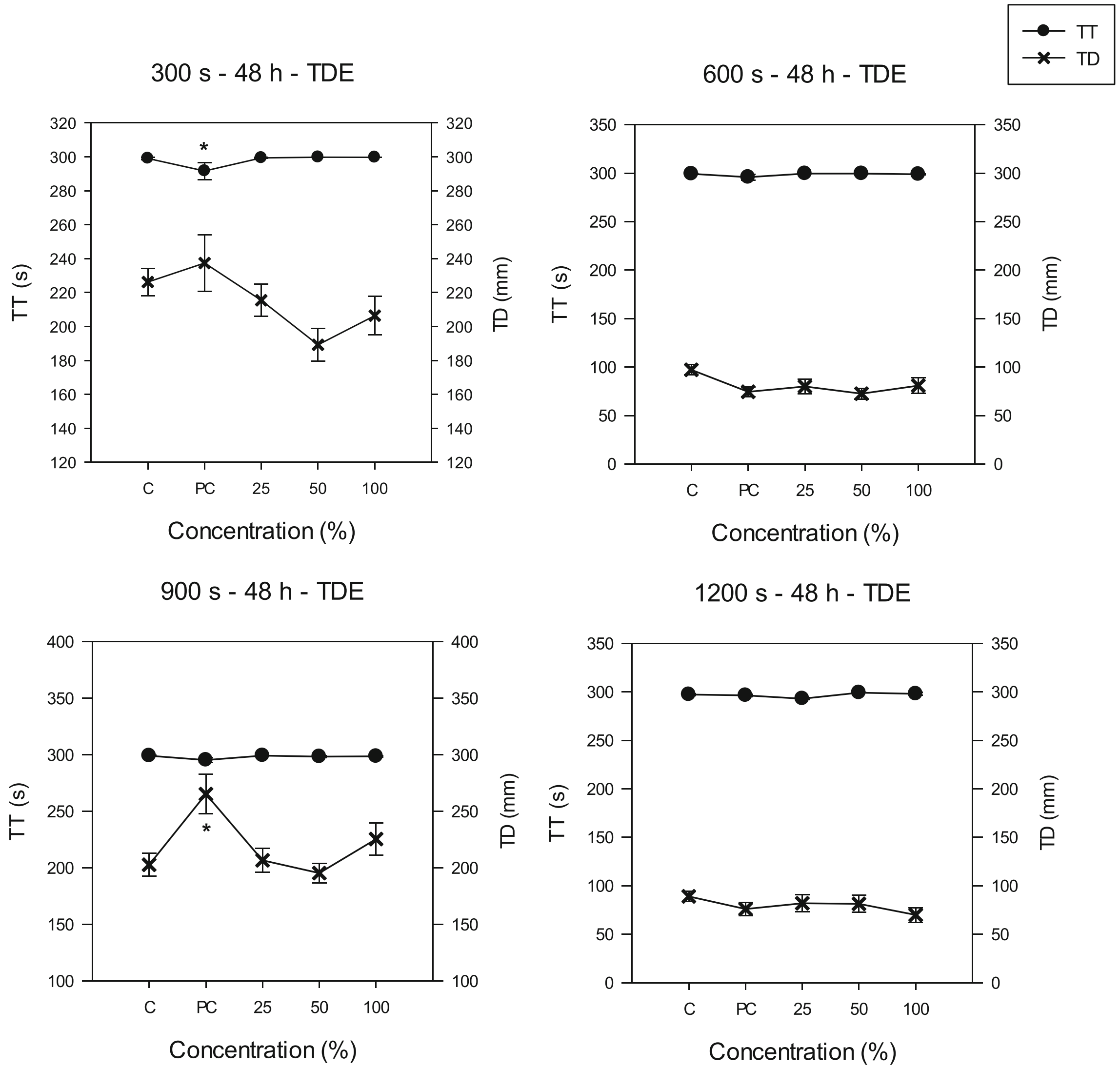

Fig. 7 Effects of treated domestic effluent (TDE—48 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. *Significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

(Mahmood et al. 2005). These results are in line with other phytoremediation studies that used macrophyte species (such as *Eichhornia crassipes* and *Chara vulgaris*) to this purpose (Saha et al. 2017; Mahajan et al. 2019).

Toxic effects on *L. minor*

Chemical environmental stress may occur due to the presence and exposure of biota to chemicals whose metabolism leads to the formation of highly unstable oxygen derivatives, known as reactive oxygen species (ROS). Their accumulation in the cells of plants may cause severe oxidative damage, thus inhibiting growth and grain yield (Caverzan et al. 2016), and to protect themselves from the deleterious effects of ROS, plants express antioxidant mechanisms (Racchi 2013). One option to prevent the occurrence of oxidative damage is through enhancement of CAT activity (Racchi 2013), an enzyme which very efficiently promotes the conversion of hydrogen peroxide (resulting from the dismutation of the superoxide anion by superoxide dismutase) to water and molecular oxygen (Valko et al. 2006). In this study, exposure to RDE was not able to cause alterations in CAT activity; on the other hand, RTE exposure increased the activity of this enzyme, suggesting the establishment of a pro-oxidative scenario. These patterns were also obtained in previous works. Singh et al. (2008) reported a CAT activity increase in *L. minor* after being exposed for 7 days to different concentrations of industrial effluents with high amount of metals. Basiglini et al.

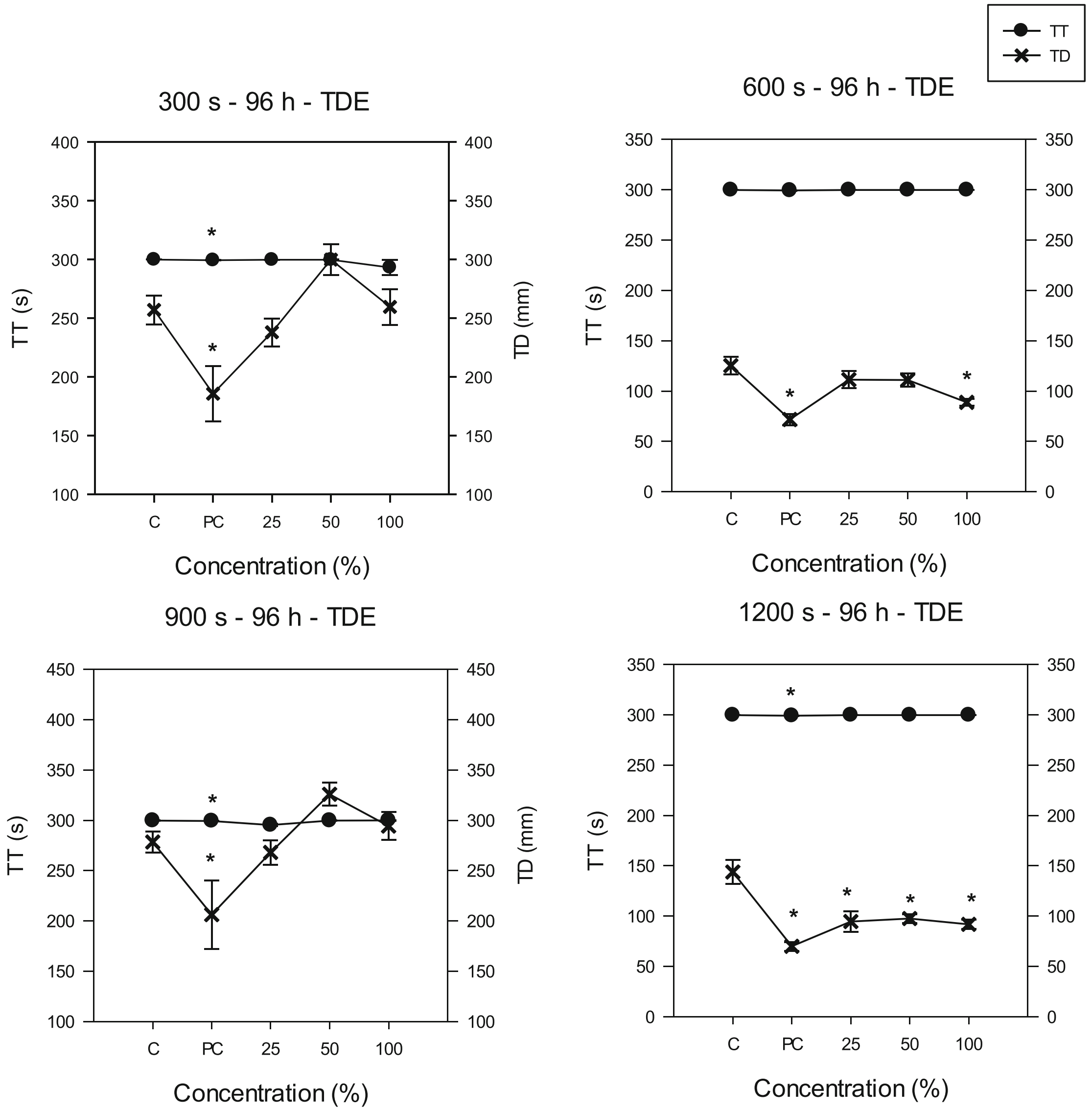

Fig. 8 Effects of treated domestic effluent (TDE—96 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. *Significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

(2018) did not find CAT activity alterations after effluent exposition, but reported significant changes in another antioxidant enzyme responsible for hydrogen peroxide degradation in plants, namely, ascorbate peroxidase (APX). Radić et al. (2010), after exposing *L. minor* to an industrial effluent for 7 days, observed a significant increase in peroxidase activity (POD), an enzymatic form also involved in the antioxidant defense mechanism of plants. In general, it is possible to suggest that the activation of antioxidant defense system linked to oxidative stress in plants occurs after exposure to industrial effluents; however, the pattern of response of the species depends on its level of tolerance, plant growth stage, and concentration of contaminants (Gill and Tuteja 2010).

The antioxidant activity of carotenoids arises primarily as a consequence of the ability of the conjugated double-bonded structure to delocalize unpaired electrons (Mortensen et al. 2001). This is primarily responsible for the excellent ability of β-carotene to physically quench singlet oxygen without degradation and for the chemical reactivity of β-carotene with free radicals (Valko et al. 2006). The here-obtained results showed that DE was not capable to cause alterations in Car content, but RTE exposure significantly decreased this pigment content. This result is in agreement with the previously discussed data concerning CAT activity, for which RDE was not capable to cause alterations in this enzyme's activity, while RTE exposure was responsible for its decrease, which may

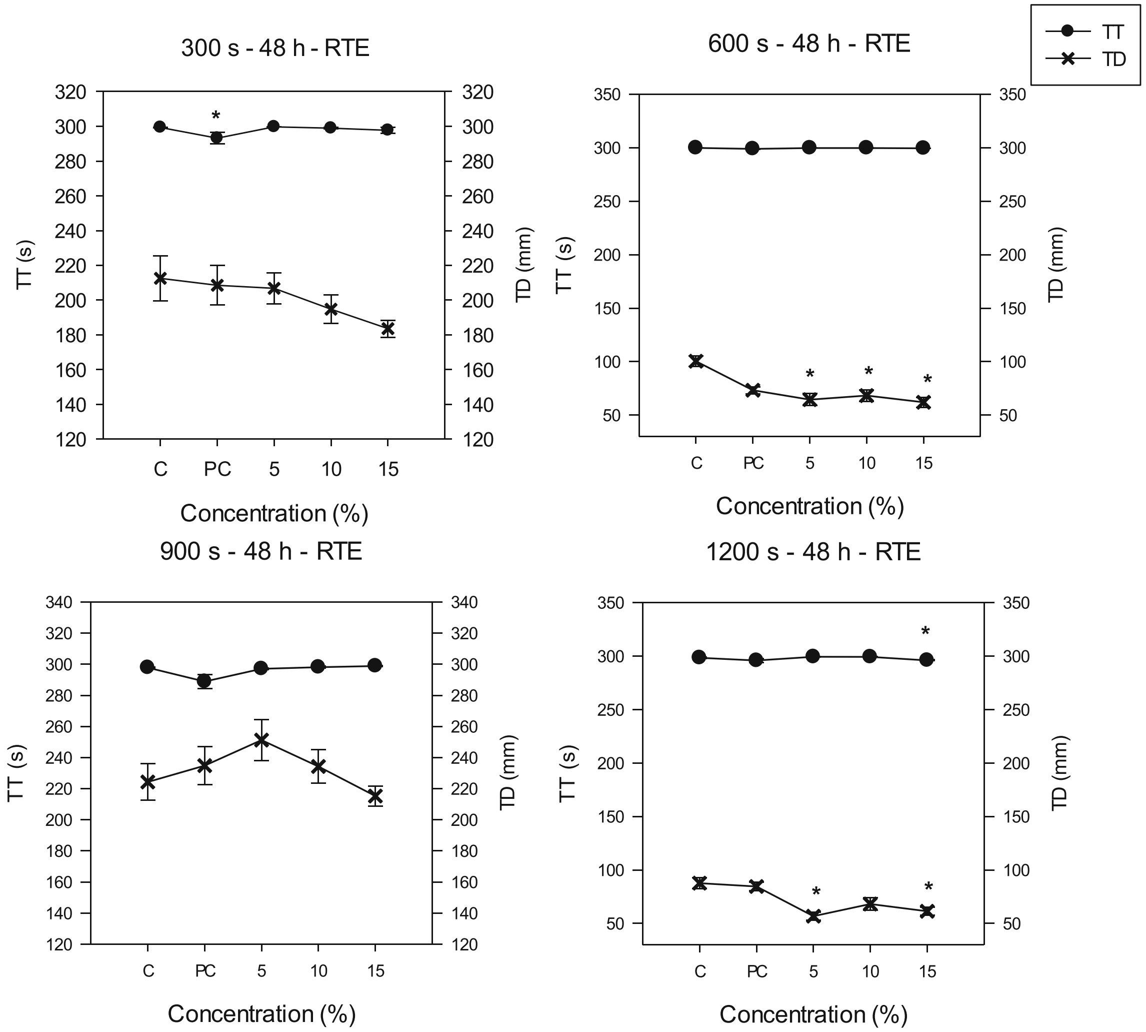

Fig. 9 Effects of raw textile effluent (RTE—48 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. *Significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

suggest a mechanism defense against ROS increase. The excess of released ROS that occurs in response to the metabolism of chemicals found in the analyzed effluents is capable of causing the oxidation of Car, thereby decreasing their overall content in plants exposed to this effluent. Decrease in Car content was also reported by Brkanac et al. (2010) after analyzing a long-term exposure of *L. minor* to different surface water samples contaminated with effluent discharge (namely municipal and industrial effluent from Croatia). Considering the here-obtained data, and from the literature, it is possible to conclude that this pigment may be considered a sensible parameter to assess abiotic environmental stress.

Anthocyanin is a secondary metabolite that can be produced in response to oxidative stress, performing important protection roles (Juszczuk et al. 2004), such as a scavenger of a wide array of reactive oxygen and nitrogen species (Dauphinee et al. 2017) and other mechanisms of defense (sunscreen and metal chelating) (Landi et al. 2015). An increase in its content might indicate the occurrence of oxidative stress; on the other hand, a decreasing trend of its levels means a failure of the (antioxidant) defense system (Miguel 2011). In this study, *L. minor* exposed to RDE had an increase in AA content indicating the occurrence of pro-oxidative alterations that were, however, effectively counteracted by this defensive mechanism. On the contrary, plants exposed to RTE had decreased levels of these pigments suggesting that the oxidant process was capable of significantly oxidizing these compounds. This set of results regarding AA levels suggests a potential failure on the antioxidant defense system. Since the Car content in these RTE-exposed plants was not affected, alternative mechanisms of defense (e.g., CAT) must have been activated—

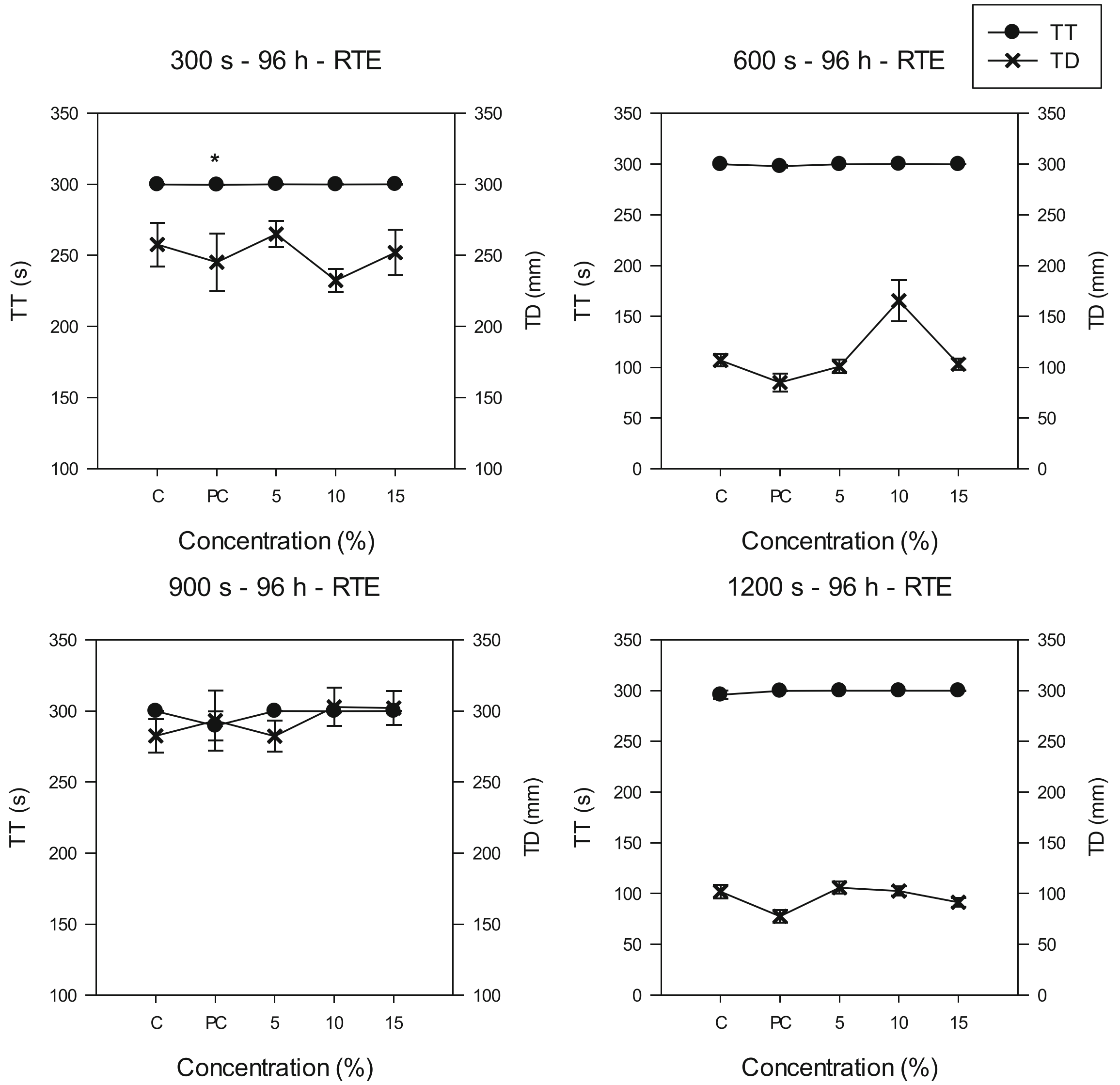

Fig. 10 Effects of raw textile effluent (RTE—96 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. *Significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

indeed, this parameter was significantly increased following exposure to this effluent. Normally, AA is not a pigment commonly analyzed to determine effluent toxicity, but this study demonstrated its usefulness and sensitivity to address the toxicity of both domestic and/or industrial effluents.

However, under oxidative stress, if it is mild or intermediate, organisms usually block general programs of their life cycle (such as reproduction or extensive biosynthesis), to develop responses to prevent or neutralize negative ROS effects (Lushchak 2014). Photosynthetic pigments (namely chlorophylls) are included in the group of physiologically important substances whose biosynthesis may be compromised by chemical insult, as demonstrated by some studies addressing the effects of different classes of chemical contaminants. Roy et al. (2015), after exposing *L. minor* for 96 h to 10–25% of tannery industrial effluent, found a significant decrease in Chl content. Tkalec et al. (2008) obtained similar results with the same species after 6 and 12 days of cadmium exposure, and a decrease in *L. minor* Chls was reported by Fekete-Kertész et al. (2015) after exposure to chemicals such as benzophenone and bisphenol A during 7 days. This inhibitory effect in terms of Chlor levels was reported after exposing *L. minor* to RTE, but not to RDE; a failure of the antioxidant defense mechanisms was closely followed by significant decreases in the contents of both Chlor a and b. On the other hand, and despite oxidative damages, the exposure to effluents caused an increase in root size, which may be associated with a large amount of nutrients, normally present in effluents, that favored their own uptake and enhanced the plant's metabolism and growth, characteristics described in the literature for some

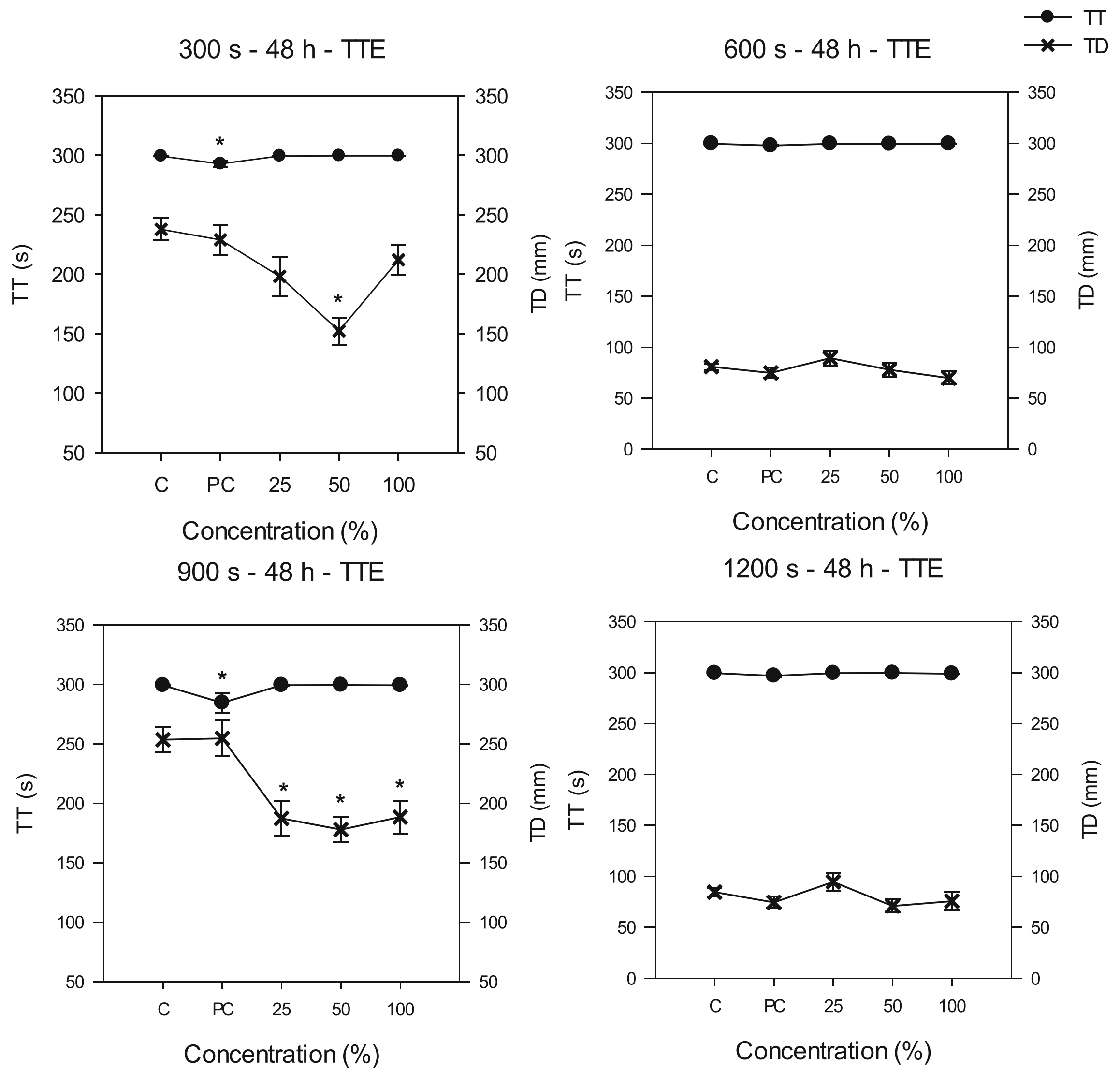

Fig. 11 Effects of treated textile effluent (TTE—48 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance

plant genera/species, including *Lemna* spp. (Cedergreen and Madsen 2002). This response has already been reported after exposure to industrial effluents (Patel and Kanungo 2010). The results about the increase of the root size in plants exposed to the effluents may be in conflict with the decrease of pigments levels, an effect that was observed in these plants as well, as shown above. However, stressed plants (as those analyzed in this study, which were subjected to stressful biotic and/or abiotic factors) are capable to allocate energy to different physiological systems according to their necessities (Nguyen et al. 2016; Mundim and Pringle 2018). This can be an explanation for our findings since plants spent more energy in favoring their root growth to enhance nutrient absorption, in relation to the energy invested to produce pigments. This assumption is explained by Bazzaz et al. (1987) since, at an ecological level, energy budget allocation includes the relationship between investment in one function and investment in others, such as the relationship between defense and growth; at a physiological level, energy allocation entails the partitioning of resources within the plant and the consequences of this partitioning to favor gain or loss of critical resources.

Toxic effects on *D. magna*

The effect of dilutions of the effluent (raw and post-treatment) on daphnids was examined with acute (mortality and behavior) and sub-chronic (reproduction) toxicity tests. Acute test data showed that RDE and TDE had no significant acute toxicity. Considering these data, it is possible to suggest two possibilities: (1) the treatment applied to wastewater by plants (namely *L. minor*) is effective enough to remove most toxic

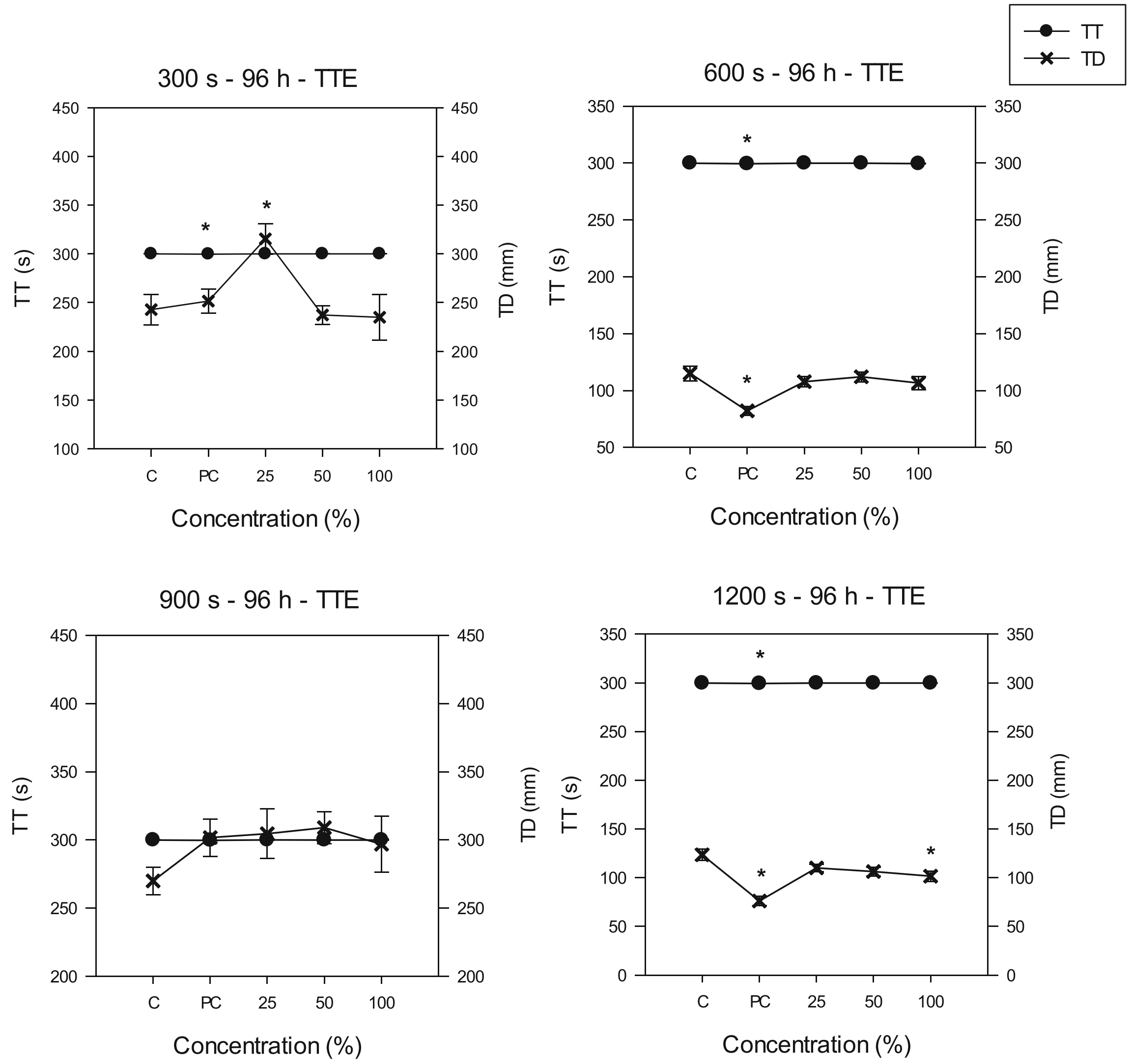

Fig. 12 Effects of treated textile effluent (TTE—96 h of exposure) on *D. magna* locomotion in dark and light periods. Values are mean values ± standard error. 300 and 900 s corresponding to light periods, 600 and 1200 s corresponding to dark periods. C: control, PC: phytoremediation control, TT: total swimming time, TD: total distance. *Significant differences between treatment and control ($p < 0.05$). For each parameter, mean and standard error are shown

compounds, or (2) this specific effluent that was here-tested did not receive a considerable load of toxic compounds. This is not in agreement with the literature since in most cases, RDE are toxic to aquatic organisms, such as *Selenastrum capricornutum* (Ra et al. 2007), *D. magna* (Ra et al. 2007; Gholami-Borujenia et al. 2018), and *Danio rerio* (Zhang et al. 2012).

The toxicity of RTE towards *D. magna* was possible to be converted into an EC_{50} value and into TUa, showing the evident toxicity of this effluent. This was a somehow expected result, considering its great complexity, as mentioned previously. In addition, these results are in agreement with those from the literature (Karthikeyan and Meyer 2006; Verma 2008; Gebrati et al. 2011), which show the correlation between EC_{50} and TUa with industrial effluent toxicity. On the other hand, after the phytoremediation process, these values were no longer possible to be calculated since TTE did not show any measurable acute toxicity, thus indicating the efficiency of the phytoremediation process with *L. minor* to treat this effluent. The increased efficacy of the treatment process, yielded a significant improvement in the quality of the effluent, which certainly can be attributed to the capacity of *L. minor* to absorb substances from the medium (Ugya 2015) and to its capacity to decrease the levels of those chemicals that were initially responsible for the higher COD values that were measured in this effluent.

These results are in agreement with the results of the reproduction test. *D. magna* is well known for its modulation of reproductive features, showing different reproduction strategies according to the environmental conditions in which they are kept, food amount and sources, presence of toxic substances, and other factors, such as genetic variability (Ebert 1993; Enserink et al. 1993; Viganò 1993). In this study, only the RTE effluent (raw and treated) caused alterations in the number of neonates. Part of this result was already expected since it was not possible to calculate values of EC_{50} and TUa for RDE. These results may, however, be indicative, but not a statement, of the absence of toxicity by this effluent. On the contrary, such parameters were indeed calculated for TE, indicating its likely toxicity. Furthermore, the composition of the here-tested effluents may be a factor influencing these results since as described in other section of this discussion, in general, RDE had a potentially higher concentration of organic compounds (such as nutrients), while RTE is most likely a complex mixture with different organic and inorganic compounds, including metals, dyes, and detergents that are toxic to the here-used model organism, *D. magna* (Westlake et al. 1983; Rodriguez et al. 2006; Gholami-Borujenia et al. 2018).

The here-tested phytoremediation process significantly decreased COD in RTE, but as a complex mixture, it may have a high variety and concentration of compounds, such as dyestuffs, salts, acids, bases, surfactants, dispersants, humectants, oxidants, and detergents, which render these waters esthetically unacceptable and unusable (Khandare and Govindwar 2015). However, these substances cannot be completely removed by phytoremediation processes (Bokhari et al. 2016; Khataee et al. 2012; Mkandawire and Dudel 2007) and may subsist in the treated effluent even after the treatment; in this case, it is plausible to find a mixture of chemicals that includes organics, metals, and azo dyes. Thus, reproduction alterations provoked by TTE on *D. magna* can be associated to these compounds. Similar results have been already demonstrated in previous studies from the literature. Flohr et al. (2012) obtained similar results (reproduction increase) after exposing *D. magna* for 21 days to the soluble fraction of different industrial wastes. This result is not entirely surprising since an increase in reproduction after exposure to an environmental contaminant may suggest a forced attempt of assuring the conservation of the species when challenged (Terra et al. 2008).

In relation to *D. magna* behavior, in general, effluent exposures (RDE, TDE, RTE, and TTE) reduced swim distance of the test organisms. Behavioral modifications are normally associated with neural disorders (Tierney 2011). A number of substances potentially present in DE and in TE are capable of causing changes in behavior of aquatic organisms, such as metals (Semsari and Megateli 2010), pharmaceuticals (Rivetti et al. 2016), and azo dyes (Barot and Bahadur 2015). Thus, behavioral traits may be considered highly integrative parameters that react to the presence of a large array of environmental chemicals, especially those that are part of a complex matrix such as treated effluents.

Conclusion

Both effluents were capable of altering several key parameters of *L. minor*, namely morphological (root length) and physiological/biochemical (pigment content and catalase activity) endpoints. Despite these findings, the here used plant species showed to be highly promising in terms of its effectiveness to be used in tertiary treatment (phytoremediation) of contaminated effluents. This efficacy was reflected by a significant decrease of COD (in both effluents) and, for RTE, a noteworthy decrease of the acute toxicity parameters measured in *D. magna*. In addition, RTE (raw and treated) was shown to have higher toxicity since it caused reproduction alterations in *D. magna*, while RDE did not. Lastly, behavior alterations in *D. magna* were elicited by both effluents pre- and post-treatment, suggesting the existence of neurotoxic chemicals in the effluents, even after treatment.

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 6:105–121. https://doi.org/10.1016/S0076-6879(84)05016-3

Al-Jlil SA (2009) COD and BOD reduction of domestic wastewater using activated sludge, sand filters and activated carbon in Saudi Arabia. Biotechnology 8:473–477. https://doi.org/10.3923/biotech.2009.473.477

Alkimin GD, Daniel D, Frankenbach S, Serôdio J, Soares AMVM, Barata C, Nunes B (2019) Evaluation of pharmaceutical toxic effects of non-standard endpoints on the macrophyte species *Lemna minor* and *Lemna gibba*. Sci Total Environ 657:926–937

Arrojo BA (2006) Advances systems for biological treatment of high nitrogen-loaded wastewater. PhD thesis. Universidad De Santiago de Compostela

ASTM (2014) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken

Barot JK, Bahadur A (2015) Toxic impacts of C.I. Acid Orange 7 on behavioural, haematological and some biochemical parameters of *Labeo rohita* fingerlings. International Journal of Scientific Research in Environmental Sciences 3:284–290. https://doi.org/10.12983/ijsres-2015-p0284-0290

Basiglini E, Pintore M, Forni C (2018) Ecotoxicology and environmental safety effects of treated industrial wastewaters and temperatures on growth and enzymatic activities of duckweed (*Lemna minor* L.). Ecotoxicol Environ Saf 153:54–59

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. BioScience 37(1):58–67

Bokhari SH, Ahmad I, Mahmood-ul-hassan M, Mohammad A (2016) Phytoremediation potential of *Lemna minor* L. for heavy metals.

International Journal of Phytoremediation 18:25–32. https://doi.org/10.1080/15226514.2015.1058331

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Brkanac, S.R., Stipaničev, D., Širac, S., Glavaš, K., Pevalek-Kozlina (2010) Biomonitoring of surface waters using duckweed (*Lemna minor* L.) BALWOIS 2010—Ohrid, Republic of Macedonia, 6p

Caverzan A, Casassola A, Patussi Brammer S (2016) Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. Abiotic Biotic Stress Plants - Recent Adv Future Perspect. https://doi.org/10.5772/61368

Cedergreen N, Madsen TV (2002) Nitrogen uptake by the floating macrophyte *Lemna minor*. New Phytol 155:285–292

Chang SH, Chuang SH, Li HC, Liang HH, Huang LC (2009) Comparative study on the degradation of I.C. Remazol Brilliant Blue R and I.C. Acid Black 1 by Fenton oxidation and FeO/air process and toxicity evaluation. J Hazard Mater 166:1279–1288

Chaudhary E, Sharma P (2014) Use of duckweed in wastewater treatment. International Journal of Innovative Research in Science, Engineering and Technology 6:13622–13624

Daniel D, Dionísio R, Alkimin GD, Nunes B (2018) Acute and chronic effects of paracetamol exposure on Daphnia magna: how oxidative effects may modulate responses at distinct levels of organization in a model species. Environ Sci Pollut Res 26:3320–3329. https://doi.org/10.1007/s11356-018-3788-y

Dauphinee AN, Fletcher JI, Denbigh GL, Lacroix CR, Gunawardena AHLAN (2017) Remodelling of lace plant leaves: antioxidants and ROS are key regulators of programmed cell death. Planta. 246:133–147. https://doi.org/10.1007/s00425-017-2683-y

Dionísio, R., Daniel, D., Alkimin, G.D., Nunes, B. (2020) Multiparametric analysis of ciprofloxacin toxicity at ecologically relevant levels: short- and long-term effects on *Daphnia magna*. Environmental toxicology and pharmacology. Accepted paper (editorial process)

Ebert D (1993) The trade off between offspring size and number in *D. magna* the influence of genetic, environmental and maternal effects. Arch Hidrobiol 4:453–473

Enserink L, de la Haye M, Maas H (1993) Reproductive strategy of *Daphnia magna*: implications for chronic toxicity tests. Aquat Toxicol 25:111–123. https://doi.org/10.1016/0166-445x(93)90023-t

EPA (1993) Method 410.4, Revision 2.0: The Determination of Chemical Oxygen Demand by Semi-Automated Colorimetry. United States Environmental Protection Agency. Address: <https://www.epa.gov/sites/production/files/2015-08/documents/method_410-4_1993.pdf>

Fekete-Kertész I, Kunglné-Nagy Z, Gruiz K (2015) Assessing toxicity of organic aquatic micropollutants based on the total chlorophyll content of *Lemna minor* as a sensitive endpoint. Periodica Polytechnica Chemical Engineering 59:262–271

Flohr L, Castilhos Júnior AB, Matias WG (2012) Acute and chronic toxicity of soluble fractions of industrial solid wastes on *Daphnia magna* and *Vibrio fischeri*. Sci World J 2012:643904. https://doi.org/10.1100/2012/643904

Gebrati L, Idrissi LL, Mouabad A, Nejmeddine A (2011) Use of Daphnia test for assessing the acute toxicity of effluents from a textile industry in Marrakech (Morocco). Phys Chem News 60:133–140

Ghaly AE, Ananthashankar R, Alhattab M, Ramakrishnan VV (2014) Production, characterization and treatment of textile effluents: a critical review. J Chem Eng Process Technol 5:182. https://doi.org/10.4172/2157-7048.1000182

Gholami-Borujenia F, Nejatzadeh-Barandozib F, Aghdasi H (2018) Data on effluent toxicity and physicochemical parameters of municipal wastewater treatment plant using *Daphnia magna*. Data in Brief 19:1–7

Gill SS, Tuteja N (2010) Plant physiology and biochemistry reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gogate PR, Pandit AB (2004) A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. Adv Environ Res 8:501–551. https://doi.org/10.1016/S1093-0191(03)00032-7

Herath I, Vithanage M (2015) Phytoremediation in constructed wetlands. In: Ansari AA et al (eds) Phytoremediation: management of environmental contaminants, V.2, pp 243–263. https://doi.org/10.1007/978-3-319-10969-5_21

Hernando MD, Fernández-Alba AR, Tauler R, Barceló D (2005) Toxicity assays applied to wastewater treatment. Talanta. 65:358–366

Ibañez SG, Oller ALW, Paisio CE, Alderete LGS, González PS, Medina MI, Agostini E (2018) The challenges of remediating metals using phytotechnologies. In: Donati, E.R. (eds.), Heavy metals in the environment: microorganisms and bioremediation. CRC Press, Taylor & Francis. pp.173–191. DOI: https://doi.org/10.1201/b22013-9

Juszczuk IM, Wiktorowska A, Malus E, Rychter AM (2004) Changes in the concentration of phenolic compounds and exudation induced by phosphate deficiency in bean plants (*Phaseolus vulgaris* L.). Plant Soil 267:41–49

Kanu I, Achi OK (2011) Industrial effluents and their impact on water quality of receiving rivers in Nigeria. Journal of applied Technology in Environmental Sanitation 1:75–86

Karthikeyan KG, Meyer MT (2006) Occurrence of Antibiotics in Wastewater Treatment Facilities in Wisconsin, USA. Science of The Total Environment 361(1-3):196–207. https://doi.org/10.1016/j.scitotenv.2005.06.030

Khandare RV, Govindwar SP (2015) Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnol Adv 33:1697–1714

Kharat DS (2015) Treatment of textile industry effluents: limitations and scope. J Environ Res Develop 9:1210–1213

Khataee AR, Movafeghi A, Torbati S, Lisar SYS, Zarei M (2012) Phytoremediation potential of duckweed (*Lemna minor* L.) in degradation of C.I. Acid Blue 92: artificial neural network modeling. Ecotoxicol Environ Saf 80:291–298

Klamerth N, Rizzo L, Malato S, Maldonado MI, Agüera A, Fernández-Alba AR (2010) Degradation of fifteen emerging contaminants at mg LL1 initial concentrations by mild solar photo-Fenton in MWTP effluents. Water Res 44:545–554

Lameira, V., 2008. Estudos dos efeitos letais e subletais (reprodução e teratogênese) do fármaco triclosan para Daphnia similis, Ceriodaphnia dubia, Ceriodaphnia silvestrii (Cladocera, Crustacea). Dissertação de Mestrado. Instituto de Pesquisas Energeticas e Nucleares—USP. DOI: https://doi.org/10.11606/D.85.2008.tde-09092009-182753

Landi M, Tattini M, Gould KS (2015) Multiple functional roles of anthocyanins in plant–environment interactions. Environ Exp Bot 119:4–17

Lima SB, Souza DC, Albuquerque FP (2015) Confection of ceramic bricks with biomass contaminated by cupper incorporation. Ambiência, [s.l.] 11(1):31–47. https://doi.org/10.5935/ambiencia.2015.01.02

Lushchak VI (2014) Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 224:164–175

Lyu J, Park J, Kumar Pandey L, Choi S, Lee H, De Saeger J, Depuydt S, Han T (2018) Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in *Lactuca sativa* L. Ecotoxicol Environ Saf 149:225–232

Mahajan P, Kaushal J, Upmanyu A, Bhatti J (2019, 2019, 11p. ID 8351272) Assessment of phytoremediation potential of *Chara vulgaris* to treat toxic pollutants of textile effluent. Journal of Toxicology. https://doi.org/10.1155/2019/8351272

Mahmood Q, Zheng P, Islam E et al (2005) Lab scale studies on water hyacinth (*Eichhornia crassipes* Marts Solms) for biotreatment of textile effluent. Caspian Journal of Environmental Science 3:83–88

Miguel MG (2011) Anthocyanins: antioxidant and/or anti-inflammatory activities. Journal of Applied Pharmaceutical Science 1:07–15

Mkandawire M, Dudel EG (2007) Are *Lemna* spp. effective phytoremediation agents? Bioremediation, Biodiversity and Bioavailability 1:56–71

Mortensen A, Skibsted LH, Truscott TG (2001) The interaction of dietary carotenoids with radical species. Arch Biochem Biophys 385:13–19. https://doi.org/10.1006/abbi.2000.2172

Mundim FM, Pringle EG (2018) Whole-plant metabolic allocation under water stress. Front Plant Sci 9:852. https://doi.org/10.3389/fpls.2018.00852

Nguyen D, Rieu I, Mariani C, van Dam NM (2016) How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. Plant Mol Biol 91:727–740. https://doi.org/10.1007/s11103-016-0481-8

OECD, 2004. Test no. 202: *Daphnia* sp. acute immobilization test, OECD guidelines for the testing of chemicals, section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264069947-en

OECD (2006) Test no. 221: *Lemna* sp. growth inhibition test. OECD guidelines for testing of chemicals, section 2. OECD Publishing, Paris. https://doi.org/10.1787/20745761

OECD (2012) Test No. 211: *Daphnia magna* reproduction test, OECD guidelines for the testing of chemicals, section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264185203-en

Olguín EJ, Sánchez-Galván G (2012) Heavy metal removal in phytofiltration and phycoremediation: the need to differentiate between bioadsorption and bioaccumulation. New Biotechnol 30:1–8

Paisio CE, Fernandez M, González PS, Talano MA, Medina MI, Agostini E (2017) Simultaneous phytoremediation of chromium and phenol by *Lemna minuta* Kunth: a promising biotechnological tool. Int J Environ Sci Technol 15:37–48. https://doi.org/10.1007/s13762-017-1368-1

Patel DK, Kanungo VK (2010) Phytoremediation potential of duckweed (*Lemna minor* L: a tiny aquatic plant) in the removal of pollutants from domestic wastewater with special reference to nutrients. The Bioscan 5:355–358

Pawęska K, Bawiec A (2017) Activated sludge technology combined with hydroponic lagoon as a technology suitable or treatment of wastewater delivered by slurry tanks. Journal of Ecological Engineering 18:29–37

Persoone G, Baudo R, Cotman M, Blaise C, Thompson KC, Moreira-Santos M, Vollat B, Törökne A, Han T (2009) Review on the acute *Daphnia magna* toxicity test—evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowl Manag Aquat Ecosyst 393:01. https://doi.org/10.1051/kmae/2009012

Ra JS, Kim HK, Chang NI, Kim SD (2007) Whole effluent toxicity (WET) tests on wastewater treatment plants with *Daphnia magna* and *Selenastrum capricornutum*. Environ Monit Assess 129:107–113. https://doi.org/10.1007/s10661-006-9431-2

Racchi M (2013) Antioxidant defenses in plants with attention to *Prunus* and *Citrus* spp. Antioxidants. 2:340–369. https://doi.org/10.3390/antiox2040340

Radić S, Stipaničev D, Cvjetko P, Mikelić IL, Rajčić MM, Širac S, Pevalek-Kozlina B, Pavlica M (2010) Ecotoxicological assessment of industrial effluent using duckweed (*Lemna minor* L.) as a test organism. Ecotoxicology. 19:216–222. https://doi.org/10.1007/s10646-009-0408-0

Rajasulochana P, Preethy V (2016) Comparison on efficiency of various techniques in treatment of waste and sewage water—a comprehensive review. Resource-Efficient Technologies 2:175–184. https://doi.org/10.1016/j.reffit.2016.09.004

Ribeiro F, Ferreira NGC, Ferreira A, Soares AMVM, Loureiro S (2011) Is ultraviolet radiation a synergistic stressor in combined exposures? The case study of *Daphnia magna* exposure to UV and carbendazim. Aquat Toxicol 102(1–2):114–122. https://doi.org/10.1016/j.aquatox.2011.01.007

Rivetti C, Campos B, Barata C (2016) Low environmental levels of neuro-active pharmaceuticals alter phototactic behaviour and reproduction in *Daphnia magna*. Aquat Toxicol 170:289–296

Rizzo L (2011) Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water Res 45:4311–4340

Rodriguez P, Martinez-Madrid M, Cid A (2006) Ecotoxicological assessment of effluents in the Basque country (northern Spain) by acute and chronic toxicity tests using *Daphnia magna* Straus. Ecotoxicology. 15:559–572. https://doi.org/10.1007/s10646-006-0091-3

Roy S, Nagarchi L, Das I, Achuthananthan JM, Krishnamurthy S (2015) Cytotoxicity, genotoxicity, and phytotoxicity of tannery effluent discharged into Palar River basin, Tamil Nadu, India. Journal of Toxicology. Article ID 504360, 9p. https://doi.org/10.1155/2015/504360

Saha P, Banerjee A, Sarkar S (2015) Phytoremediation potential of duckweed (*Lemna minor* L.) on steel wastewater. International Journal of Phytoremediation 17:589–596. https://doi.org/10.1080/15226514.2014.950410

Saha P, Shinde O, Sarkar S (2017) Phytoremediation of industrial mines wastewater using water hyacinth. International Journal of Phytoremediation 19(1):87–96. https://doi.org/10.1080/15226514.2016.1216078

Samchetshabam G, Hussan A, Choudhury TG (2017) Impact of textile dyes waste on aquatic environments and its treatment. Environment & Ecology 35:2349–2353

Semsari S, Megateli S (2007) Effect of cadmium toxicity on survival and phototactic behaviour of *Daphnia magna*. Environ Technol 28:799–806. https://doi.org/10.1080/09593332808618841

Seow TW, Lim CK, Nor MHM, Hanif M, Muhammad MMF, Yong LC, Adibah Y, Zaharah I (2016) Review on wastewater treatment technologies. International Journal of Applied Environmental Sciences 11:111–126

Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sensing Environ 81:337–354

Singh K, Misra A, Pandey SN (2008) Responses of *Lemna minor* L. (duckweed) plants to the pollutants in industrial waste water. Res Environ Life Sci 1:5–8

Sivakumar D (2014) Role of *Lemna minor* Lin. in treating the textile industry wastewater. International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering 8: 208–212

Souza CB, Silva GR (2019) Phytoremediation of effluents contaminated with heavy metals by floating aquatic macrophytes species, IntechOpen, DOI: https://doi.org/10.5772/intechopen.83645. Available from: https://www.intechopen.com/online-first/phytoremediation-of-effluents-contaminated-with-heavy-metals-by-floating-aquatic-macrophytes-species

Terra NR, Feiden IR, Fachel JMG, Lemos CT, Nunes EA (2008) Ecotoxicological evaluation of sediment and water samples from Sinos River, Rio Grande do Sul, Brazil, using Daphnia magna and V79 cells. Acta Limnologica Brasiliensia 20:63–72

Tierney KB (2011) Behavioural assessments of neurotoxic effects and neurodegeneration in zebrafish. Biochim Biophys Acta (BBA) - Mol Basis Dis 1812:381–389. https://doi.org/10.1016/j.bbadis.2010.10.011

Tkalec M, Prebeg T, Roje V, Pevalek-Kozlina B, Ljubešić N (2008) Cadmium-induced responses in duckweed *Lemna minor* L. Acta

Physiol Plant 30:881–890. https://doi.org/10.1007/s11738-008-0194-y

Türker OC, Türe C, Böcük H, Yakar A (2014) Constructed wetlands as green tools for management of boron mine wastewater. International Journal of Phytoremediation 16:537–553. https://doi.org/10.1080/15226514.2013.798620

Ugya AY (2015) The efficiency of *Lemna minor* L. in the phytoremediation of Romi stream: a case study of Kaduna refinery and petrochemical company polluted stream. J App Biol Biotech *3*: 11–14. https://doi.org/10.7324/JABB.2015.3102

Vacchi FI, Von der Ohe PC, Albuquerque AF, Vendemiatti JAS, Azevedo CCJ, Honório JG, Silva BFD, Zanoni MVB, Henry TB, Nogueira AJ, Umbuzeiro GA (2016) Occurrence and risk assessment of an azo dye—the case of disperse red 1. Chemosphere. 156:95–100. https://doi.org/10.1016/j.chemosphere.2016.04.121

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40. https://doi.org/10.1016/j.cbi.2005.12.009Chem Biol Interact

Verma Y (2008) Acute toxicity assessment of textile dyes and textile and dye industrial effluents using *Daphnia magna* bioassay. Toxicology and Industrial Healt 24:491–500. https://doi.org/10.1177/0748233708095769

Viganò L (1993) Reproductive strategy of *Daphnia magna* and toxicity of organic compounds. Water Res 27:903–909

Wellburn AR, Lichtenthaler H (1984) Formulae and program to determine total carotenoids and chlorophyll a and b of leaf extracts in different solvents. In Advances in Photosynthesis Research 2:9–12

Westlake GF, Sprague JB, Hines RJ, Brown IT (1983) Sublethal effects of treated liquid effluent from a petroleum refinery. III. Avoidance and other locomotor responses of rainbow trout. Aquat Toxicol 4:235–245. https://doi.org/10.1016/0166-445x(83)90020-6

Wu GQ, Bi WH, Lui JM, Fu GW (2011) Determination of chemical oxygen demand in water using near-infrared transmission and UV absorbance method. Chin Opt Lett 9:310705

Zhang W, Liu W, Zhang J, Zhao H, Zhang Y, Quan X, Jin Y (2012) Characterization of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish. J Environ Sci (China) 24: 2019–2027

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