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Uptake of Cadmium by *Lemna minor*, a (hyper?-) accumulator plant involved in phytoremediation applications

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Abstract. Metal pollution in waters and soils is a major environmental and human health problem. Cadmium (Cd^{2+}) is a heavy metal displaying toxic effects in plants. In this work we studied the potentiality of *Lemna minor*, a monocotyledonous aquatic macrophyte, to phytoremediate cadmium-polluted waters. The plants were exposed to different cadmium concentrations 0, 13, 22 and 46μM $CdSO_4$ for a period of 24, 48 and 72 hours. Relative growth rates (RGR), bioconcentration factor (BCF), tolerance index (T_i), cadmium uptake in whole plant and maximum efficiency of PSII (Fv/Fm) were measured under controlled climate conditions. RGR, T_i and Fv/Fm declined with increasing exposure time and cadmium concentrations, while the BCF and cadmium uptake showed an opposite behavior. Data analysis of RGR, BCF, T*i* and F_V/F_M indicates that *L. minor* maintains a good capacity of growth, metal bioconcentration, tolerance and efficiency of PSII up to 48h in plants exposed to 13 and 22μM $CdSO_4$. Our results exhibited that *L. minor* is a good cadmium accumulator and is able to remediate Cd-polluted waters, especially at low Cd concentrations.

Key words: *Lemna minor*, cadmium (Cd), phytoremediation, chlorophyll *a* fluorescence.

Introduction

Heavy metal pollution of soils and waters is a very serious environmental problem with potentially harmful consequences for agriculture and human health. The modern agricultural practices and the industrial activities have polluted soils and waters with great amounts of heavy metals (Rascio and Navari-Izzo 2010). Cadmium (Cd) was selected in this study since this metal ion, is toxic to living organisms and is a widespread, naturally occurring, element that is present in soils, rocks, waters, plants and animals. Cd is included among the top twenty worst polluting chemicals (Harris et al. 2011) Cd is harmful because it can replace some essential elements that play key roles in active sites of enzymes, and also because of its high affinity for sulfhydryl groups (Pietrini et al. 2005). Furthermore, European Directive in the field of water policy has established that cadmium with mercury, nickel and lead are priority hazardous substances in aquatic environment and has introduced severe measures to prevent or limit inputs of these pollutants into groundwater (Directive 2000/60/EC). There is a considerable interest in developing cost effective and environmentally friendly technologies for the remediation of wastewater polluted with toxic trace elements. Cd extraction from water can be carried out *in situ*, at rates much higher than in soils, and is one of the effective and promising phytoremediation technologies (Prasad et al. 2010). The ideal plants for phytoextraction should possess the ability to tolerate and accumulate high levels of heavy metals in their harvestable parts, while producing high biomass. Many species of macrophytes are used for phytoremediation research for wastewater treatment (Khellaf and Zerdaoui 2009). One of the most commonly used aquatic plant is *Lemna minor* belonged to the family of Lemnaceae. In particular, *Lemna minor* is a free-floating, aquatic perennial plant that forms a rapidly-expanding mat of foliage (to 1/4" tall) on water surface, able to remove and accumulate large amounts of cadmium, principally through the fronds (Zayed et al. 1998). Thus, this study was carried out to evaluate the ability of *Lemna minor* to tolerate, remove and bioconcentrate cadmium in a short-term experiment.

Materials and Methods

The experiments reported in this paper were conducted on a geographically isolated clone of *Lemna minor*. Before metal treatment, plants (30 fronds corresponding to a leaf surface of about 3 cm^2) were acclimatized in plates (diameter 3 cm) for 20 days under controlled climate conditions (23/19°C and 14-h photoperiod, photon flux density of 350 µmol m^2 s^{-1}). The plants were then treated with Hoagland mineral solution (Forni et al., 2001) containing 0, 13, 22 and 46µM $CdSO_4$ under the aforementioned conditions for periods of 24, 48 and 72h. In response to metal exposure, relative growth rate (RGR), bioconcentration factor (BCF), tolerant index (T*i*), cadmium uptake in whole plant, and maximum efficiency of photosystem II (Fv/Fm) were analysed.

L. minor relative growth rates were calculated according to Hunt's equation:

$$R = \ln W_2 - \ln W_1 / T_2 - T_1$$

where R is the relative growth rate (g g^{-1} day^{-1}), and W_1, T_1 and W_2, T_2 are the initial and final dry weights and times for each day treatment, respectively (Hunt 1978).

The bioconcentration factor (BCF) was calculated as follows (Rahmani and Stenberg 1999):

$$BCF = \text{Cd in plant biomass (mg } kg^{-1}) / \text{Cd in solution (mg } l^{-1})$$

Tolerance index (T*i*) was calculated as follows (Lux et al. 2004):

$$Ti = \text{dry weight of plants grown in Cd solution} / \text{dry weight of plants grown in control solution}$$

At the end of each day of treatment, plants were harvested and oven-dried at 105°C for 48h. Each sample was then digested with pure HNO_3:H_2O (1:1) for 72h. After digestion, the volume of each sample was adjusted to 25 ml using double deionized water. Determinations of Cd concentration in plant were carried out by atomic absorption spectrometry (AAnalyst-300, HGA-800, Perkin Elmer)

Maximum efficiency of photosystem II (PSII) was characterized by variable to maximum chlorophyll *a* fluorescence ratio (F_V/F_M) measured by means of chlorophyll fluorescence imaging (FluorCam MF700, PSI, Brno, Czech Republic). Plants were first dark-adapted for 20 min. During the measurement, the plants were exposed first to low irradiation (5 µmol $m^{-2}s^{-1}$) for the determination of basal chlorophyll fluorescence (F_0). Then a saturating light flash (1500 µmol m^{-2} s^{-1}) was applied to determine maximum fluorescence (F_M). The maximum efficiency of PSII (F_V/F_M) value was calculated according to the equation :

$$F_V/F_M = (F_M - F_0)/ F_M.$$

All data were subjected to ANOVA and LSD test was used to separate the means.

Results and Discussion

Relative growth rates (RGR) of *L. minor* declined with increasing exposure time and Cd concentrations (Fig.1). The highest RGR reduction was found in plants exposed to 46µM $CdSO_4$ while no significant differences were observed in the other two concentrations. Our results are in line with Uysal and Taner (2007) that found a growth reduction in *L. minor* during the exposure to similar Cd concentrations for 7 days.

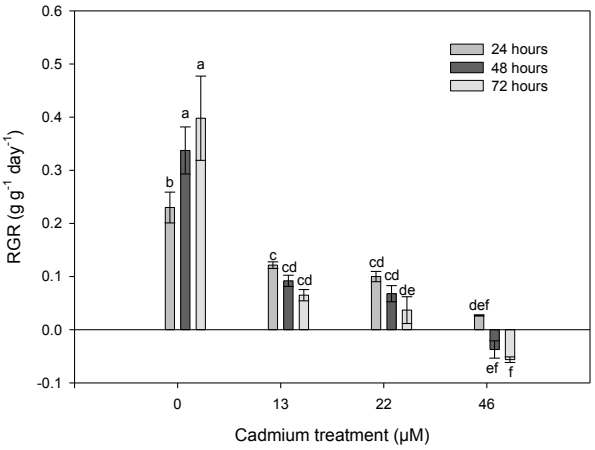

Fig. 1. Relative growth rate of *Lemna minor* treated with 0 (Control), 13, 22 and 46 µM $CdSO_4$ during the 3 days of experiment. Data are given as means of six replicates ± standard error (SE). Bars with the same letter were not significantly different ($P < 0.05$, ANOVA; LSD mean comparisons test).

To evaluate the ability of plants to concentrate Cd from the external solutions in their tissues, the bioconcentration factor (BCF) was calculated. As reported in table 1, BCF of *L. minor* showed an increase in all tested Cd treatments and in response to time exposure. In particular, all Cd treatments showed the highest values of BCF at the end of the experiment (72h). However the highest BCF values were measured in plants exposed to 13µM $CdSO_4$. Our results confirmed the indication suggested by Zayed et al. (1998) for whom a plant is considered a good accumulator when reaches a BCF over 1000. In fact, we found values of BCF very close to 1000 after 48h of treatment and upper to 1000 after 72h in plants exposed to 13 and 22µM $CdSO_4$.

Table 1. Bioconcentration factor (BCF), tolerance index (T*i*) and cadmium uptake measured during the 3 days of experiment in fronds of *Lemna minor* treated with 13, 22 and 46 µM $CdSO_4$. Values are means of six replicates ± standard error (SE). Within columns, values with the same letter were not significantly different ($P < 0.05$, ANOVA; LSD mean comparisons test).

Conc (µM)	Time (h)	BCF	T*i*	Cd uptake (µg/plate)
13	**24**	**476**±21 f	**0.78**±0.03 a	**2.12**±0.17 f
	48	**875**±26 c	**0.61**±0.02 bc	**4.43**±0.18 e
	72	**1275**±32a	**0.43**±0.02 d	**6.75**±0.18 c
22	**24**	**367**±6 g	**0.82**±0.02 a	**2.83**±0.21 f
	48	**721**±22 d	**0.63**±0.02 b	**6.20**±0.42cd
	72	**1075**±38b	**0.44**±0.02 d	**9.57**±0.64 b
46	**24**	**354**±5 g	**0.80**±0.01 a	**5.25**±0.47de
	48	**599**±4 e	**0.55**±0.01 c	**8.76**±0.42 b
	72	**845**±3 c	**0.35**±0.01 e	**12.28**±0.38a

To assess the capability of plants to grow in the presence of a given concentration of metal, the tolerance index (T*i*) was calculated. As reported in table 1, T*i* of *L. minor* showed a decrease in all tested Cd treatments and in response to time exposure. Plants with T*i* higher than 0.6 are considered tolerant (Lux et al. 2004). Our results showed a good tolerance to Cd in plants exposed to 13 and 22µM $CdSO_4$ after 48h, since we found values of T*i* equal and/or higher than 0.6.

These results are well correlated with the Cd uptake detected in *L. minor* plants and reported in table 1. Plants accumulated high amounts of Cd in a concentration- and time-dependent manner. The highest Cd uptake was observed in plants exposed to 46µM $CdSO_4$ after 72 h. The lower Cd content was measured in plants exposed to 13µM and 22µM $CdSO_4$ after 72 h, 50% and 25% lower, respectively, compared to that one measured in plants grown at 46µM $CdSO_4$ after 72h.

To analyse the effect of cadmium treatments at physiological level, the maximum efficiency of PS II (F_V/F_M) was measured. The F_V/F_M values showed reduction in all tested Cd treatments and in response to time exposure (Fig.2). The lowest value of F_V/F_M was observed after 72 hours in plants exposed to 46µM $CdSO_4$. The increased amount of Cd uptake affected the efficiency of photosystem II with a corresponding significative decrease of F_V/F_M. Our results agree with Zhang et al. (2007) that found a reduction of F_V/F_M and other fluorescence parameters in *L. minor* during the exposure to similar Cd concentrations for 2 days.

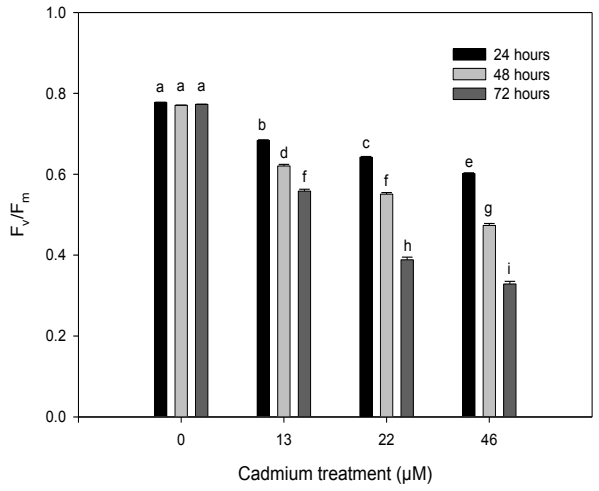

Fig. 2. Effect of cadmium on maximum efficiency of PSII (F_v/F_m) in fronds of *Lemna minor* treated with 0 (Control), 13, 22 and 46µM $CdSO_4$ during the 3 days of experiment. Data are given as means of six replicates ± standard error (SE). Bars with the same letter were not significantly different ($P < 0.05$, ANOVA; LSD mean comparisons test).

Conclusions

In this study, we investigated the toxic effects of Cd and its bioaccumulation on *L. minor*. Lemna showed to be a plant with great capability of Cd absorption and accumulation in the fronds, with a potential use in phytoremediation field.

The combined data analysis of RGR, BCF, T*i* and F_V/F_M indicates that *L. minor* showed a good capacity of growth, metal bioconcentration, tolerance and photosynthetic efficiency up to 48h in plants exposed to 13 and 22µM $CdSO_4$. In particular, under the aforementioned conditions, *Lemna* was able to accumulate up to 1800 mg/kg (data not shown) on dry matter basis higher than 0.01% defined as threshold Cd concentration for hyperaccumulator plants (Baker and Brooks 1989). Furthermore, in front of such high accumulation, *Lemna* showed a good protection of its photosynthetic apparatus as indicated by its Fv/Fm maintenance, up to 46µM CdSO4 after 24h.

According to obtained results, *L. minor* can be considered a good accumulator of Cd especially at low Cd concentrations. Infact, *L. minor* has potential for the remediation of Cd-polluted waters because of its high accumulation capacity, rapid growth rate and the ease of culture and harvest.

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