International Journal of Phytoremediation
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bijp20

Ceratophyllum demersum L. and Potamogeton alpinus Balb. from Iset′ River, Ural Region, Russia Differ in Adaptive Strategies to Heavy Metals Exposure - A Comparative Study

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Accepted author version posted online: 10 Jun 2013.Published online: 18 Oct 2013.

To cite this article: Galina Borisova , Nadezhda Chukina , Maria Maleva & M. N. V. Prasad (2014) Ceratophyllum demersum L. and Potamogeton alpinus Balb. from Iset′ River, Ural Region, Russia Differ in Adaptive Strategies to Heavy Metals Exposure - A Comparative Study, International Journal of Phytoremediation, 16:6, 621-633, DOI: 10.1080/15226514.2013.803022

To link to this article: http://dx.doi.org/10.1080/15226514.2013.803022

International Journal of Phytoremediation, 16:621–633, 2014

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226514.2013.803022

CERATOPHYLLUM DEMERSUM L. AND *POTAMOGETON ALPINUS* BALB. FROM ISET' RIVER, URAL REGION, RUSSIA DIFFER IN ADAPTIVE STRATEGIES TO HEAVY METALS EXPOSURE – A COMPARATIVE STUDY

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We examined the uptake of five heavy metals (Cu, Fe, Ni, Zn, and Mn) in* Ceratophyllum demersum *L. (hornwort) and* Potamogeton alpinus *Balb. (pondweed) from Iset' river, Ural region, Russia. This study was conducted in a territory that is highly urbanized where the surface waters are contaminated by a wide spectrum of pollutants. The environmental situation in this territory drastically deteriorated due to anthropogenic activity. The water quality in most of the water bodies in the Ural region is rather poor. In a comparative study of* C. demersum *and* P. alpinus, *differential accumulation pattern was noted for heavy metals (HMs). Higher amounts of HMs accumulated in* C. demersum *compared to* P. alpinus. *Also it was shown that in leaves of* C. demersum *there were high amount of total phosphorus, nitrogen, organics acids and ash; high activity of guaiacol peroxidase; high content of non-enzymatic antioxidants viz., flavonoids, ascorbate, glutathione and proline; high amount of thiols (soluble and membrane bound) compared to* P. alpinus.

KEY WORDS: aquatic macrophytes, heavy metals, bioconcentration factor, non-enzymatic antioxidants, enzymatic antioxidants, *in situ* phytoremediation

INTRODUCTION

The present study deals with comparative adaptive strategies of two aquatic macrophytes from Iset' river (Ural region, Russia) which is known for its aquatic plant diversity (Figure 1). The water quality of this river is poor due to different kinds of anthropogenic activity. The most commonly reported pollutants are heavy metals, organic and nitrogenous compounds, petroleum products (Chukina and Borisova 2010). The significant role of aquatic macrophytes in water renovation and ecosystem functioning has been highlighted in several publications (Malec *et al*. 2011; Mazej and Germ 2009; Mishra and Tripathi 2008; Basile *et al*. 2012).

Different kinds of pollutants that are released into aquatic systems which interact with rooted and free floating macrophytes. Aquatic macrophytes either absorb the bioavailable

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Figure 1 The map of investigation region (Middle Ural). The arrow indicates the position of water sampling and investigated aqutic plants (color figure available online).

pollutants and/or accelerate the sedimentation of the suspended particulate matter, which ultimately settle down and deposit as sediments. Aquatic macrophytes are the most important components of water bodies and take up metals from water due to their direct contact with the aquatic environment. They are able to accumulate heavy metals (HMs) several fold higher than their surroundings (Keskinkan *et al*. 2004; Kumar and Prasad 2004; Prasad *et al*. 2001; Rozentsvet *et al*. 2003). Thus, they play a significant role in biogeochemical cycling of HMs (Malec *et al*. 2011).

Aquatic plants are of special interest unlike the terrestrial plants, because they are capable of bioconcentrating many metals in large quantities (Pratas *et al*. 2012; Monferrán *et al*. 2012; Vardanyan and Ingole 2006). Therefore, submerged and free-floating plants growing in polluted water bodies play beneficial role for scavenging HMs. Further these thickets of aquatic macrophytes reduce the velocity of water currents by accelerating the sedimentation of the suspended fine particulates of metals, which otherwise are toxic to the biota when present in the inland waters in bioavailable form. The free-floating, submerged or emergent plants have different accumulation abilities (Chukina and Borisova 2010). At the same time, data on changes in the physiological and biochemical parameters of macrophytes with in response to different loads of anthropogenic pollution are rather limited.

It has been recognized that some of the aquatic macrophytes can be used as indicators of low level environmental contamination (Vardanyan and Ingole 2006; Zurayak 2001). Plants that are able to concentrate large amounts of elements in their tissues are considered as accumulators. The bioavailability of trace elements for plants is dependent on many environmental factors such as the concentration of the elements in the environment, abiotic factors, exposure time, growth form of the plant, type of absorption mechanism, affinity

of trace elements for the adsorption sites, trace element speciation, sampling period etc. (Kumar and Prasad 2004; Mishra and Tripathi 2008).

Aquatic macrophytes differ not only in their capacity to take up trace elements into root tissues but also in the proportion of trace elements transferred to above-ground parts, although uptake through the leaves may also be of significance (Guilizzoni 1991; Penget *et al*. 2008). Some rooted submerged plants absorb trace elements directly from water when they are not readily available in sediments and/or are in high concentrations in the surroundings.

The toxicity of HMs is generally thought to be due to inactivation of enzymes and functional proteins by directly binding to them. The numerous studies show that the HMs toxicity may be due to oxidative damage by the generating high concentrations of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$), all of which can react with nucleic acids, lipids, pigments, proteins and cause lipid peroxidation, membrane damage and inactivation of enzymes (Mittler 2002; Aly and Mohamed 2012; Huang *et al*. 2010).

In response to HMs stress, aquatic plants as well as terrestrial have evolved different detoxification mechanisms and adaptive strategies (Prasad *et al*. 2001; Titov *et al*. 2007). Notable are synthesis of different thiol compounds such as phytochelatins, molecular chaperons as well as antioxidant inductions and cellular compartmentation by low molecular weight metal binding complexes etc. (Malec *et al*. 2009; Esposito *et al*. 2012; Aly and Mohamed 2012).

To minimize the damaging effects of ROS, plants have envolved an effective defence system including enzymatic antioxidants such as superoxide dismutase (SOD) catalase (CAT), different peroxidase and low molecular non-enzymatic antioxidants (ascorbate, gluthatione, proline, flavonoids etc.) that paticipate in scavenging ROS (Márquez-García *et al*. 2012; Kachout *et al*. 2009; Maleva *et al*. 2012).

Unlike terrestrial plants where a root is the primary organ for uptake of HMs, in the aquatic plants entire organism is exposed to metal contaminated water and evolve specific mechanisms of adaptations (Lukina and Smirnova 1988). Further, accumulation of metals by aquatic plants from natural conditions open opportunities for phytoremediation and bioindication (Favas *et al*. 2012). Growth of aquatic macrophytes under multi-pollutant waste water conditions, the degree of accumulation of metals and the variations in bioconcentration are crucial for applying the services of these beneficial plants for phytoremediation and ecosystem engineering (Kearney and Zhu 2012; Klumpp *et al*. 2012; Martinez *et al*. 2011; Miretzky *et al*. 2004; Monferran *et al*. 2012). The use of aquatic macrophytes for the removal of inorganic and organic waste from water bodies has gained considerable attention. Submerged plants play important role in maintaining the health of aquatic ecosystems through the accumulation and/or decomposition of toxins (Prasad 2007).

The aim of this research—the comparative analysis of two prominent aquatic macrophytes *viz*., *C*. *demersum* and *P. alpinus* that are implicated in phytoremediation and toxicity bioassays. In this investigation we have adapted some of the physiological and biochemical parameters to determing the tolerance and indicator value for environmental monitoring. Therefore, the objective of this study is: a) to quantify the selected metals in submerged macrophytes *viz*., *C*. *demersum* and *P. alpinus*; b) to compare the bioconcentration factors of studying plants; c) to investigate the total phosphorus, nitrogen, organic acids and ash; d) to determine the non-enzymatic and enzymatic antioxidants.

MATERIALS AND METHODS

Study Area and Sampling

Iset' River is located in the Ural Mountains. The length of the Iset River is 606 km. The area of its basin is 58.900 km^2. The study area is located in the Urals Federal District. Its administrative center is the city of Ekaterinburg, formerly known as Sverdlovsk (Figure 1). The Iset' River flows through the marshland and heavily populated territory. It is a cascade of ponds and reservoirs, connected by small segments of the natural riverbed. The water resources of the Iset' River are strongly modified under the influence of reservoirs, diversion flow from other basins and under the influence of industry. The water quality does not meet the desirable international standards. Various water quality parameters are higher than the mininum acceptable concentrations. Heavy metals, organic compounds, ammonium and nitrite nitrogen and petroleum products are the most widely spread pollutants in the waters of Iset' River. Earlier studies on aquatic plants revealed their different ability to accumulate HMs (Chukina and Borisova 2010) that gave the opportunity to choose two species of aquatic macrophytes with different accumulativ strategies—*Ceratophyllum demersum* L. (hornwort) and *Potamogeton alpinus* Balb. (pondweed).

The plants of *P. alpinus* and *C. demersum* were sampled from the Iset' River in the blooming period (July). To determine the basic physiological and biochemical parameters leaves of plants were used immediately. Composite samples of plants leaves were collected not less than 10 plants. The water samples were simultaneously collected to determine HMs. The water for samples was collected at depths of up to 0.5 m (not less than 10 points) and mixed.

Species Description

Ceratophyllum demersum L. is a submerged aquatic plant, widely distributed all over the world. It has no roots and grows in still or very slow-moving water. Stem thin, smooth, up to 60 cm in the upper part highly branched. Leaves needle-like, in whorls of 6–12. The leaves are rich in the respiratory cavities, mesophyll cells are large with large chloroplasts.

Potamogeton alpinus Balb. is a submerged aquatic perennial plant anchoring in the substrate by rhizomes. It grows in water bodies such as ponds, lakes, rivers and slow-moving streams. It produces long and unbranching stem with thin lance-shaped leaves. Two layers of epidermal cells and one layer of mesophyll present with leaves internal structure.

Determination of Heavy Metals

To determine of HMs the macrophytes leaves (approximately 10 g of fresh weight) after sampling were washed 0.01% Na-EDTA and twice with distilled water for the removal of metal adsorbed on the surface of leaves. The plant material was dewatered on tissue paper and dried at 55°C for 24 h and subsequently 24 h at 75°C. Non-filtered samples of water were evaporared.

Heavy metals in the leaves of plants and water samples were determined using atomic absorption spectrometry (AAS Vario 6, "Analytik Jena", Germany) after digesting with 70% HNO_3 (analytical grade) (Ermachenko and Ermachenko 1999). The bioconcentration factor (BCF) was calculated as ratio concentration in plant (mg/kg): concentration in water (mg/L).

Characterization of Leaf Structural Parameters

Parameters of the leaf mezostructure were determined in 30 replicates according to Mokronosov and Borzenkova (1978). The leaf thickness was measured by inspecting the leaf cross sections with a Meiji Techno light microscope (Japan) and an eyepiece micrometer AM 9–2 (GSZ, Russia). A computer-assisted protocol based on Simagus Mesoplant software (OOO Siamz, Russia) was used to determine the quantitative parameters of mesophyll (cell projection, cell surface area, cell volume). Leaves were preliminary macerated with 5% chromic acid dissolved in 1 N HCl.

Determination of Total Phosphorus, Nitrogen, Organic Acids and Ash

The total phosphorus and nitrogen contents were measured by colorimetric methods after the wet digestion of plant material with the mixture of acids H_2SO_4 and $HC1O_4$. Total phosphorus was determined by standard methods based on reactions with ammonium molybdate in the acid medium (Fiske and Subbarow 1925); total nitrogen was determined using the Nessler reagent (Polley 1954). Organic acids content was determined according to Welschen and Bergkotte (1994). The ash content was determined after the burning of the weighed sample of plant material in the muffle furnace for 8 hours at 550°C. All parameters were calculated as units per 1 g dry wight (DW). One unit was defined as 1 mg of measured compounds (total phosphorus, nitrogen, organic acids or ash).

Extraction and Determination of Non-Enzymatic Antioxidants

Flavonoids content was determined in 96% ethanol extract with citrate boron reagent spectrophotometrically at 420 nm (Rogozhin 2006). Leaf samples (0.5 g) were extracted with 1% Triton X-100 (Sigma) solution in 96% ethanol in ratio 1:5 for 24 hours. In the experimental samples extract (0.5 ml) was reacted with 2.5 ml of citrate-boron reagent (20% ethanolic solution of citric acid mixed with 20% ethanolic solution of boric acid—1:1). In blank samples extract (0.5 ml) was reacted with 2.5 ml of 20% citric acid solution in 96% ethanol. After 15 min mixing the optical density was measured at 420 nm with a PD-303 UV spectrophotometer (Apel, Japan). The standard curve for flavonoids was prepared with rutin solution in citrate-boron reagent. The flavonoids content was calculated by subtracting the blank samples from the experimental samples and expressed in units per 1 g DW. One unit was defined as 1 mg of flavonoids.

Ascorbic acid and glutathione were determined in one integrated weighed sample using trilonometric method by parallel titration with 2.6-dichlorphenol-indophenol and potassium iodate (Pett 1936). Content of ascorbic acid and glutathione was calculated as units per 1 g DW. One unit was defined as 1 μg of ascorbic acid or glutathione.

The proline content was determined by a commonly accepted technique (Bates 1973) based on proline's reaction with ninhydrin. The chromophore-containing toluene optical density was measured at 520 nm with a PD-303 UV spectrophotometer (Apel, Japan). Content of proline was calculated as units per 1 g DW against standard proline. One unit was defined as 1 μg of proline.

Enzyme Extraction and Determination

To obtain the enzyme extract 0.5 g of fresh leaves was homogenized on ice in 5 ml of cold sodium–phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged for 20 min at 15000 g and 4°C and the resulting supernatant was used for the enzyme analyses.

Activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by inhibition of photochemical reduction of nitroblue tetrazolium (NBT) (Sigma) at 560 nm (Beauchamp and Fridovich 1971). The SOD activity was expressed in enzyme units per 1 g DW. One unit of enzyme activity corresponded to the amount of protein inhibiting the NBT reduction by 50%.

Activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was determined by the increase in the optic density of reaction medium for 1 min at 470 nm as a result of guaiacol oxidation (Chance and Maehly 1955). We calculated the enzyme activity using the extinction coefficient of 22.6/(mM cm) and expressed in enzyme units per 1 g DW. One unit of enzyme activity was defined as amount of enzyme producing 1 mM of tetraguaiacol for 1 min at 30°C.

Extraction and Determination of Thiols

Plant material (1 g) was homogenized on ice with a mortar and pestle in a 5 ml of cold Tris-HCl buffer (0.1 M, pH 7.8). The homogenate was centrifuged for 25 min at 15000 g in a refrigerated centrifuge at 4°C (centrifugation I) and the supernatant was collected. The pellet was resuspended in 5 ml of cold buffer and centrifuged again in the same conditions (centrifugation II). The supernatants from centrifugations I and II were pooled (total volume ca.10 ml) and used to estimate of soluble protein and total soluble thiols.

To isolate the membrane bound thiols, the pellet from centrifugation II was solubilized through its stirring with 5 ml of 0.1% triton X-100 in the extraction buffer (see above) for 15 min at 4°C. The fraction was clarified by centrifugation (30 min, at 15000 g). The pellet was solubilized again with a fresh buffer volume under the same conditions and clarified by centrifugation. The supernatants, containing the membrane bound fraction, were pooled and used for the estimation of membrane bound protein and thiols.

The concentration of sulfhydryl groups was estimated according to Ellman (1959) with some modifications. The reaction mixture contained 300 μl of the supernatant, 300 μl 10% SDS, a 2.4 ml Tris-HCl buffer (100 mM, pH 7.8), 300 μl of 10 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Sigma) and it was incubated for 1 h at 37°C. After incubation, the absorbance was measured at 412 nm. Reduced glutathione (GSH, Sigma) was used as a standard. Content of soluble and membrane bound thiols was calculated as units per 1 mg of protein (soluble or membrane, respectively). One unit was defined as 1 μM of SH.

Protein content was determined according to Bradford (1976), using bovine serum albumin (Sigma) as a standard.

Reagents. Chemicals whose manufactures are not specified above were produced in Russia.

Statistics

All experiments were repeated three times with three replicates. Data presented in the figures are the average means ± standard error. The data were compared using Student's *t*-test (Statistica 6.0); differences between two species were significant at $p < 0.05$, except the data marked with an asterisk (*).

Table 1 Heavy metals (HMs) in Iset' river water, in the *C. demersum* and *P. alpinus* leaves and bioconcentration factor (BCF)

Parameter investigated	Cu	Fe	Ni	Zn	Mn
HMs in Iset' river water ($*10^{-3}$ mg/L)	7.0	351.2	3.9	25.5	67.4
		C. demersum			
HMs in leaves (mg/kg)	43.5	1706.2	16.6	372.6	2977.3
BCF	6214	4858	4256	14612	44174
		P. alpinus			
HMs in leaves (mg/kg)	13.2	577.3	11.3	84.1	378.4
BCF	1886	1644	2897	3298	5614

RESULTS AND DISCUSSION

A comparison of data on the accumulation of heavy metals in the studied species revealed that the order of the values of biological accumulation in leaves of *C. demersum* was as follows: Ni < Cu < Zn< Fe < Mn and in leaves of *P. alpinus* – Ni < Cu < Zn< Mn < Fe (Table 1).

The content of the investigated metals in the leaves of *C. demersum* were several times higher than in leaves of *P. alpinus*. The maximum difference observed for manganese content. In the leaves of *C. demersum* it was 7.9 times higher than in the leaves of *P. alpinus*. The minimum difference was found for nickel. The amount of Ni was only 1.5 times greater than the concentration of this metal in the leaves of *P. alpinus*.

Bioconcentration factors of the investigated metals in the leaves of *C. demersum* and *P. alpinus* were higher compared to terrestrial plants. However the values of bioconcentration factor in hornwort were significantly higher than in pondweed leaves. Presumably this fact can be explained by the lack of roots in hornwort.

Apparently plants resistance to HMs can be achieved by an avoidance mechanism, which includes the immobilization of a metal in roots and in cell wall. The intercellular detoxification mechanisms against HMs are based on their sequestration in cell vacuoles, on binding the metals by appropriate ligands like organic acids, proteins and peptides and on the presence of enzymes that can function at high levels of metals (Inze and van Montagu 1995; Garbisu and Alkorta 2001).

High accumulation capacity of *C. demersum* probably related to its anatomical features such as dissected blade, a large portion of aerenchyma and large mesophyll cells. These features allow this plant specie to absorb heavy metals (Table 2).

Large cells were observed also in aquatic plant *Lemna minor* (duckweed) which is exposed to a large amounts of pollutants (Kapitonova 2002). Duckweed can remove the exess of pollutants either by binding in tissues in the form of some nontoxic or less

Table 2 Structural characteristics of *C.demersum* and *P. alpinus* leaves

Plant species	Leaf thickness (μm)	Cell projection area (μm^2)	Cell volume (10^3 μm^3)	Cell surface area (10^3 μm^2)
C. demersum	388.0 ± 17.0	7485.1 ± 541.6	458.5 ± 47.2	29.9 ± 2.2
P. alpinus	64.7 ± 5.3	574.0 ± 28.6	9.1 ± 0.7	2.3 ± 0.1

Data represent average means $\pm$ SE (n = 3).

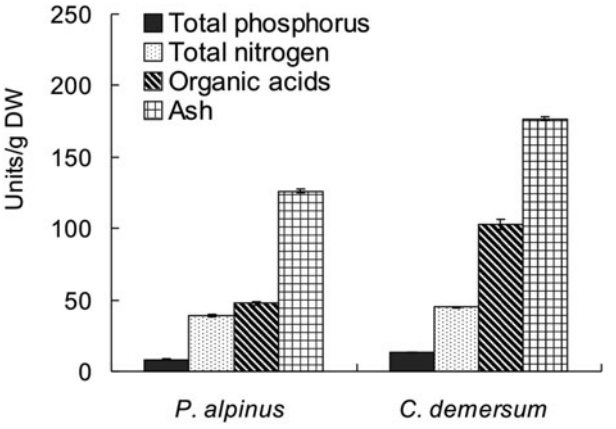

Figure 2 The content of total phosphorus, total nitrogen, organic acids and ash in the leaves of *P. alpinus* and *C. demersum* from Iset' River, Middle Ural, Russia. Data represent mean values and standard errors determined from three replicate experiments. Differences between two species were significant at $p < 0.05$.

toxic complexes or by "diluting" them in to a large amount of phytomass. This feature is apparently important for normalizing vital activities of plants and provides for succussful growth of this species in heavily polluted water bodies. Obviously, for the rooting pondweed (*P. alpinus)* rhisomes are an essential barriers to prevent the penetration of pollutants inside the leaves.

It was shown that the content of total phosphorus and others mineral elements in *C. demersum* leaves was on average 1.5 times higher than in *P. alpinus* (Figure 2). The small differences of total nitrogen content were observed (in 1.2 times). A significant difference in organic acids amount between the studied species was found. Its content in *C. demersum* was 2.2 times higher compared to *P. alpinus* (Figure 2).

There is a view that the increase of total phosphorus in plants can contribute to their adaptability. It is known that phosphorus play a important role in the anabolic and catabolic processes and energy cells. It was found that living organisms are able to form polyphosphates in the cells, which can bind metal ions, performing, so a regulatory role in stress conditions (Kornberg *et al*., 1999).

Organic acids also play an important role in the resistance of plants to elevated concentrations of HMs. There is a hypothesis according to which the metal complexes formed with organic acids are submitted later in the vacuole that provides isolation of metals (Jocsak *et al*., 2005).

In plants, HMs are known to induce oxidative stress such as overproduction of ROS can caused cellular damage. The formation of ROS is prevented by well-organized enzymatic and non-enzymatic antioxidant system (Mittler 2002; Noctor and Foyer 1998). According to some authors, the antioxidant system comprises also thiol-enriched proteins and peptides (metallothioneins and phytochelatins), which not only bind the excess of HMs but also help to neutralize toxic radicals (Cobbett and Goldsbrough 2002; Thornalley and Vasak 1985).

Analysis of the antioxidant system parameters has shown that macrophytes species with high accumulation capacity had more higher values of antioxidant enzymes activity. For example in *C. demersum* peroxidase activity was 8 times higher than in *P. alpinus* (Figure 3). The higher levels of GPX activity were detected in *Lemna minor* under increased

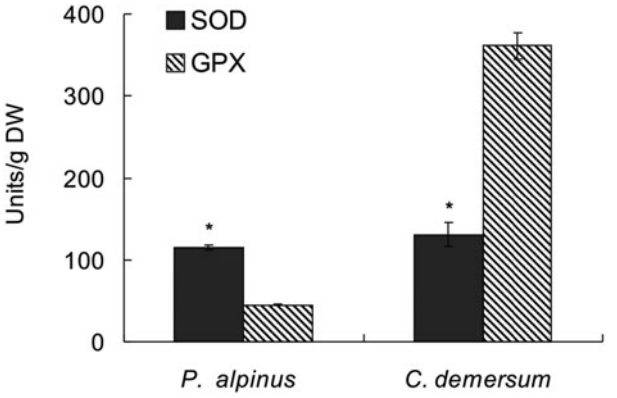

Figure 3 The activity of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) in the leaves of *P. alpinus* and *C. demersum* from Iset' River, Middle Ural, Russia. Data represent mean values and standard errors determined from three replicate experiments. Differences between two species were significant at $p < 0.05$, except the data marked with an asterisk (*).

cadmium and copper concentrations (Paczkowska *et al*. 2007; Teisseire and Guy 2000) and in rice leaves in response to excess iron, copper and zinc (Fang and Kao 2000). The significant increased in peroxidase activity was also observed in *Erica andevalensis* shoots treated by Tinto River (Spain) water highly contaminated by different HMs (Oliva *et al*. 2009).

The non-enzymatic antioxidants such as ascorbic acid, glutathione, proline and flavonoids are also very important in defense against oxidative stress. For examle, flavonoids have ideal structure and redox properties for free radical scavenging and can chelate transition metal ions thus preventing the formation of ROS (Montoro *et al*. 2004). The content of flavonoids in the leaves of *P. alpinus* was more than 6 times lower than in the leaves of C. *demersum*. Also the content of glutathione, ascorbate, and proline was significantly (1.5–2 times) higher in the C. *demersum* (Figure 4).

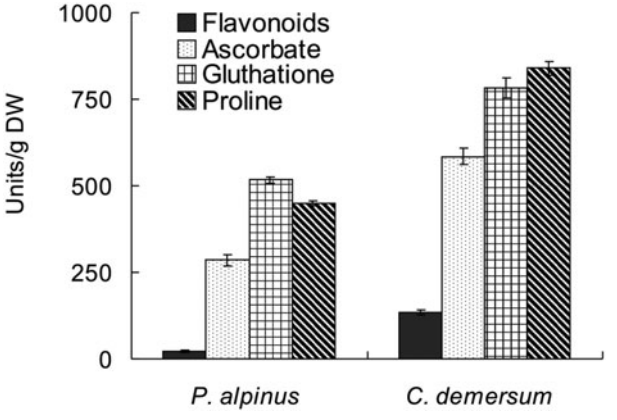

Figure 4 The content of non-enzymatic antioxidants in the leaves of *P. alpinus* and *C. demersum* from Iset' River, Middle Ural, Russia. Differences between two species were significant at $p < 0.05$.

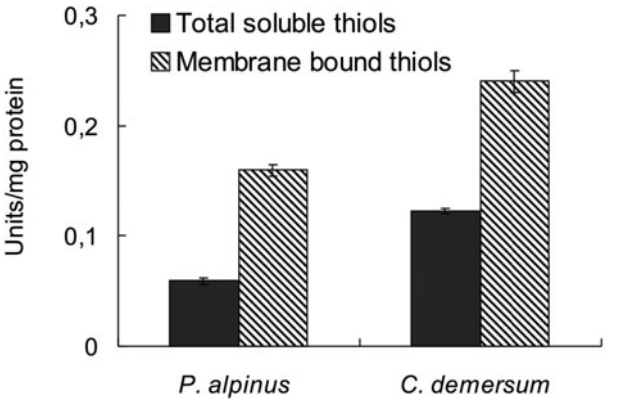

Figure 5 The content of total soluble thiols and membrane bound thiols in the leaves of *P. alpinus* and *C. demersum* from Iset' River, Middle Ural, Russia. Differences between two species were significant at $p < 0.05$.

Glutathione (GSH) is an important antioxidant produced by the cell and is responsible for maintenance of antioxidative machinery of cells integrity under stress. It protects thiols of enzymes and react with with hydrogen peroxide and hydroxyl radical together with ascorbate (Noctor and Foyer 1998). In additional, GSH can directly bind HMs and serves as a phytochelatin precursor, which plays a major role in HMs sequestration (Cobbett and Goldsbrough 2002).

To minimize the detrimental effects of HMs accumulation, plants have evolved detoxification mechanisms, mainly based on chelation and subcellular compartmentalization. Apparently thiols play a significant role in HMs detoxification not only because of their chelation but their ability to scavenge of ROS (Wong 2004).

The significant differences between two investigated species in the content of thiols were found (fig. 5). The content of total soluble and membrane bound thiols in *C. demersum* leaves was in 1.5–2.0 times higher than in *P. alpinus*.

It is revealed that species with high accumulative ability have a high antioxidant status as evidenced by their high level of enzymatic and non-enzymatic antioxidants. In contrast, for species with low HMs adsorption properties a protective mechanism to prevent the pollutants entrance play a large role in adaptation to environmental conditions.

Results of this study have shown that investigated macrophytes differed by their accumulation strategy. The species with maximal accumulation ability (*C. demersum*) have increased guaiacol peroxidase activity, high content of mineral elements, organic acids, flavonoids, glutathione, ascorbate, and proline as well as thiols (soluble and membrane bound). Presumably, the most efficient functioning the antioxidant system favors a better tolerance of plants to the impact of HMs.

CONCLUSION

The physiological and metabolic functions *viz*., photosynthetic apparatus structure, activity of guaiacol peroxidase, rate of assimilation of N, P, production of organic acids and total ash content, the amount of non-enzymatic antioxidants such as ascorbic acid, glutathione, proline and flavonoids and total soluble and membrane bound thiols content of *C. demersum* provided precise information on its adaptive strategies. It was shown that

a broad spectrum of antioxidant components are involved in plant adaptations to the water pollution.

Although *P. aplinus* is exposed to similar environment its metabolic capabilities are less compared to *C. demersum*. Besides that *C. demersum* photosynthetic apparatus structure favoured accumulation of metals than *P. alpinus*. The elucidated features allowed *C. demersum* to accumulate high concentrations of HMs.

Study of plants with different accumulative ability from nature could serve as potential and novel tools for biomonitoring and phytoremediation. Results of the present research would be helpful for planning, designing *in situ* phytoremediation strategies for polluted aquatic ecosystems.

ACKNOWLEDGMENT

This work was supported by the Federal Program "Scientific and Scientific-Pedagogical Personnel of the Innovative Russia" (state contracts 14.A18.21.0203). MNVP is thankful to the Ural Federal University named after the first President of Russia B.N. Yeltsin Ekaterinburg**,** Russia for invitation as "Visiting Professor" to participate in the on going research activities.

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