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The impact of humic acid on chromium phytoextraction by aquatic macrophyte *Lemna minor*

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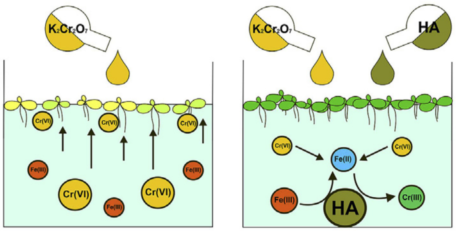
HIGHLIGHTS

- Cr(VI) decreases growth rate and content of photosynthetic pigments in *Lemna minor*.
- Cr(VI) is reduced into Cr(III) in presence of humic acid.
- Cr(III) is less toxic and less bioavailable.
- Presence of humic acid affects the efficiency of Cr phytoextraction.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies assessing chromium phytoextration from natural waters rarely consider potential implications of chromium speciation in the presence of ubiquitous humic substances. Therefore, the present study investigated the influence of environmentally relevant concentration of humic acid (TOC $= 10$ mg L^{-1}) on chromium speciation (Cr $= 0.15$ mg L^{-1}) and consequently on phytoextraction by aquatic macrophyte duckweed *Lemna minor*. In absence of humic acid, only hexavalent chromium was present in water samples and easily taken up by *L. minor*. Chromium uptake resulted in a significant reduction of growth rate by 22% and decrease of chlorophyll *a* and chlorophyll *b* contents by 48% and 43%, respectively. On the other hand, presence of humic acid significantly reduced chromium bioavailability (57% Cr uptake decrease) and consequently it did not cause any measurable effect to duckweed. Such effect was related to abiotic reduction of hexavalent chromium species to trivalent. Hence, findings of our study suggest that presence of humic acid and chromium speciation cannot be neglected during phytoextraction studies.

1. Introduction

It is well recognized, that the presence of different anthropogenic pollutants results in an overall degradation of the aquatic environment with heavy metals being considered as one of the most dangerous pollutants (MEA, 2005; Uysal, 2013). Among other heavy metals, chromium represents a major environmental and health concern due to its toxicity, multifarious use and widespread applications. Although, there are natural sources of chromium, the majority of chromium pollution originates from anthropogenic sources as tanning industry, production of steel and alloys, wood preservation, paint pigment manufactures and many others (Kimbrough et al., 1999). From these sources, chromium has been released into the environment *via* leakage, poor storage, improper disposal practices or with polluted wastewaters (Palmer and Wittbrodt, 1991). For instance, during tanning between 30 and

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50% of chromium applied in conventional chromium tanneries is lost with the wastewater (Montañés et al., 2014) and thus insufficient wastewater treatment makes tannery effluents a direct route of chromium into a watercourse (Shakir et al., 2012).

The problem of chromium pollution consists in a fact that chromium as well as other heavy metal cannot be degraded in any reasonable period of time and they must be extracted from the polluted site (Uysal, 2013). Common remediation techniques include precipitation, ion exchange, membrane separation and adsorption (Mohan and Pittman, 2006). Such remediation methods suffer from limitations like high cost, manpower and some of them can also create secondary pollution problems. Besides, none of them are suitable for *in situ* remediation of the aquatic systems (Azizur et al., 2015). Considering all the aspects, efforts are underway to use more environmentally friendly and low cost methods to treat large volumes of contaminated waters. One of the possible methods is phytoextraction which is a phytoremediation technique utilizing naturally occurring processes by which plants extract metals and concentrate them in a plant body. In water media, submerged plants accumulate metals by their whole body while floating aquatic plants absorb or accumulate contaminants by its roots. However, floating duckweed from a family Lemnaceae has a considerable ability to accumulate metals from an aqueous medium (Uysal, 2013), because the entire bottom surface of the plant is in a permanent contact with the water surface (Ben-shalom et al., 2014). Further advantages of duckweeds are their rapid growth, cold tolerance, easy harvesting and a good tolerance for a wide range of pH values (Priya et al., 2012).

The success of chromium phytoremediation is controlled by several factors which influence the chromium bioavailability and consequently remediation efficiency of a plant. However, majority of studies on phytoextraction of chromium have been aimed at assessing chromium removal efficiency without taking into account the chromium redox speciation. In the aquatic environment, chromium exist in two stable oxidation states: Cr(III) and Cr(VI) (Kimbrough et al., 1999). Although Cr(III) should occur only under anoxic or suboxic conditions (Kotas and Stasicka, 2000), it has been found in significant quantities in many oxygenated surface waters (Kaczynski and Kleber, 1993) where Cr(VI) is predicted to be the predominant species. The reason for this is that the interconversion and thus presence and ratio between both oxidation states is affected not only by concentration of oxygen but also by other natural factors (Kotas and Stasicka, 2000). Cr(VI) is readily reduced to Cr(III) by ubiquitous natural organic matter as humic acids (Bartlett, 1991) which also exhibits a tendency to form complexes with Cr(III) (Masscheleyn et al., 1992). Chromium oxidation states considerably differ from one another and are also taken up by plants by different mechanisms (Shanker et al., 2005). Cr(VI) is a strong oxidizing agent, toxic and a carcinogenic element (Khorsandi and Rabbani-Chadegani, 2013), soluble in water, well bioavailable and highly mobile (Kimbrough et al., 1999). In contrast, Cr(III) has a low toxicity to plants (Pereira et al., 2013) and is poorly mobile (Kotas and Stasicka, 2000). Therefore the key knowledge on chromium phytoextraction is the understanding of uptake of chromium by aquatic macrophytes in presence of humic substances and the impact assessment of naturally occurring oxidation states of chromium on phytoextraction efficiency. However, the previous investigation of chromium phytoextraction has practically dismissed natural chromium speciation and in this context, the aim of the study was to investigate how the presence of humic acid influences the oxidation state, mobility and bioavailability of chromium in water and consequently affects its toxicity and bioconcentration in duckweed *Lemna minor*.

2. Materials and methods

2.1. Duckweed *L. minor*

The environment, where plants vegetate, considerably affects diversity within species (Henry, 2005) and consequently alter species' tolerance or sensitivity to stressors (Orcutt and Nilsen, 2000). Therefore three duckweeds *L. minor*, diverging in vegetation conditions were compared and one of them was chosen for the phytoextraction experiment. First duckweed *L. minor* L. (LM1) originated from a laboratory culture of Institute of Chemistry and Technology of Environmental Protection (Faculty of Chemistry, Brno University of Technology, Czech Republic). In our laboratory, it has been successfully cultivated under controlled conditions (temperature 23 ± 2 °C, photoperiod 16/8 h) for more than six years. The second duckweed (LM2) was collected from a small pond in Ljubljana marsh in Slovenia (45°56′57.5″N 14°20′17.4″E) while the third duckweed (LM3) was collected in University Botanical Gardens in Ljubljana, Slovenia (46°02′24.1″N 14°30′52.7″E) from a slowly running stream fed by groundwater. After collection, healthy fronds were gently washed by tap water and transported to plastic containers where they were allowed several weeks to acclimatize prior to the experiments. The acclimatization of duckweed proceeds by weekly addition of Steinberg medium (ISO 20079, 2005) into natural water (1:1) collected with duckweed, temperature and photoperiod was alike for laboratory culture. During the acclimatization, no problems with growth or presence of fungi and algae were noticed. Growth inhibition test with standard compound KCl (ISO 20079, 2005) was used for assessment of duckweeds' sensitivities. Definitive tests (3–12 g L^{-1}) were carried out twice in triplicates. Results are given as mean values of 168 h EC_{50} with 95% confidence interval (95% CI).

2.2. Water quality parameters

A sample of water where duckweeds *L. minor* grew was submitted for analyses comprehending pH, TOC, concentrations of ammonium nitrogen, alkalinity (expressed as HCO_3^-), orthophosphates, and chlorides following standard procedure (Rice et al., 2012). Analyses were performed in duplicates and repeated three times. For determination of total metal concentrations, the water samples were decomposed in triplicates by HNO_3:H_2O_2 = 1:1 in Teflon vessels (561B, Savillex, Minnesota, USA) at 200 °C for 2 h, including six blank samples. Samples were quantitatively transported to polypropylene tubes and diluted to certain volume by Milli-Q water. Metal concentrations in diluted digested water samples were determined by ICP-MS (Agilent 7500ce). Limit of detection was calculated as concentration corresponding to three fold standard deviation (3s, N = 6) of blank determinations. Results were calculated as mg of metal per L. The data are presented as means (N = 3) with standard deviations (SD).

2.3. Chromium and humic acid preparation and analysis

Stock solution of chromium Cr(VI) was prepared by dissolution of 100 mg of $K_2Cr_2O_7$ (Kemika, Croatia, p.a.) in one liter of Steinberg medium (ISO 20079, 2005). Concentration of total chromium (CrT) in water samples was determined by ICP-MS (see procedure at section 2.2). Concentration of hexavalent chromium (Cr(VI)) in water samples was determined spectrophotometrically according to ISO 11083 (1996). The data are presented as means (N = 6) in mg of Cr(VI) per L with standard deviations (SD).

Humic acid (HA) (Sigma–Aldrich) was diluted in the Steinberg