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Accumulation of chromium and interaction with other elements in *Chlorella vulgaris* (Cloroficeae) and *Daphnia magna* (Crustacea, Cladocera)

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Abstract: *Assays with Chlorella vulgaris Beijerinck Novakova, 1890 and Daphnia magna Straus, 1820 were performed to determine Cr and other elements concentration in tissues of both species by instrumental neutron activation analysis (INAA), after being exposed to 150, 280 and 350 $\mu g\ l^{-1}$ Cr (VI). Interaction among Cu, Zn, Fe and Cr were also registered. In the control of C. vulgaris, the amount of Cr was < 4 $\mu g\ g^{-1}$; in the treatments with Cr (VI) the values were 47, 82 and 100 folds greater than the control for the lowest, intermediate and highest concentrations tested respectively. In the control of D. magna, the amount of Cr was <3 $\mu g\ g^{-1}$; in the treatments with Cr (VI) the values were 14, 13 and 27 folds higher than the control for the lower, intermediate and higher Cr (VI) concentrations respectively, and from 3 to 9 times less than for C. vulgaris These results show that C. vulgaris is very efficient accumulator of Cr (VI) from polluted waters, and in consequence, it is proposed to be used in phytoremediation procedures.*

Key words: *Chromium, Bioconcentration, Oligoelements, Chlorella vulgaris, Daphnia magna, INAA*
PDF of full length paper is available with author ***(*amgagneten@gmail.com)***

Introduction

Heavy metals are considered as extremely toxic contaminants when present in aquatic systems. The most studied heavy metals in ecotoxicology are Hg, As, Cr, Pb, Cd, Ni and Zn. Chromium is an important contaminant because it is widely used in the process of leather tanning. Chromium is also used in the stainless steel production and in the manufacture of glass, electroplating, pigments, fungicides and batteries (Rinderhagen *et al*., 2000). Ions of heavy metals usually enter the cells through the same transport channels used by physiological important cations *e.g.* Ca, Mg, Cu and Zn (Luoma and Rainbow, 2006). They interfere with physiological activities such as photosynthesis, gaseous exchange and nutrient absorption, and cause reduction in plant growth, dry matter accumulation and yield (Rajesh and Madhoolika, 2005; Sahu *et al.,* 2007).

In the trophic chain, photosynthetizing organisms constitute the main access routes for heavy metals to consumers organisms (Moreno Sanchez and Devans, 1999). Zooplankton plays a key role in aquatic systems because its main food source are microalgae and particulated organic matter. On the other hand, it is ingested by larvae and juvenile fishes (Escalante, 1982, 1983) participating, in this way, in the transference of heavy metals from producers to superior trophic levels.

The great surface of exposure of planktonic organisms per mass unit, toghether with their high reroduction rates, determine a rapid adsorption and absorption of several contaminants. The fraction of zooplankton which feeds through filtration accumulates many metals and other contaminants diluted in water or associated with bacteria, algae and particulated organic matter. In this sense, the quantification of toxic elements is very important in order to investigate the uptake and transfer along the trophic chain and to monitor the levels of contamination of different aquatic environments (Ravera, 2001).

The small size or zooplanktonic organisms are the most important constraint to carry out bioaccumulation analysis. However, the Instrumental neutron activation analysis (INAA) overcomes this limitation, because this technique analyze very small amounts of biomass. It can determine simultaneously the concentration of several elements and is a very selective and precise method (Langstom and Spence, 1994). Althoug Cr (VI) removal by some algae have been recently studied (Baran *et al*., 2005; Ruangsomboon *et al.,* 2006), surveys on accumulation of heavy metals in plankton by INAA are very scarce. Campanella *et al.* (1999), analyzed macrominerals and trace elements of *Spirulina platensis* by INAA and Mosulishvili *et al*. (2004) applied the epithermal neutron activation analysis to investigate accumulation and adsorption of mercury by *S. platensis*, showing that this species has potential to be used in the remediation of sewage waters at Hg concentrations ~100 $\mu g\ l^{-1}$.

The aim of this work was to study the potential of planktonic organisms belonging to two trophic levels as Cr accumulators, to compare the bioconcentration of Cr and other elements in tissues of *C. vulgaris* and *D. magna* and to analyze interaction between Cr and other oligoelements by INAA in both organisms after being exposed to three Cr (VI) concentrations.

Materials and Methods

We measured the concentration of Cr (VI) in the tissues of *C. vulgaris* and *D. magna* and the concentration of Cr remaining in the culture medium. We also analyzed possible interactions between Cr and other oligoelements.

Both species were exposed by triplicate to a control (without Cr (VI), T0) and three different Cr (VI) concentrations: 150, 280 and 350 µg l^{-1} (T1, T2 and T3 respectively). Cr was added as $K_2Cr_2O_7$. The concentrations were selected according to Kungolos and Aoyama (1993) and Gagneten (2006).

C. vulgaris was cultured according to Borowitzka (1988). Algae were exposed to the three mentioned Cr (VI) concentrations and three controls for 24 hr. Each replica consisted of beakers with 300 ml Cr (VI) solution and *C. vulgaris* suspension (absorbance=1.5 at 650 nm). After homogenization, the vessels were transferred to a culture chamber with constant temperature and photoperiod (20 ± 1°C; 16 hr light: 8 hr dark). Afterwards, they were centrifuged (6,000 rpm) to separate the culture medium. Prior to Cu, Zn, Fe, Cr, Ag and Br determination by INAA , algae samples were dried at 35°C to constant weight. For each replica we obtained 144.8 ± 21.4 mg dry weight (d.wt.).

Adult organisms of *D. magna* were exposed to identical Cr (VI) concentrations and three controls for 48 hr. Each replica consisted of 50 adult *D. magna* per test chamber (beakers of 500 ml) randomly separated. Sorted adults were acclimatized to culturre medium (2 g $NaHCO_3$; 2.24 g $CaCl_2$; 0.26 g K_2SO_4 in 10 l distilled water; conductivity: 0.05 mS cm^{-1}; dissolved oxygen: 7.8 mg l^{-1}; pH: 7.6); temperature and photoperiod were constant (20 ± 1°C; 16 hr light: 8 hr dark). Animals were fed 24 hr before the exposition to Cr (VI) but no food was given to the test organisms during the experiment.

After 48 hr, animals were carefully washed twice with distilled water, frozen and dried at 60°C up to constant weight, to latter determine by INAA the amount of Zn, Fe, Cr, Ag and Br absorbed; 1.86 ± 0.9 mg d.wt. *D. magna* were obtained for each replica.

Cr, Ag, Br: Cr and other oligoelements (Cu, Zn, Fe, Cr, Ag, Br) concentrations in tissues of *C. vulgaris* and *D. magna* were determined by INAA. For this analysis, a RA6 reactor placed in Laboratories of the Bariloche Atomic Center (CAB) at the Atomic Energy National Comition (CNEA) has been used. Samples were irradiated, together with standard reference material: *Scenedesmus obliquus* 208 (IAEA-392, 2005) in order to improve the precision of the analysis. After irradiation, g-spectrometry measurements at proper times were carried out using a solid state HPGe type N detector equipped with a suitable software program.

Remaining concentration of Cr in *C. vulgaris* and *D. magna* culture medium was determined by atomic absorption spectrophotometry (Perkin Elmer 403). According to ASTM (2003) Standards – Water and Environmental Technology, chromium concentrations were determinated in Centralized Service of Great Instruments (CERIDE-CONICET), Laboratory in the Proficiency Testing Program Canadian Association for Environmental Analytical Laboratories (CAEL).

The concentration of heavy metals in both organisms were tested with the nonparametric Kruskal-Wallis test to check for significant differences between treatments ($p < 0.05$).

Results and Discussion

In *C. vulgaris,* the concentration of Cr in the control was < 4 µg g^{-1} d.wt. In the treatments with Cr (VI), the concentrations were 148.3, 330.3 and 399.6 µg g^{-1} d.wt. for T1, T2 and T3 respectively (Table 1 and Fig.1). In *D. magna*, the amount of Cr was < 3 µg g^{-1} d.wt. in T0; in the treatments with Cr (VI), the concentrations were 42.3, 40.4 and 82.3 µg g^{-1} d.wt., for T1, T2 and T3 respectively (Table 1 and Fig. 2) showing that algae absorbed a greater amount of Cr than cladocerans.

When *C. vulgaris* was exposed to Cr (VI), the Cr concentration were 47; 82 and 100 folds greater than the control for T1, T2 and T3 respectively. In *D. magna*, the values were 14, 13 and 27 folds higher than the control for the lower, intermediate and highest concentrations tested respectively, and 3 to 9 times less than for *C. vulgaris*.

The remaining Cr concentration in the culture medium after the exposure of algae was < 50 µg l^{-1} in all the treatments. *C. vulgaris* accumulated 66.7, 82.2 and 85.8% Cr for T1, T2 and T3 respectively (Fig. 3), while *D. magna* accumulated only 3% in T3 and the half of this, in T1 and T2 (Fig. 4). These results show that *D. magna* is not a very active accumulator of Cr, at least for the Cr (VI) concentrations tested.

Yan and Pan (2002) reported that *Chlorella pyrenoidosa, Closterium lunula* and *Scenedesmus obliquus* accumulated 95, 79 and 67% respectively after exposure to 50 g l^{-1} Cu. The Cu concentration accumulated reached a maximum value after the first day of exposure. Moreover, they found that *C. pyrenoidosa*, the smallest of the species tested, accumulated more Cu probably because they had more binding sites available for the union with metals. These resullts are in accordance with the obtained in the present study with *C. vulgaris* after 24 hr of Cr (VI) exposure. The amount of remanent Cr in the growing media was lower than 50 µg l^{-1}, even for the treatment with the higher Cr (VI) addition. This result confirms that *C. vulgaris* is an extremely efficient bioaccumulator. Other algae species were found suitable for removing Cr from aqueous solution, as was showed by Baran *et al*. (2005) . According to Hooda (2007), phytoremediation is an emerging technology, which uses plants and microorganisms to remove pollutants from contaminated sites. Despite several advantages, phytoremediation has not yet become a commercially available technology.

In contrast, the difference between the amount of Cr recorded before and after growing *D. magna* in the culture medium was very low. This fact demonstrates that, even considering that *D. magna* constitutes an excellent test organism to determine if a specific media is toxic or not, it accumulates significatly lower amounts of Cr than *C. vulgaris* ($p<0.05$). However, the fact that *D. magna* accumulated Cr means that at least part of it was bioavailable.

In *C. vulgaris* exposed to Cr (VI) (Fig. 1), Cu and Zn augmented for increasing Cr concentrations. This would indicate that

Table - 1: Cr and other elements concentrations recorded in *C. vulgaris* and *D. magna* tissues after exposure to the three Cr (VI) concentrations tested: Control (T0), 150 (T1), 280 (T2) and 350 µg l^{-1} (T3)

	Cr (µg l^{-1})	Cu (µg g^{-1} d.wt.)	Zn (µg g^{-1} d.wt.)	Fe (µg g^{-1} d.wt.)	Cr (µg g^{-1} d.wt.)	Ag (µg g^{-1} d.wt.)	Br (µg g^{-1} d.wt.)
Chlorella vulgaris	Control (T_0)	670	252	2230	< 4	3.23	0.361
	150 (T_1)	914.6 (220.2)	304.3 (44.7)	3180 (12375)	148.3 (0.5)*	2.64	0.53 (0.03)
	280 (T_2)	943.3 (153.0)	320.3 (34.0)	2890 (477.9)	330.3 (5.0)*	2.37 (1.0)	0.51 (0.1)
	350 (T_3)	1003.3 (140.1)*	798.3 (380.5)*	1706.6 (714.7)	399.6 (47.0)*	3.0 (0)	0.35 (0.1)
Daphnia magna	Control (T_0)	nd	178 (24.1)	422 (147.9)	<3.0 (0.9)	13.26 (19.3)	33.5 (9.6)
	150 (T_1)	nd	195 (61.5)	301 (60.6)	42.3 (13.03)*	13.54 (21.4)	28.7 (12.2)
	280 (T_2)	nd	175 (48.5)	289 (138.6)	40.4 (11.3)*	3.40 (1.1)	17.4 (6.3)
	350 (T_3)	nd	168 (50.6)	249 (173.4)	82.3 (19.4)*	0.889 (1.1)	16.2 (3.6)
	Reference material (µg g^{-1} d.wt.)	< 800	124.6	487	4.33	< 1	1.82

The values correspond to the media and 1 standard error, (*) Significant differences between control and exposures ($p<0.05$) ($n=3$), d.wt. = Dry weight

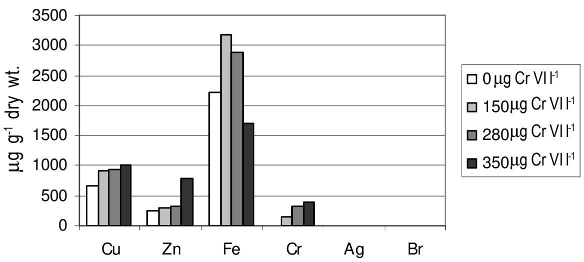

Fig. 1: Cr and other oligoelements in *Chlorella vulgaris* tissues exposed to different Cr (VI) concentrations. Measurements performed through INAA. The consigned value corresponds to the average over three replicates for each Cr (VI) treatment

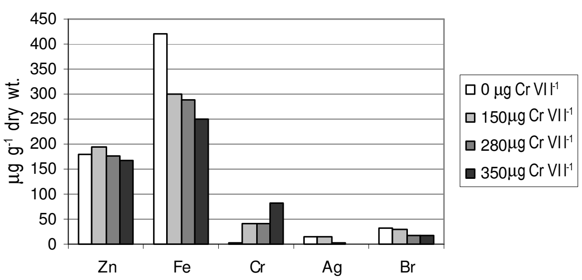

Fig. 2: Cr and other elements concentrations in *D. magna* tissues after exposure to three different Cr (VI) concentrations. Measurements performed through INAA. The consigned value corresponds to the average over the three replicates for each Cr (VI) treatment

these elements were favoured by the presence of Cr in microalgae. Fe was more variable: compared with T0 (2230 µg g^{-1} d.wt.), we observed a decrease to 1706.6 µg g^{-1} d.wt. in T3. *C. vulgaris* retained a big amount of Fe in T1 (3180 µg g^{-1} d.wt.) and T2 (2890 µg g^{-1} d.wt.). On the other side, values obtained for Ag and Br were very much lower with respect to values obtained for other metals and didn´t show important differences with the control (Table 1 and Fig.1).

In *D. magna* Cr may also interact with other elements because many oligoelements varied in this species (Fig. 2): Zn, Fe and Br diminished with the increment in Cr (VI) concentration, if compared with the control. In contrast, Ag was similar in T0 (13.26 µg g^{-1} d.wt.) and in T1 (13.54 µg g^{-1} d.wt.), but dropped in T2 (3.40 µg g^{-1} d.wt.) and T3 (0.889 µg g^{-1} d.wt.) (Table 1 and Fig. 2). The different scale in the "y" axis in Fig. 1 and Fig. 2 indicates that in *C. vulgaris*, the concentrations of oligoelements were higher than in *D. magna.* The Kruskal-Wallis test showed that the concentrations of Cu, Zn and Cr in the tissues of the algae revealed significant differences ($p<0.05$) among the treatments with Cr (Table 1, Fig.1). On the other hand, we found significant differences ($p< 0.05$) only for Cr in *D. magna* tissues (Table 1, Fig. 2).

A possible cause of the little amount of Cr recorded in *D. magna* is that microcrustaceans accumulate some pollutants in their exoskeleton but moulting releases them: as a result, crustaceans are periodically detoxificated by moulting, which occurs several times during their life-span. In this sense, after a few days of exposure, Robinson *et al.* (2003) found that *D. magna* rapidly accumulated Cd on their carapaces, but it dropped drastically after ecdisis. Another cause that could influence the low Cr accumulation by *D. magna* is the high alkalinity and water hardness of the culture medium. Janssen *et al.* (2003) suggest that the competition between Ca, Mg, Na and metal ions, results in decreasing toxicity to the organisms tested. In this sense, Karthikeyan *et al.* (2007) reported that Ni accumulation by *Cirrhinus mrigala* was significantly influenced by pH water.

Other elements (mainly Zn and Cu) varied in *C. vulgaris* tissues in relation to the control when exposed to different Cr (VI) concentrations, indicating the influence of Cr on the amount of other oligoelements, and probably showing an interaction with them. Control values showed that Cr, Ag and Br were the elements less abundant in *C. vulgaris* and *D. magna*, while Fe, Cu and Zn were the most abundant.

Previous work developed by Luoma and Rainbow (2006), showed that the union of a metal to the surface of a cell membrane may induce a direct biological response or, alternatively, it may go through the membrane and join intracellularly the target site. A variation could be the competition of the metal for a transportation system used as an essential micronutrient. As a consequence, the union of the metal to the site in the surface would inhibit its supply,

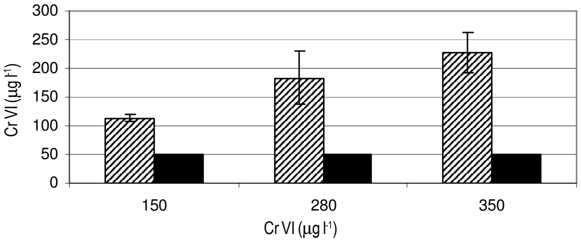

Fig. 3: Cr concentration in the growing media before (dashed bars) and after (dark bars) the incorporation of *Chlorella vulgaris* to the assays. The error bars indicate 1 standerd error

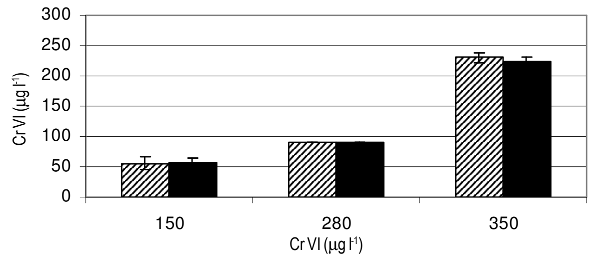

Fig. 4: Cr concentration in the growing media before (dashed bars) and after (dark bars) the incorporation of *D. magna* to the assays. The error bars indicate 1 standard error (1SE)

inducing, in this way, a deficiency of the nutrient. In *C. vulgaris* and *D. magna* the variation in oligoelements could be due to the interaction with Cr and they could compete against Cr to occupy the same toxicity sites.

There are many variables that influence the bioconcentration of a specific toxic, such as the contaminant concentration in water, the physico-chemical form of the contaminant, the permeability of the membrane, the type and amount of food, its degree of contamination and the features of the physical and chemical environment. All these factors influence both the organism and the contaminant (Rinderhagen *et al*., 2000).

The relevance of the present work stands out in the simultaneous examination of many elements in organisms with scarce biomass belonging to two trophic levels with INAA and their interaction with Cr, a heavy metal poorly studied. We also propose *C vulgaris* for future phytoremediation research.

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