

Studies on Bioaccumulation of ^{51}Cr by *Ulva* sp. and *Ruppia maritima*

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Abstract—This study aims at contributing to the characterization of the process of biological incorporation of chromium by two benthonic species, the macroalgae *Ulva* sp. and the aquatic macrophyte *Ruppia maritima*, to subsidize future activities of monitoring the contamination of aquatic biota. This study is based on laboratory experiments to characterize the incorporation kinetics of the radiotracer ^{51}Cr in two oxidation states (III and VI), under different salinities (7, 15, and 21 ‰). Samples of two benthonic species were collected on the margins of Rodrigo de Freitas Lagoon (Rio de Janeiro, Brazil), acclimated in the laboratory and subsequently subjected to experiments. In tests with ^{51}Cr (III and IV), it was observed that accumulation of the metal in *Ulva* sp. has inverse relationship with salinity, while for *R. maritima*, the maximum accumulation occurs in salinity 21‰. In experiments with Cr(III), increases in the uptake of ion by both species were verified. The activity of Cr(III) was up to 19 times greater than the Cr(VI). As regards the potential for accumulation of metals, a better sensitivity of *Ulva* sp. for any chromium tri or hexavalent forms was verified, while for the Cr(VI) it will require low salinities and longer exposure (>24h). For *R. maritima*, the results showed the uptake of Cr(VI) increase along with time (>20h), because this species is more resistant for the hexavalent form and useful for any salinity as well.

Keywords—Chromium, Cr-51, macroalgae, macrophyte, uptake.

I. INTRODUCTION

THE increasing pollution that reaches the water bodies in different localities, mainly in estuarine regions, near the large urban concentrations or in areas of intense agricultural and industrial practice, had motivated the search for biological and physic-chemical indicators in order to monitor and measure the loads of pollutants and their effects on the biota [1], [2]. Radionuclides may be used as radiotracers in studies of metal uptake, distribution and retention in marine flora [3]-[5].

Macroalgae are widely used as indicators of toxic substances' presence such as metals, and had been employed in numerous studies of bioaccumulation of metals because they incorporate these elements directly from water [6], [7].

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Algae are capable of concentrating specific metal ions traces (essential and non-essential metals) at very high levels, due to functional sites on their surface and intracellular ligands, participating in the dynamics of these pollutants in coastal environment [8], [9].

Metal accumulation in algae appears to be controlled by an initial absorption (e.g. with polysaccharide wall cell), followed by uptake to the membrane vacuoles containing polyphenols at high concentration [10], but the mechanisms of metal uptake are likely to vary for different metals and under different ecological conditions [11]. Furthermore, physico-chemical parameters, such as salinity or nutrients, also affect seaweed photosynthesis and thus metal accumulation [12].

In small concentrations, some metallic ions are essential for maintenance and growth of all organisms as many molecules, enzymes, and reactions require metallic elements [13]. However, when in excessive quantities, metals can interfere with normal cellular metabolism and become toxic to plants and animals [13], [14].

Chromium is considered to be a serious environmental pollutant due to its wide industrial use [15]. Chromium salts are used extensively in electric industry for anti-corrosive treatment and electric shielding of some components. Also, it is used in the manufacture of pigments, in fungicides, and in wood preservatives. The toxicity of Cr depends on its valence state. The most stable oxidation states are Cr(III) and Cr(VI) [16], [17], the latter being the more toxic due to its ability to cross biological membranes, acting as an oxidant, interfering with the absorption of nutrients and in photosynthesis process [18], and also because of its high mobility in the soil and aquatics environments [19], [20]. Chromium (III) is an essential trace element for human and animal health and is more stable than Cr(VI).

Owing to the fact that there are few studies of dynamic incorporation of chromium trace metals ions in controlled conditions by Brazilian local macroalgae and macrophyte, and due to the lack of knowledge concerning their kinetics uptake in the present work, taking advantage of a radiotracer technique with ^{51}Cr , an experimental study was carried out on the uptake kinetics of this element in two oxidation state (III and VI) by coastal macroalgae *Ulva* sp. and macrophyte *Ruppia maritima* under different salinities (7, 15, and 21 ‰).

II. MATERIALS AND METHODS

Samples of green algae *Ulva* sp. and the macrophyte *R. maritima* were collected between February and October 2013 in two points on the margins of Rodrigo de Freitas Lagoon (see Fig. 1) located in the urban area of Rio de Janeiro

(Brazil). Samples of *Ulva lactuca* were collected in control station, located at the Bananal Bay, Parque Nacional da Serra da Tiririca (22°58'30.27"S and 43° 1'26.79" W), a natural area

characterized by the presence of rocky coastlines exposed to the action of waves.

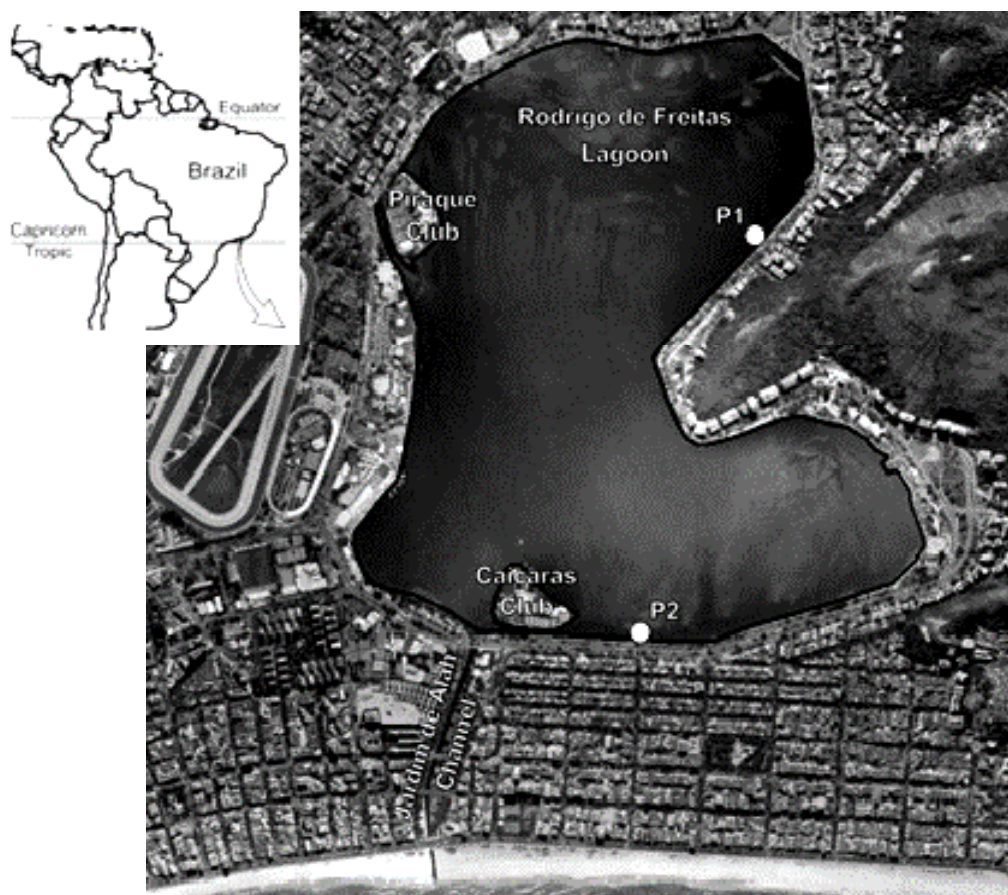


Fig. 1 Collection points of *Ulva sp.* and *R. maritima* in Rodrigo de Freitas Lagoon

A. Experiments with Hexavalent Chromium

Algae and macrophyte with 4.0 g and 3.0 g, respectively, (wet weight) were separated and placed in nylon bags, and later, transferred to beakers of 1 L, containing 500 mL of artificial sea water salinity with 7‰, 15‰, and 21‰. The radiotracer ^{51}Cr ($T_{1/2} = 27.7$ d) was supplied by the Institute of Nuclear Energy Research (IPEN-CNEN/SP), and so was sodium chromate (Na_2CrO_4). It was then diluted with deionized water so that the final average spiked activity was 274×10^3 Bq. To adjust the pH of the solution around 6.0-7.0, some amount of 0.5 N NaOH and 1.0 N HCl solutions were used.

After different time intervals of 2, 6, 10, and 24h, three aliquots were withdrawn and transferred to 50 mL plastic bottles, then washed with 30 mL of deionized water. The sample activities were measured by using a high purity Germanium detector coupled to 8192 multichannel analyzers. At the end of the experiments, the biological material was transferred to a watch glass and then to the oven for drying at 50 °C for three days, for dry weight measurements.

B. Experiments with Trivalent Chromium

Experiments with Cr(III) were performed after chemical reduction of sodium chromate (Na_2CrO_4) according to the methodology proposed by [21], which consists in separating aliquots of standard ^{51}Cr diluted in 10 mL of water, adjusting the pH to 2.0 with HCl 1M, later, adding some microliters of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) 1.57 mol/L solution to maintain the reduction potential at 280 mV Ag/AgCl. Afterwards, the solution was passed into a micro-column filled with ion exchange resin Dowex 1X8 (300-400 mesh), to remove any chromate and ensure the trivalent state of the ion.

The same routine previously described for experiments with hexavalent chromium was conducted. After different time intervals of 2, 6, 20, and 44 hours, three aliquots were withdrawn and transferred to plastic bottles and then analyzed at detector. However, for this experiment, only the species collected at Rodrigo de Freitas Lagoon was considered.

The statistical treatment of data was performed by the use of the OriginPro 8.0 Software with the non-parametric method, Kruskal-Wallis test.

III. RESULTS AND DISCUSSION

Fig. 2 shows the curves of bioaccumulation of Cr(VI) by *Ulva lactuca* collected at control station and *Ulva sp.* and *R. maritima* collected at Rodrigo de Freitas Lagoon as a function of the three salinities considered in this work. A high accumulation of hexavalent form by *Ulva* species in salinity 7‰ decreasing the chromium incorporation rate was found as the salinity increases. On the other hand, for aquatic macrophyte, the opposite behavior was found observing that the kinetic of incorporation is quite similar for salinities 7 and 15‰ (Fig. 2 (C)).

For *Ulva sp.*, up to 10h, it was noticed that there was almost no uptake of Cr(VI) by the algae tissues considering higher salinities. The results obtained with the Kruskal-Wallis test indicated that these species have significant differences in the Cr(VI) incorporation kinetics ($p < 0.05$) over the exposure time. Regarding salinity, differences were only found in the Cr(VI) incorporation kinetics for *R. maritima* within 2h of exposure (Fig. 2 (C)). The genus *Ulva* also showed differences in the kinetics of chromium accumulation among collection points of algae ($p < 0.05$).

The range of optimal salinity (e.g. osmotic balance and photosynthetic activity) for the genus *Ulva* is 18 to 27‰ [22]-[24]. However, in low salinities, there are fewer ions (Na^+) in solution, reducing the possibility of chloro-metal complexes formation and by increasing the ions abundance of metal-free water, thus favoring metals bioaccumulation by algae [25], [26]. In experiments of Cr, Cd, Zn accumulation and by *Ulva lactuca* [11], it was also observed that the metals uptake by the algae were extremely dependent on the salinity, reaching rates 2 to 3 times higher when the salinity decreases from 28‰ to 10 ‰.

According to [11], both physico-chemical change (e.g. free ion concentration, activity coefficient or ionic strength) and physiological change (e.g. membrane permeability) are likely responsible for the observed salinity effects.

R. maritima belongs to the euryhaline group and occurs in waters with an unstable salinity, such as mixo- and hyperhaline brackish waters, continental salt waters. In hyperhaline waters, it tolerates salinities up to 3 times the salinity of the sea [27] because *R. maritima* has the widest known salinity tolerance of any submerged angiosperm. One of the reasons why *R. maritima* can survive in a wide range of salinities is its ability to osmoregulate [28]. Possibly due to this adaptation, a reduction in the metal accumulation in higher salinity was verified.

According to [28], specimens of the macrophyte of Florida (United States), whose maximum salinity is 27.7‰, reached greater abundance when the salinity was around 14‰, but when the salinity dropped to 5-10 ‰, the populations of *R. maritima* were almost terminated. In experiments with Cu, Cd, Pb, and Zn considering *Potamogeton natans* and *Elodea canadensis* submerses macrophytes, it was verified that concentrations of Cu, Zn, and Cd increased with decreasing salinity. Lead concentration, however, was unaffected by salinity [29].

From Fig. 2, greater uptake of Cr(VI) for all the studied

species after 24h of exposure time was verified. The *Ulva* species collected in Rodrigo de Freitas lagoon (Fig. 2 (A)), showed the high bioaccumulation, 1235%, at 7‰ salinity, whereas under the same conditions, for the macroalgae collected at the station control it was 845% (Fig 2 (B)). For the *R. maritima* species, the activity accumulated was 380% at the end of the experiment, showing no different behaviour along the experiment for the two lowest salinities (7‰ and 15‰). However, at the highest salinity, the uptake increased rapidly from 250% to 640% after 10h of exposure.

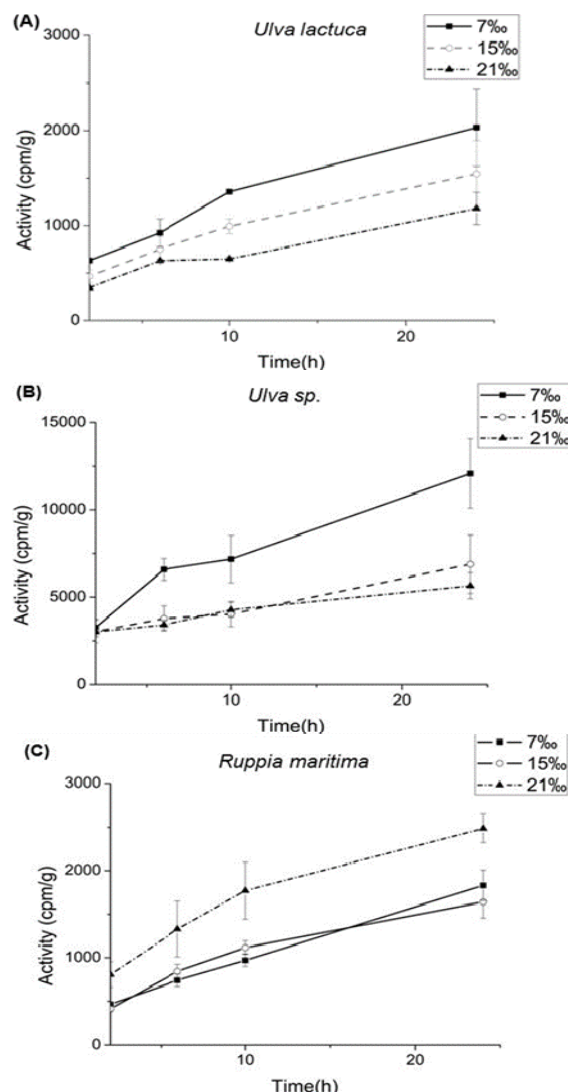


Fig. 2 Activity of hexavalent ⁵¹Cr in *Ulva lactuca* (n=3) collected in control station and *Ulva sp.* and *R. maritima* collected in Rodrigo de Freitas Lagoon placed in solutions prepared with artificial sea water salinity 7‰, 15‰, and 21‰. Mean ± standard error (n=3)

Fig. 3 shows the accumulation of Cr(III) in two species collected at the Rodrigo de Freitas lagoon. For the lower toxicity of Cr(III), an overall increase was noticed in the uptake of this metal ion by both species. Being, as expected, the highest bioaccumulation for the *Ulva sp.* at lower salinity in 6h of experiment (14.500 %), but at the end of the

experiment, it decreased to 10.400% (Fig. 3 (A)), corresponding to 8 times higher when comparing to Cr(VI) under the same experimental conditions. Though, at high salinity 21‰ in 6h, it reaches its maximum uptake that corresponds to approximately 19 times the value found for Cr(VI) and, it dropped to 16 times at the end of the experiment as time goes by. For the *R. maritima* species, the same bioaccumulation behaviour was found for the two lower salinities (Fig. 3 (B)), considering the hexavalent state. However, to the higher salinity, at 6h of exposure time, the Cr(VI) activity was 13 times higher, and by the end of the experiment, it was only 4 times higher, pointing out that the Cr(III) activity remained constant along time. The results obtained with the Kruskal-Wallis Test indicated that these species have significant differences in the Cr(III) incorporation kinetics ($p < 0.05$) over the exposure time.

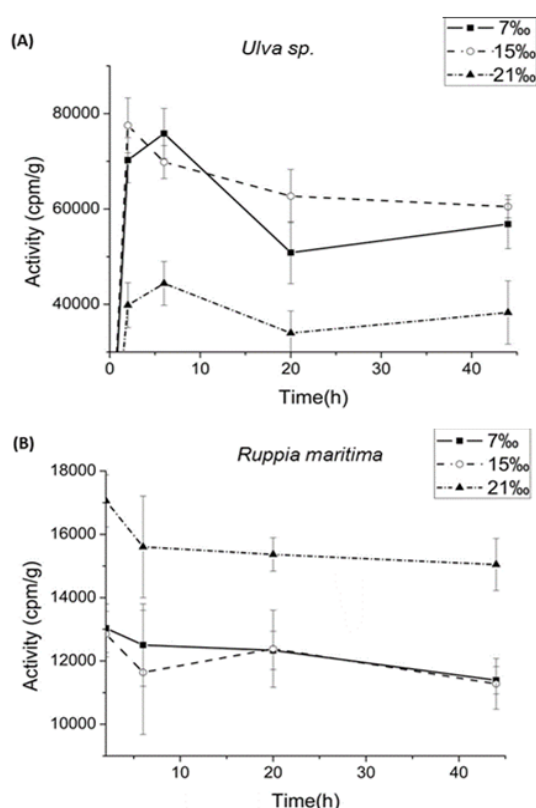


Fig. 3 Comparison of the activity of trivalent ^{51}Cr in *Ulva sp.* and *R. maritima* ($n=3$) collected in Rodrigo de Freitas Lagoon placed in solutions prepared with artificial sea water salinity 7‰, 15‰, and 21‰, mean \pm standard error ($n=3$)

The recent topic in environmental science is biomonitoring, and the most accepted definition for this methodology is the use of systematic answers of biological indicators to assess and monitor the changes in the environment. The biomonitoring can be species, group of species, or biological communities, and also their vital functions that are so closely related with certain environmental factors, which can be used as evaluation indicators for a given area [30].

One criterion traditionally adopted for biomonitoring of

indicator species is the comparison of the bioaccumulation potential, being considered a good biomonitor for species that have metal higher levels in their tissues [31]-[33]. Many studies suggest that *Ulva sp.* has a great potential as biomonitor due to its wide distribution, large size and because its metal concentrations reflected the bioavailable levels of metals in the water [34], [35]. Studies with *Ulva rigida* showed that changes in biochemical composition under different nutrient regimes may result in different bioaccumulation potentials of some metals ions by macroalgae [36]. Therefore, it appears from the analysed results that, even at higher salinities, the *Ulva* species could be successfully used as biomonitor for any chromium tri or hexavalent form, with an excellent response just for a couple of hours of exposure for Cr(III), although for the hexavalent form low salinities and at least one day of exposure will be required. The potential for good display of metals, especially in the case of the *Ulva sp.*, is reinforced by a comparison with studies on bio-accumulation of ^{51}Cr by *Piper nigrum* conducted by [19] where it was found that the accumulation of $^{51}\text{Cr(VI)}$ on the black pepper was negligible, good powers of accumulation being obtained only in trials using $^{51}\text{Cr(III)}$ solutions. On the other hand, the *R. maritima* species is known to be more resistant than the *Ulva* species for higher salinities. Our results showed that it would be the best choice to use as a bioindicator and, since the uptake of Cr(VI) increases with time ($>20\text{h}$), it is clear that this species is also more resistant to the hexavalent form as well, and useful in any salinity.

IV. CONCLUSION

The results of this study showed different behaviours of bioaccumulation of chromium species at different salinities (7 to 21‰). For Cr(VI) it was confirmed that the increment of uptake by macroalgae *Ulva sp.* at lower salinities (mainly for 7‰), whereas for macrophyte *R. maritima*, more resistant to saline stress, the best bioaccumulation potential is obtained at high salinity (21‰).

In experiments with Cr(III), for the studied algae and macrophyte species, increments in activity in tissues between 4-19 times higher than for the Cr(VI) were recorded. Regarding the potential to bioaccumulation, greater sensitivity of *Ulva sp.* rather than *R. maritima* at any salinity (7-21‰) was verified.

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