Uptake and Release of Divalent Zinc Ions from Aqueous Solutions by Aquatic Moss *Fontinalis Antipyretica*

R. Martins and R. Boaventura

LSRE – Laboratory of Separation and Reaction Engineering
Faculty of Engineering; University of Porto
Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Aquatic mosses are able to accumulate zinc and some other heavy metal ions from aqueous solutions and partially release them when exposed to metal-free water. They play an important role in the assessment of toxic elements in water. The advantage of mosses over direct water sampling is that the use of the former lessens spatial and temporal variations, enhances the level of contaminant identification by concentrating toxic elements, and provides information relative to the bioavailable species. However, to make the concentration of metals that can be measured in mosses a reliable indicator of the concentration of toxic elements in the water, we need to model the bioaccumulation phenomenon. Laboratory experiments were conducted to determine zinc uptake and release kinetics by the aquatic moss *Fontinalis antipyretica*, as this species is widely spread in Portuguese rivers and the majority of the European countries. Zinc was chosen for this study because (i) it acts as micro-nutrient for plant growth in low concentration values; (ii) it is toxic when in excess inhibiting the growth and (iii) it is present in many industrial wastewaters and mine drainage waters discharged into rivers and lakes.

INTRODUCTION

The ultimate disposal of wastewater can only be onto land or into water. But whenever watercourses are used for ultimate disposal, the wastewater is treated to prevent any injury to aquatic life in the receiving water. Different types of industrial wastes have been aggravating the problem of water pollution. This problem becomes complex because of the qualitative and quantitative differences in pollution according to the industries involved, and due to the non-degradability of inorganic pollutants like heavy metals which are hazardous when discharged into a water body.

To attain the full goal of zero pollution the immediate problems have to be solved by adopting alternative technologies that suit the situation of low capital availability, minimum manpower and can also save on energy consumption.

There has been considerable interest in the use of aquatic plants for removal various pollutants, like heavy metals, because of their fast growth rate and simple growth requirements. Data from laboratory [1] and field studies [2] have shown that heavy metal uptake by aquatic bryophytes depends on the nature and amount of aquatic plants, their stage of development, earlier treatment as well as the volume of feeding water and its metal ion content and the presence of other dissolved substances. They immobilize, mobilize, or transform metals by extracellular precipitation reactions, intracellular accumulation, oxidation-reduction reactions, methylation and demethylation, and extracellular binding and complexation.

The quantitative determination of pollutants in the several compartments of aquatic ecosystems plays an important task for the identification of pollution sources, the evaluation of contamination or decontamination trends and ecological quality control.

Aquatic mosses show a high capacity to assimilate nutrients, toxics organic composites and heavy metals, leading to a concentration inside the plants several times higher than in the surrounding water [2, 3]. This allows an integration of casual fluctuations in metal concentration in the water during long periods of time [4, 5]. Reports of 30 to 50 fold concentrations are common, and levels of 1000 to 100000 times have been reported [6, 7, 8, 9, 10]. Laboratory experiments had shown that the uptake of zinc by *Rhynchostegium riparioides* is higher than by *Fontinalis antipyretica* at least by a factor of two [11].

Metal uptake by plants consists of two processes: a fast metabolism-independent surface reaction, and a metabolism-dependent slow uptake. The first can be considered an adsorption process onto the cell wall of the organism. The second process occurs when the metal moves from the wall or water into the cell body.

The overall biosorption is most effective in treatment of waters containing low concentrations, 1 to 20 mg l^{-1}, of heavy metal cations [9, 13].

Many biosorption studies use dead, chemical-treated biomass for heavy metal cations removal [3], a process similar to ion exchange, though with better removal results. An advantage of using living organisms (plants, microorganisms) over dead biomass is that they grow and hence produce a regenerating supply of metal removal material.

The purpose of this work is to evaluate the heavy metal removal potential and to determine metal uptake and release kinetics by the aquatic moss *Fontinalis antipyretica*, as this species is widely spread in Portuguese rivers and the majority of the European countries. Since genetically engineered organisms and/or exotic organisms are socially, politically, and environmentally unacceptable, a native strain of aquatic moss was used in the experiments. Moreover, the effect of water hardness on zinc biosorption by mosses was also assessed.

Zinc was selected as the target metal because it: a) acts as micro-nutrient for plant growth in low concentrations; b) is toxic when in excess inhibiting the growth and c) is present in many industrial effluents and mine drainage waters directly discharged into rivers and lakes. It is a regulated metal and poses serious health hazard to humans, even at concentrations of 5 mg l^{-1}.

MATERIALS AND METHODS

Materials

Aquatic mosses, *Fontinalis antipyretica*, were collected at Aldão, not far from the source of the Selho River, a tributary of the Ave River. The moss is shown in figure 1.

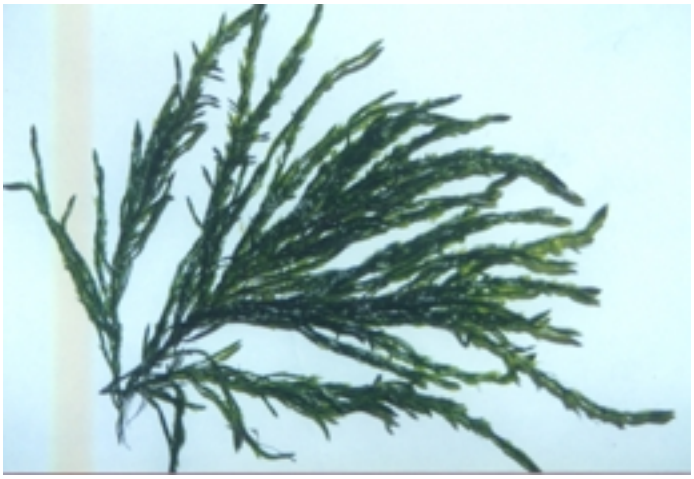

Figure 1 : Aquatic moss *Fontinalis antipyretica*.

The sampling site is located at an unpolluted river stretch, so the metal content in the plants is assumed to be of natural origin and represents the background level. The plants were rinsed in situ, directly in the river water to remove sediments and invertebrates settled in mosses. In the laboratory the mosses were washed with distilled water, selecting the plant green parts and kept for some hours in a refrigerator prior to starting the experiments.

Collecting can be made in whatever moment but the better one is at the beginning of spring. The low temperatures of winter “damage” the plants, and these present a brown coloration. This can be due to a minor quantity of chlorophyll. Mosses must be collected in sites where they are totally submerged and attached to a stable substrate, that is, they can’t move when the speed or level of water stream changes.

Experimental set-up

Zinc uptake and release rates by mosses were investigated in four tanks operating in perfectly mixed conditions. Mosses were exposed to zinc contaminated water and then to metal free water. The experimental set-up is shown in figure 2. The system includes four acrylic-glass tanks, where perfectly mixed conditions are achieved by using recirculating pumps. Tanks are supplied from a constant-level reservoir with tap water

previously dechlorinated with activated carbon (GAC). Water is pumped to the four tanks from this reservoir. The metal solution is introduced in the water lines (one for each tank), by using a multi-channel peristaltic pump, at flow rates calculated to give different metal concentrations in each tank. Zinc (Zn) as $ZnCl_2$ was added to the water stream to give concentrations from 1.05 to 3.80 mg l^{-1}. Flow rate was set at 600 ml min^{-1} and the water level was maintained constant in the tanks. The experimental set-up is under a metallic structure 1.20 m length, supporting two cool fluorescent lamps (a 40 W white light lamp and a 36 W rose light one). Lamps are about 0.90 m above water and the illumination at the water surface is 723 Lux. Lights were operated continuously. Experiments were carried out at ambient temperatures, between 15 and 20 ºC.

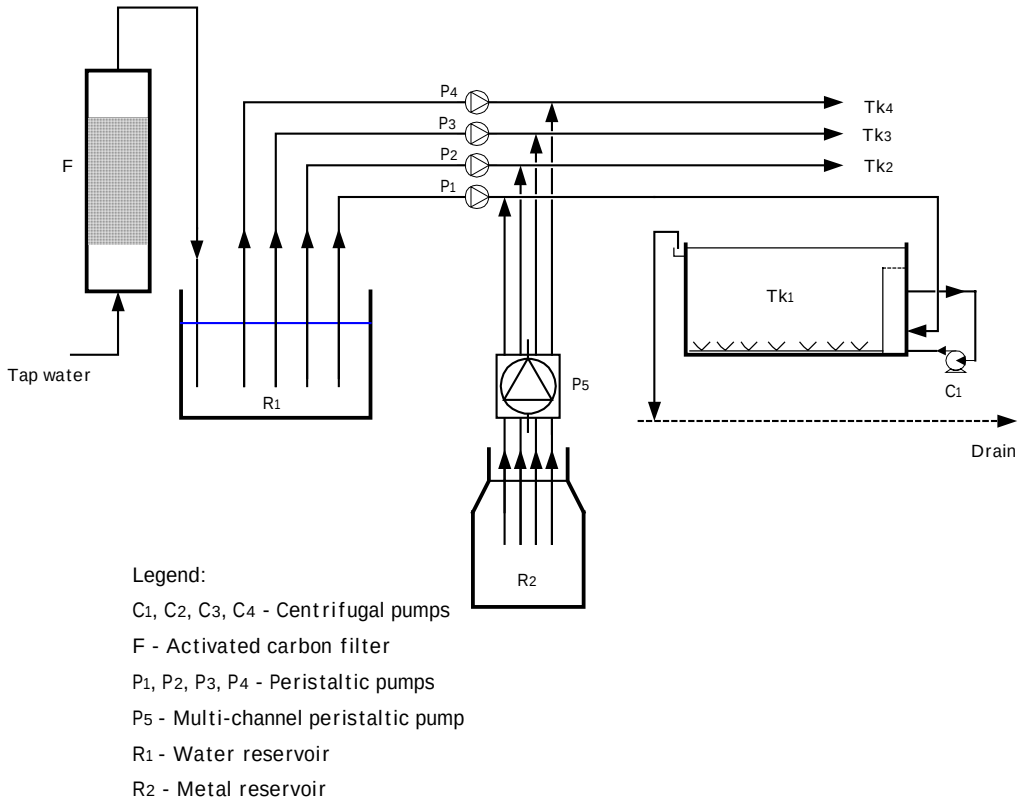

Figure 2 : Experimental set-up diagram.

Parallelepiped plastic net bags were filled with about 10 g (wet wt) aquatic mosses together with a marble to keep them immersed when introduced in the tanks. The mosses in each bag were sufficient to run two replicates of all analyses.

The experiment included an uptake period of 144 h. followed by a release stage of 144 h. too. Aquatic mosses were removed at selected times, in the course of the experimental run, for metal analysis.

The water composition (pH, total alkalinity, total hardness) was checked from time to time during each experiment.

For the study of the effect of water hardness on the sorption process, total hardness varied between 104 and 150 mg $CaCO_3$ l^{-1}.

Methods

Moss samples were rinsed thoroughly in distilled water and dried at 70ºC for 24 h. Then they were ground in a mill (ultra-centrifugal mill RETSCH ZM 100) for 90 seconds at 1400 rpm.

The moss samples were divided in two fractions for duplicate analysis. For the acid digestion [9] an acid-washed Teflon box was used. About 100 mg of ground plants were then placed in each box and added 4 ml of HNO_3 65%. The box was inserted in a Parr digestion bomb and placed in a microwave furnace during 60

seconds. After that, the digest was cooled for 2 hours and made up to a final volume of 50 ml with distilled water. The liquid was filtered under vacuum through 0.45 μm filter paper and analysed for Zn by Atomic Absorption Spectrophotometry (AAS).

KINETIC MODEL

The transfer of a metal ion M^{n+} to and from aquatic mosses is assume to be described by a first-order mass transfer model [10, 18], represented as:

$$C_W \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_m - C_{m0} \tag{1}$$

with,

C_w - metal concentration in the water, mg l^{-1}

C_m - metal concentration in the plant, μg g^{-1}

C_{m0} - initial metal concentration in the plant, μg g^{-1}

Overall concentration changes within the plant for the uptake period may be described by the equation:

$$\frac{dC_m}{dt} = k_1 C_W - k_2 (C_m - C_{m0}) \tag{2}$$

where,

k_1 - uptake rate constant, h^{-1}

k_2 - release rate constant, h^{-1}

For the condition $C_m=C_{m0}$ at t=0 and assuming C_w=constant, the integration of equation (2) gives:

$$C_m = C_{m0} + \frac{k_1}{k_2} C_W (1 - e^{-k_2 t}) \tag{3}$$

C_m tends to C_{me}, when t tends to infinity, given by the expression:

$$C_{me} = C_{m0} + \frac{k_1}{k_2} C_W \tag{4}$$

C_{me} is the metal concentration in the plant at equilibrium, μg g^{-1}.

Bioconcentration at steady-state may be described [14] by a bioconcentration factor (BCF) defined as:

$$BCF = \frac{C_{me} - C_{m0}}{C_W} = \frac{k_1}{k_2} \tag{5}$$

If plants are further exposed to metal-free water (C_w < 0.03 mg l^{-1}) at t = t_d (t_d; time at the end of uptake period), the contamination is interrupted and a decontamination period starts. Changes of metal concentration in the plant with time are then described by the equation:

$$\frac{dC_m}{dt} = -k_2 (C_m - C_{mr}) \tag{6}$$

Where C_{mr} is the residual metal concentration, μg g^{-1}.

Integrating the equation (6) with the initial condition

$$t = t_d;\ C_m = C_{mu} \tag{7}$$

yields

$$C_m = C_{mr} + (C_{mu} - C_{mr}) * e^{-k_2 (t - t_d)} \tag{8}$$

As $t \to \infty;\ C_m \to C_{mr}$ and a biological elimination factor (BEF) may be defined for the decontamination stage:

$$BEF = \frac{C_{mu} - C_{mr}}{C_{mu}} = 1 - \frac{C_{mr}}{C_{mu}} \tag{9}$$

If there is no decontamination when the plants are exposed to clean water, then BEF=0. Otherwise, BEF=1 corresponds to total metal release.

RESULTS AND DISCUSSION

The mass transfer model previously described was fitted to the experimental results in order to determine the uptake and release rate constants, k_1 and k_2, the zinc concentration at the end of the uptake phase, C_{mu},

and the equilibrium concentrations, C_{me} and C_{mr}, for the contamination and decontamination stages, respectively.

During the experimental work the feeding water was regularly analysed and its composition remained stable throughout the study. Zinc concentration in the tanks was also checked daily (figure 3).

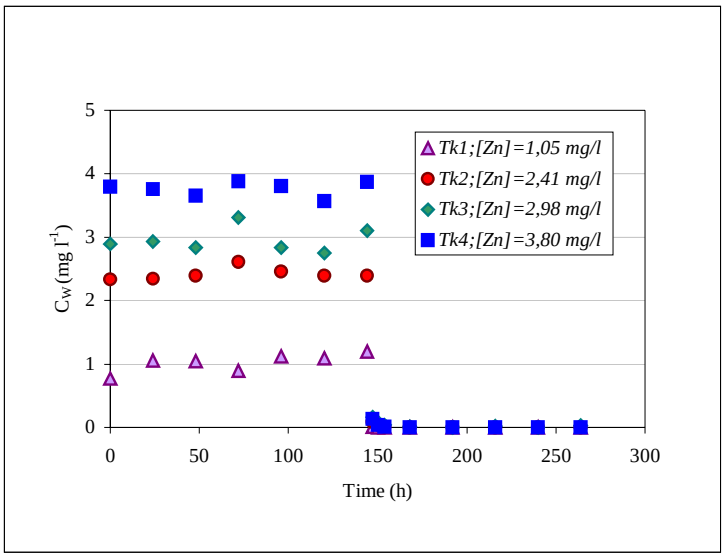

Figure 3 : Zinc concentration in the tanks throughout the experiments.

The experimental data for the contamination phase were fitted to equation 3 in order to determine the kinetic constants k_1 and k_2. Equilibrium concentration, C_{me}, was calculated using equation (4) and residual concentration after decontamination, C_{mr}, was determined by fitting equation (8) to the experimental data. Table 1 shows the kinetic constants and equilibrium concentrations for the accumulation and release phases.

Table 1 : Kinetic constants and equilibrium concentrations for zinc uptake and release

C_w (mg l^{-1})	Total hardness (mg $CaCO_3$ l^{-1})	k_1 (h^{-1})	k_2 (h^{-1})	C_{me} (µg g^{-1})	C_{mr} (µg g^{-1})
Experiment 1					
1.05	101	145	0.032	5030	656
2.41	101	85	0.025	8447	1288
2.98	101	82	0.025	10035	1455
3.80	101	59	0.020	11459	2342
Experiment 2					
3.40	104	61	0.042	5013	1110
4.85	142	96	0.790	6060	1057
3.49	150	139	0.103	4815	1648

Figure 4 to 10 show the variation of the sorbed zinc, predicted by the model for the contamination and decontamination phases, as well as the experimental data.

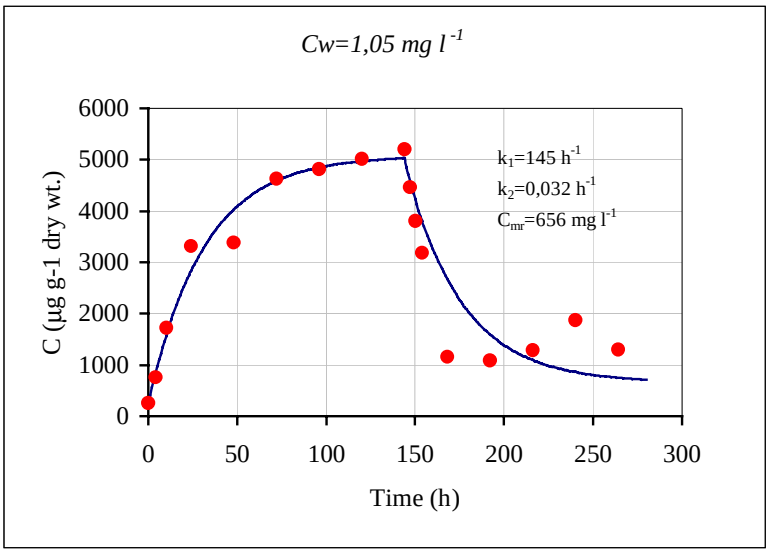

Figure 4 : Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 1.05 mg l^{-1}; total hardness of 101 mg $CaCO_3$ l^{-1} (— model; • experimental data).

The experimental results show that, k_1 decreased from 145 to 59 h^{-1} as metal concentration increased from 1.05 to 3.80 mg l^{-1}, which suggests a toxic effect on the plant and a lesser ability for metal uptake by the cells. The release rate constant, k_2, shows a similar behaviour although in a lesser extent. Increasing the water hardness from 104 to 150 mg $CaCO_3$ l^{-1} and maintaining zinc water concentration at about 3.4 mg l^{-1}, a marked increase of k_1 and k_2 was observed. At experiment 2 for equal hardness, the uptake kinetic constant varies of 139 for 96 h^{-1} when zinc concentration in water increases, what it comes to confirm the previous results.

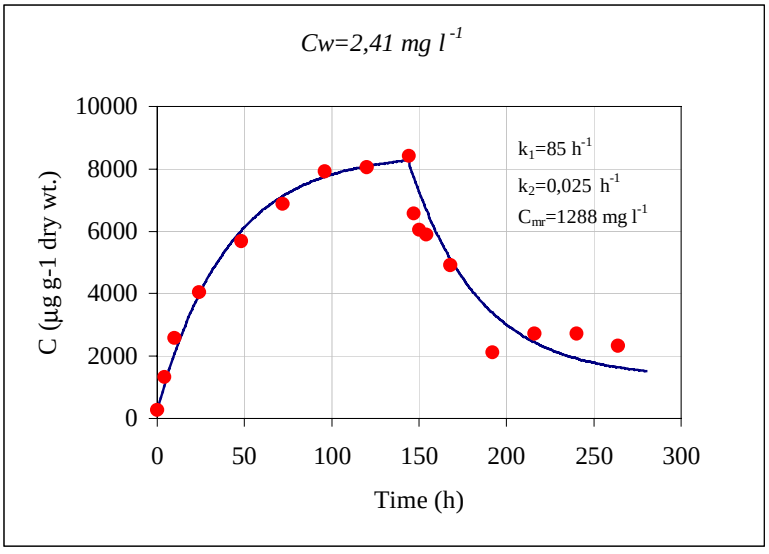

Figure 5 : Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 2.41 mg l^{-1}; total hardness 101 mg $CaCO_3$ l^{-1} (— model; • experimental data).

As higher calcium concentrations increase values of k_1, lesser time is needed to reach equilibrium, only about 24 hours or less in same cases. For k_2, we got similar results. An explanation could be the calcium and

zinc competition, being this metal mainly accumulated in the Donnan free space of the cellular membrane (more peripheral zone), and not in a more internal zone, in the protoplast or vacuoles of the cells. Comparing the theoretical values of equilibrium concentration and the concentrations observed at the end of the accumulation phase, it can be show that C_{mu} is close to C_{me} after 144 hours exposure, which confirms that the uptake period was correctly chosen.

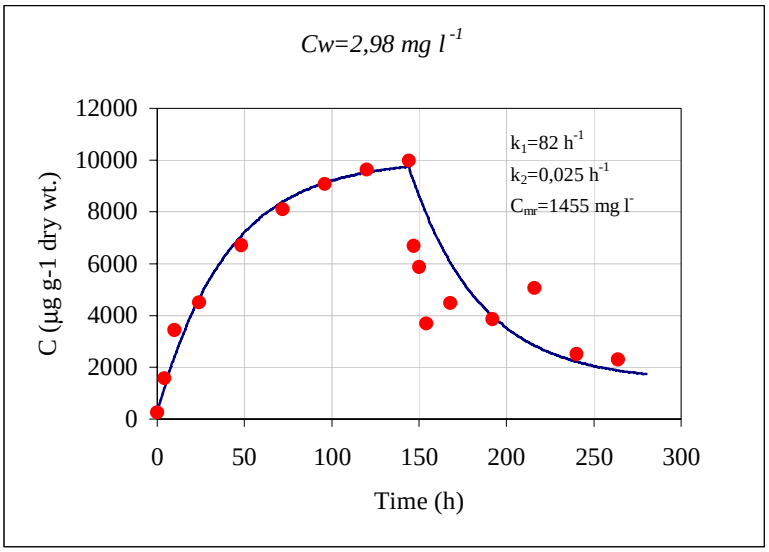

Figure 6 : Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 2.98 mg l^{-1}; total hardness 101 mg $CaCO_3$ l^{-1} (— model; • experimental data).

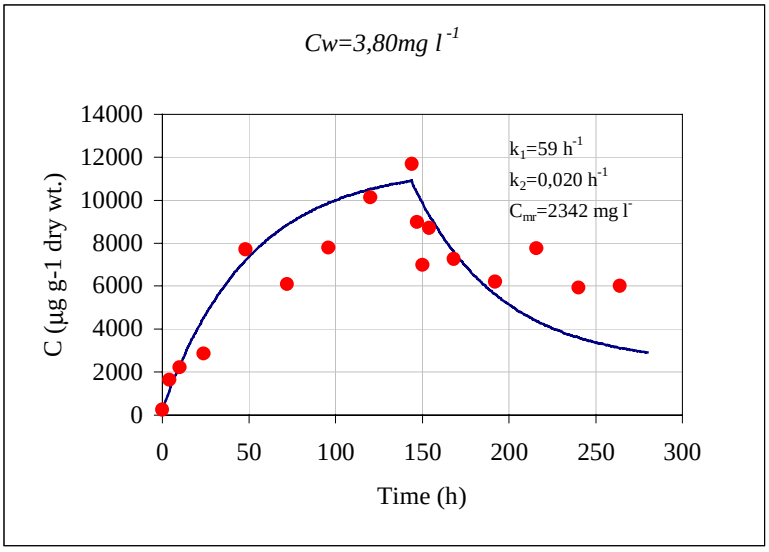

Figure 7 : Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 3.80 mg l^{-1}; total hardness 101 mg $CaCO_3$ l^{-1} (— model; • experimental data).

The fitting line in the proximity of the 144 hours indicates saturation of a finite number of binding sites on the moss cell surface after exposure to heavy metal and possibly the advent of metabolism dependent on the transport of metal to the inner cell mass.

All samples showed bright green color with bubbles trapped in the filamentous mass suggesting that respiration and photosynthesis in the mosses occurred during the experiment.

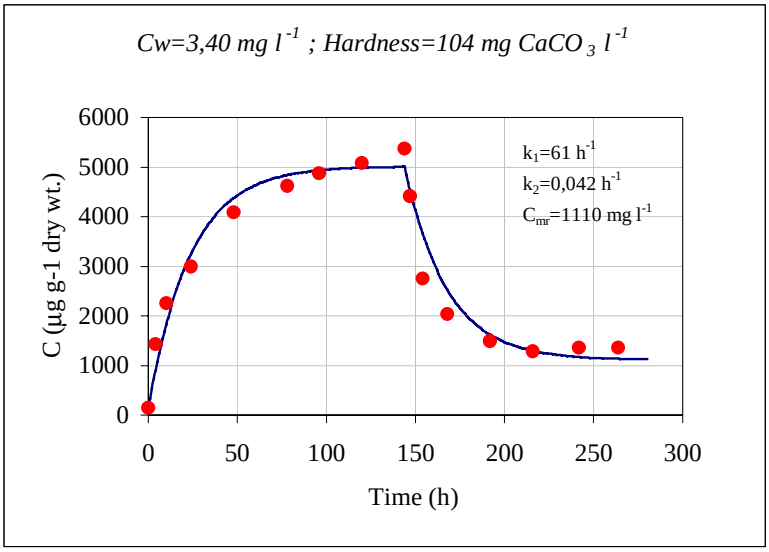

Figure 8: Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 3.40 mg l^{-1}; total hardness 104 mg $CaCO_3$ l^{-1} (— model; • experimental data).

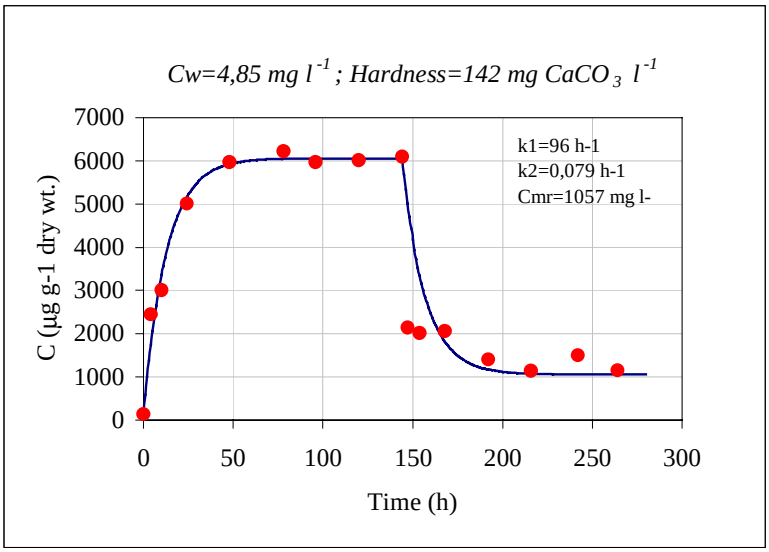

Figure 9: Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 4.85 mg l^{-1}; total hardness 142 mg $CaCO_3$ l^{-1} (— model; • experimental data).

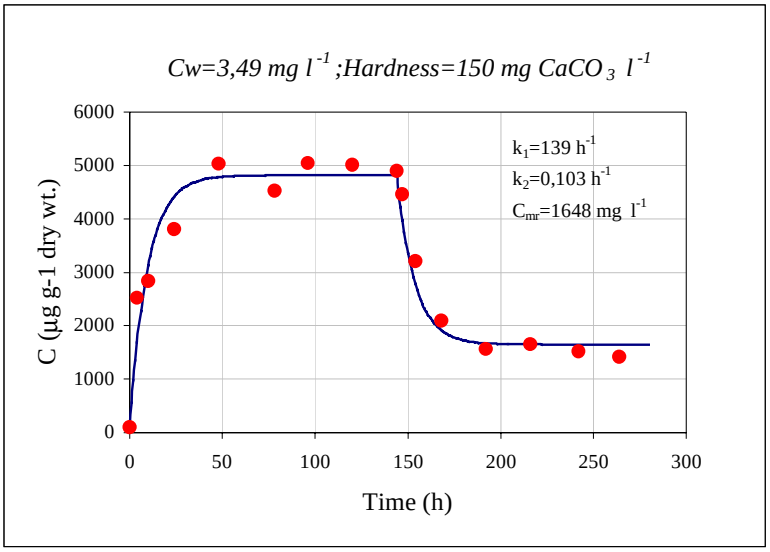

Figure 10: Uptake and release of zinc by *Fontinalis antipyretica*: metal concentration in the water 3.49 mg l^{-1}; total hardness 150 mg $CaCO_3$ l^{-1} (— model; • experimental data).

Table 2 displays the BCF and BEF values. BCF values ranged between 4531 and 2950 (water hardness = 101 mg $CaCO_3$ l^{-1}). The higher BCF value was obtained for the lower metal concentration. This indicates that constant flow of a small amount of heavy metal could result in higher uptake compared with a large amount. Lower BCF values were obtained when water hardness was increased.

BEF remained approximately constant and averaged 0.80.

Table 2 : Bioconcentration (BCF) and Biological elimination (BEF) factors

C_w (mg l^{-1})	BCF	BEF
Experiment 1		
1.05	4531	0.87
2.41	3400	0.84
2.98	3280	0.85
3.80	2950	0.78
Experiment 2		
3.40	1435	0.78
4.85	1220	0.83
3.49	1352	0.66

CONCLUSIONS

Within the zinc concentration range used in this study (1.05 to 4.85 mg l^{-1}), the aquatic moss *Fontinalis antipyretica* can accumulate, at equilibrium, the metal ion by a factor 4531 to 1220 (Zn concentration in the moss, mg Kg^{-1}, dry wt. / Zn concentration in water, mg l^{-1}).

The metal uptake rate, for identical water hardness, tends to decrease as the Zn concentration in the water increases, suggesting a toxic effect in mosses and a subsequent deterioration of their physiological state.

Competitive adsorption exists between zinc and calcium ions. For similar Zn concentrations the bioaccumulation tends to decrease as the total hardness of water increases.

At the end of the decontamination stage, plants only retain on average about 20% of the metal accumulated at the end of the uptake period.

A first-order mass transfer model adequately describes the metal uptake and release stages, then permitting the calculation of the kinetic constants and equilibrium concentrations.

The model permits to calculate the time required to reach the metal concentration in the mosses close to the equilibrium concentration; this parameter is useful for monitoring purposes.

NOMENCLATURE

BCF Bioconcentration factor;
BEF Biological elimination factor.
C_m metal concentration in the plant, $\mu g\ g^{-1}$;
C_{m0} initial metal concentration in the plant, $\mu g\ g^{-1}$;
C_{mr} residual metal concentration in the plant, $\mu g\ g^{-1}$;
C_{mu} metal concentration in the plant at the end of uptake period, $\mu g\ g^{-1}$;
C_w metal concentration in the water, $mg\ l^{-1}$;
k_1 uptake rate constant, h^{-1};
k_2 release rate constant, h^{-1};
t_d time at the end of uptake period, h.

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