

Uptake and release of zinc by aquatic bryophytes (*Fontinalis antipyretica* L. ex. Hedw.)

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Abstract

The zinc uptake and posterior release by an aquatic bryophyte—*Fontinalis antipyretica* L. Ex Hedw.—was experimentally studied in laboratory exposing the plants to different zinc concentrations in the range, 1.0–5.0 mg l⁻¹, for a 144 h contamination period, and then exposed to metal-free water for a 120 h decontamination period. The experiments were carried out in perfectly mixed contactors at controlled illumination, using mosses picked out in February 1997, with a background initial zinc concentration of 263 mg g⁻¹ (dry wt.). A first-order mass transfer kinetic model was fitted to the experimental data to determine the uptake and release constants, k_1 and k_2 , the zinc concentration in mosses at the end of the uptake period, C_{mu} , and at the equilibrium, for the contamination and decontamination stages, C_{me} and C_{mr} , respectively. A bioconcentration factor, $BCF = k_1/k_2$ (zinc concentration in the plant, dry wt./zinc concentration in the water) was determined. A biological elimination factor defined as $BEF = 1 - C_{mr}/C_{mu}$ was also calculated. BCF decreases from about 4500 to 2950 as Zn concentration in water increases from 1.05 to 3.80 mg l⁻¹. BEF is approximately constant and equal to 0.80. Comparing Zn and Cu accumulation by *Fontinalis antipyretica*, it was concluded that the uptake rate for Zn (145 h⁻¹) is much lower than for Cu (628 h⁻¹) and the amount retained by the plant decreased by a factor of about seven. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

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

Aquatic mosses show a high capacity to assimilate nutrients, toxic organic compounds (e.g. pesticides) and heavy metals, leading to a concentration inside the plants several times higher than in the surrounding environment [1,2]. Due to their physiological and

environmental characteristics and the fact they are widespread in most European rivers [3] aquatic mosses have been successfully used as biological indicators of surface waters (rivers, lakes) contamination by heavy metals [4–10]. Their accumulation capacity allows an integration of casual fluctuations in metal concentration in the water during long periods of time [11].

Particularly, aquatic bryophytes have been shown to satisfy the basic requisites for a good bioindicator [12], and so they can be used as a low cost methodology for monitoring water quality [8].

The accumulation capacity of aquatic mosses has been also explored to remove heavy metals from polluted waters [13,14].

Nomenclature

BCF	bioconcentration factor
BEF	biological elimination factor
C_m	metal concentration in the plant, $\mu\text{g g}^{-1}$
C_{m0}	initial metal concentration in the plant, $\mu\text{g g}^{-1}$
C_{mr}	residual metal concentration in the plant, $\mu\text{g g}^{-1}$

C_{mu}	metal concentration in the plant at the end of uptake period, $\mu\text{g g}^{-1}$
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
ρ	water density, kg l^{-1}

In order to get a correct and effective interpretation of biomonitoring results, several studies were carried out to establish heavy metal uptake and release kinetics either through laboratory experiments [9] or from field surveys [15].

Kinetics depends on several parameters concerning the plant itself and environmental factors (temperature, light intensity, metal concentration, presence of other compounds and physical-chemical water characteristics).

Heavy metal accumulation in bryophytes has been studied by several authors [16,17] to elucidate the uptake and/or release mechanisms and the uptake rate from metal-enriched solutions.

Experimental results in batch systems suggest that two [18] or three stages [17] are identifiable during metal uptake by plant cells. However, in some cases, a global first-order mass transfer kinetic model corresponds to a sufficiently satisfactory approach [9].

The equilibrium concentration can be determined by exposing the plant to metal bearing water for a sufficiently long period until steady-state conditions are achieved. Alternatively, equilibrium concentration may be calculated from the uptake and release kinetic rate constants, experimentally determined by contaminating the plants during a short period and then exposing them to non-contaminated water [19].

This methodology was applied to determine bioconcentration factors of zinc by *Gammarus pulex* (L.) [20], and to investigate the uptake and release kinetics of copper by *Fontinalis antipyretica* [9].

Data from field [21] and laboratory [14] studies have shown that heavy metal uptake by aquatic bryophytes depends on the selected species. Laboratory experiments showed that the uptake of zinc by *Rhynchostegium riparioides* is higher than by *Fontinalis antipyretica* at least by a factor of two [22].

However, *Fontinalis antipyretica* has been recognized as a good bioindicator for heavy metal contamination, which is confirmed by several studies reported in literature [15]. Assuming an accidental discharge of a metal bearing effluent into a river, the knowledge of the uptake and release rates is essential to predict, after a

short exposure period, the metal concentration in the water since metal concentrations in the bioindicator are known.

Moreover, it is possible, from those rates to verify if the elapsed time between the beginning of the discharge and the moss sampling for analysis is long enough to assume that equilibrium conditions were reached.

The main objective of this work is to study the uptake and release of Zn by an aquatic bryophyte—*Fontinalis antipyretica*—having in mind a future application of aquatic mosses to the decontamination of industrial effluents. Kinetic and equilibrium parameters will be determined by using a simple mass transfer model.

2. Materials and methods

2.1. Mosses

Experiments were carried out using an aquatic bryophyte, *Fontinalis antipyretica* (Hedw.) collected in the Selho River, at Aldão, in the Ave River basin. The samples were taken from a river stretch without metal contamination upstream, so the heavy metals present are assumed to be of natural origin. Mosses were rinsed in the sampling site, directly in the river water to remove sediments and invertebrates. In the laboratory the mosses were then washed with distilled water, selecting the plant green parts, which were kept for some hours in a refrigerator prior to starting the experiments. The mosses used in this experimental work were collected in February 1997.

2.2. Experimental set-up

The experimental set-up is a continuous flow system, including four 20 l—rectangular (250 mm \times 400 mm) tanks in acrylic (Fig. 1). Water recirculation centrifugal pumps promote the agitation and homogenization in order to get perfectly mixed contactors, as confirmed by the analysis of the residence time distribution using the tracer (KCl) technique.

Each tank was supplied from a reservoir containing previously dechlorinated water (by adsorption of residual chlorine onto activated carbon), using peristaltic pumps. The metal solution was introduced in the feed line of each tank by a multi-channel peristaltic pump.

From a stock solution of $ZnCl_2$, different zinc concentrations in the range $1.0\text{--}5.0\text{ mg l}^{-1}$, were obtained in the tanks. Flow rate was set at 600 ml min^{-1} and the water level remained constant in the tanks. Experiments were carried out at ambient temperature, in the range $17\text{--}20^\circ\text{C}$.

Illumination was supplied by two fluorescent lamps (a 40 W white light lamp and a 36 W rose light one) that remained switched on during all the experiments.

Lamps were about 0.9 m above water level and illumination at the surface registered an average value of 1723 lx.

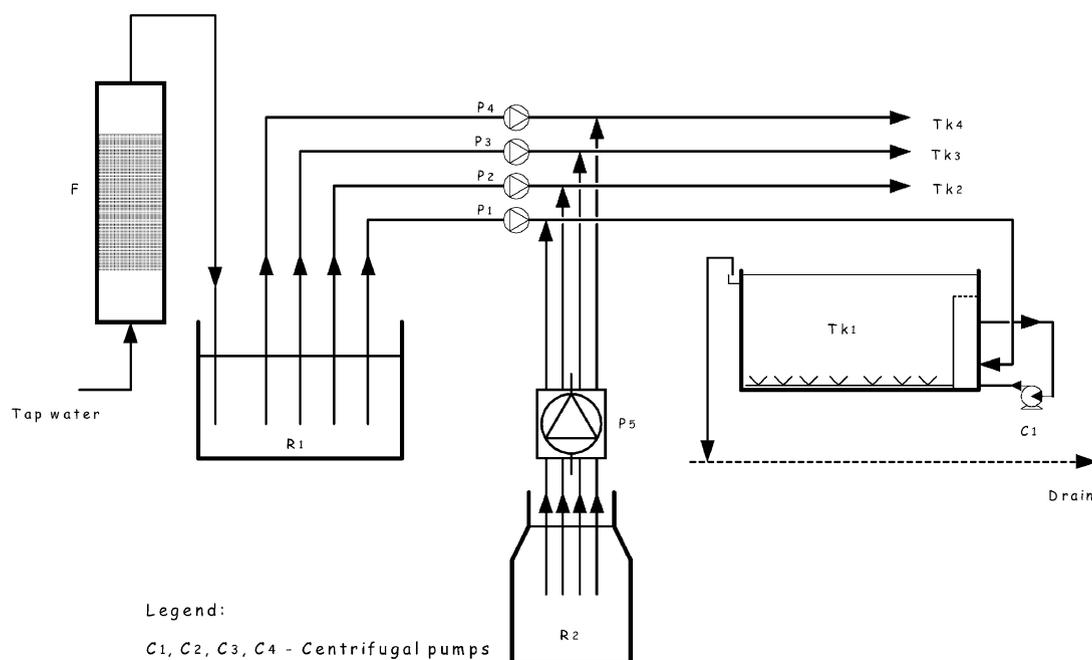
Moss samples in enough amounts for analyses in duplicate, were placed in parallelepiped plastic net bags and immersed in each tank. Experiments consisted of a

contamination period of 144 h followed by a decontamination stage of 120 h. Mosses and water samples were removed from each tank, at time intervals previously defined.

Biomass remained active during all the experiments as indicated by the oxygen bubbles release, due to photosynthesis. Although some plant growth could be expected it was negligible for the contact period within the tanks.

2.3. Analytical procedures

Moss samples from each tank were rinsed with distilled water and dried at 70°C for 24 h. Then, they were ground in a steel-chromium rings mill at 1400 rpm for 90 s. Each moss sample was analyzed in duplicate after acid digestion. Approximately 100 mg of moss were placed in boxes of teflon (23 ml capacity) previously washed with 10% HNO_3 and then digested with 4 ml of 65% HNO_3 . Each box was inserted in a Parr bomb,



- Legend:
- C1, C2, C3, C4 - Centrifugal pumps
 - F - Activated carbon filter
 - P1, P2, P3, P4 - Peristaltic pumps
 - P5 - Multi-channel peristaltic pump
 - R1 - Water reservoir
 - R2 - Metal solution reservoir
 - Tk1, Tk2, Tk3, Tk4 - Water mosses contacting tanks

Fig. 1. Experimental set-up.

which was placed in a microwave oven at 600 W for 60 s. After digestion, the bomb was left to rest during 2 h, being the solution transferred to a 25 ml volumetric flask and diluted with distilled and deionized water. After vacuum filtration through a 0.45 µm membrane, the solution was analyzed for zinc by atomic absorption spectrophotometry (AAS). The zinc content in mosses was expressed in µg g⁻¹ dry weight.

3. Kinetic model

For a two-compartments system (water–plant), the metal ions transfer from and to aquatic bryophytes is assumed to be described by a first-order mass transfer model [9,19], represented as

$$C_W \begin{matrix} \xrightarrow{k_1} \\ \xleftarrow{k_2} \end{matrix} C_m - C_{m0}, \quad (1)$$

where C_W is the metal concentration in the water, mg l⁻¹, C_m the metal concentration in the plant, µg g⁻¹, C_{m0} the initial metal concentration in the plant, µg g⁻¹, k_1 the uptake rate constant, h⁻¹, k_2 the release rate constant, h⁻¹.

The metal concentration variation in the plant along the uptake period is given by the differential equation:

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

where t is the time (h) and ρ the water density (kg l⁻¹).

Integrating Eq. (2), with the initial condition $C_m = C_{m0}$ at $t = 0$ and assuming $C_W = \text{constant}$, gives

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

When t tends to ∞ , the metal concentration in the plant tends to equilibrium (C_{me}), then

$$C_{me} = C_{m0} + \frac{k_1 C_W}{k_2 \rho}. \quad (4)$$

Replacing t by t_d (t_d = time at the end of uptake period) in Eq. (3), we can calculate the metal concentration at the end of the contamination period (C_{mu}):

$$C_{mu} = C_{m0} + \frac{k_1 C_W}{k_2 \rho} (1 - e^{-k_2 t_d}). \quad (5)$$

At steady-state conditions, the bioaccumulation rate may be represented by a bioconcentration factor (BCF) defined as

$$BCF = \frac{(C_{me} - C_{m0})\rho}{C_W} = \frac{k_1}{k_2}. \quad (6)$$

Interrupting the addition of metal to the water at $t = t_d$, a decontamination period starts up. Experimental studies have shown that in this period the metal elimination is not complete, i.e. the metal accumulated tends to a residual value greater than C_{m0} . In this phase, the metal concentration varies with time according to the equation:

$$\frac{dC_m}{dt} = -k_2(C_m - C_{mr}), \quad (7)$$

where C_{mr} is the residual metal concentration in plant, µg g⁻¹.

Integrating Eq. (7) with the initial condition

$$t = t_d; \quad C_m = C_{mu} \quad (8)$$

it becomes

$$C_m = C_{mr} + (C_{mu} - C_{mr})e^{-k_2(t-t_d)}. \quad (9)$$

As t tends to ∞ , C_m tends to C_{mr} and so a biological elimination factor (BEF) may be defined for the decontamination period:

$$BEF = \frac{C_{mu} - C_{mr}}{C_{mu}} = 1 - \frac{C_{mr}}{C_{mu}}. \quad (10)$$

The BEF can take values between zero (no decontamination when mosses are exposed to metal-free water) and one (total metal release).

4. Results and discussion

Table 1 shows the physico-chemical characteristics of the dechlorinated tap water throughout the experimental work. The evolution of zinc concentration in the tanks can be observed in Fig. 2. The initial zinc concentration in the mosses was 263 µg g⁻¹, which can be considered the background concentration for aquatic mosses at non-polluted sites [23].

Table 1
Water quality parameters throughout the experimental work

Parameter	Range
pH	6.5–7.0
Conductivity (µS cm ⁻¹)	220–240
Alkalinity (mg CaCO ₃ l ⁻¹)	50.0–58.2
Total hardness (mg CaCO ₃ l ⁻¹)	95.5–106.0
Nitrates (mg l ⁻¹)	2.3–2.5
Chloride (mg l ⁻¹)	13.4–13.8
Zinc (mg l ⁻¹)	< 0.03
TOC (mg l ⁻¹)	14.4–14.7

Experimental data for the accumulation stage were fitted to Eq. (3) to determine the kinetic constants k_1 and k_2 . The metal concentration at equilibrium, C_{me} , and the metal concentration at the end of the uptake period, C_{mu} , were calculated using Eqs. (4) and (5), respectively. Fitting the experimental data of the decontamination period to Eq. (8) it was determined the residual metal concentration, C_{mr} . Table 2 shows the values of the kinetic constants and the equilibrium concentrations, both at the uptake and release stages. The evolution of the zinc concentration as predicted by the model, as well as the experimental values are plotted in Fig. 3.

The kinetic constant 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 that reduces the metal uptake rate by the cells. Plant uptake capacity, expressed as C_{me} or C_{mu} , increases with metal concentration in water (Table 2). A limit to the amount of metal bound by the plant seems to exist, as the maximum amount of metal retained by the plant depends on the number of binding sites. At the end of the 144-h-contamination period, the maximum uptake capacity was not achieved for the metal concentration range used in this work. Uptake kinetics, however, are not dependent on the number of binding sites, but on

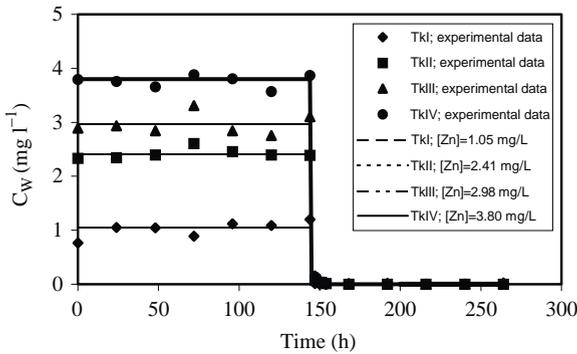


Fig. 2. Zinc concentration in the tanks throughout the experiment.

Table 2
Kinetic constants and equilibrium concentrations for zinc uptake and release

C_w ($mg\ l^{-1}$)	k_1 (h^{-1})	k_2 (h^{-1})	C_{me} ($\mu g\ g^{-1}$)	C_{mr} ($\mu g\ g^{-1}$)	C_{mr}/C_{mu}
1.05	145	0.032	5030	656	0.13
2.41	85	0.025	8447	1288	0.16
2.98	82	0.025	10 035	1455	0.15
3.80	59	0.020	11 459	2342	0.22

C_w , so the decrease in the kinetic constant k_1 as C_w increases suggests a toxic effect on the plant. For the decontamination phase, k_2 shows a similar behavior although in a lesser extent. At equilibrium the zinc concentration in plant increases, as could be expected, with the concentration in water. After decontamination, the residual zinc concentration in equilibrium with metal-free water is also proportional to the amount accumulated at the end of the uptake period.

In all cases, the Zn^{2+} uptake increased rapidly in the first hours and remained nearly constant after 100 h of accumulation, suggesting that bioaccumulation is a very fast process and about 85% of the total uptake is reached within 100 h. This behavior is compatible with the mechanism of the uptake in three stages. The first stage (exchange adsorption) corresponds to a rapid surface binding; a large amount of zinc is taken up in this stage and it is limited to the Donnan-free-space of the cell wall [17]. The second stage is slower and it is the intracellular diffusion (penetration into the protoplast including the cell organelles) that governs the process. The slow third stage results from the active accumulation of metal within the plant cells. This stage is dependent upon factors that affect the metabolism, such as temperature and light intensity. Experimental results and model fitting show that the contribution of the two last stages compared with the first one can be neglected as regards uptake kinetics.

Comparing the theoretical equilibrium concentrations and the concentrations observed at the end of the accumulation phase, the period of 144 h was suitably chosen. The extent of the decontamination period lasts more than 120 h, which it is evidenced in Fig. 3.

Table 3 shows the values of the BCF and BEF calculated from Eqs. (6) and (10), respectively. BCF values ranged between 4531 and 2950, and they vary in inverse relation to the zinc concentration in the water. For lower zinc concentrations ($1.052\ mg\ l^{-1}$), the mosses can accumulate approximately 4500 times more than the zinc concentration in the water. In the working range of zinc concentration in water it was observed an exponential relationship between BCF and C_w (Fig. 4):

$$BCF = 4600.9 * C_w^{-0.3281} \quad (r^2 = 0.99)$$

$$\text{for } 1.05 < C_w < 3.80\ mg\ l^{-1}.$$

In the decontamination period, the zinc released by the aquatic mosses reached high values. The BEF remained approximately constant and averaged 0.80. The fraction of zinc retained by the plant at equilibrium with metal-free water (C_{mr}/C_{mu}) increases with the maximum accumulated at the end of the uptake period (C_{mu}), as can be observed in Table 2.

Exposing the aquatic moss *Fontinalis antipyretica* to a $0.6\ mg\ l^{-1}$ copper solution in similar conditions, Gonçalves and Boaventura [9] obtained a Cu concentration

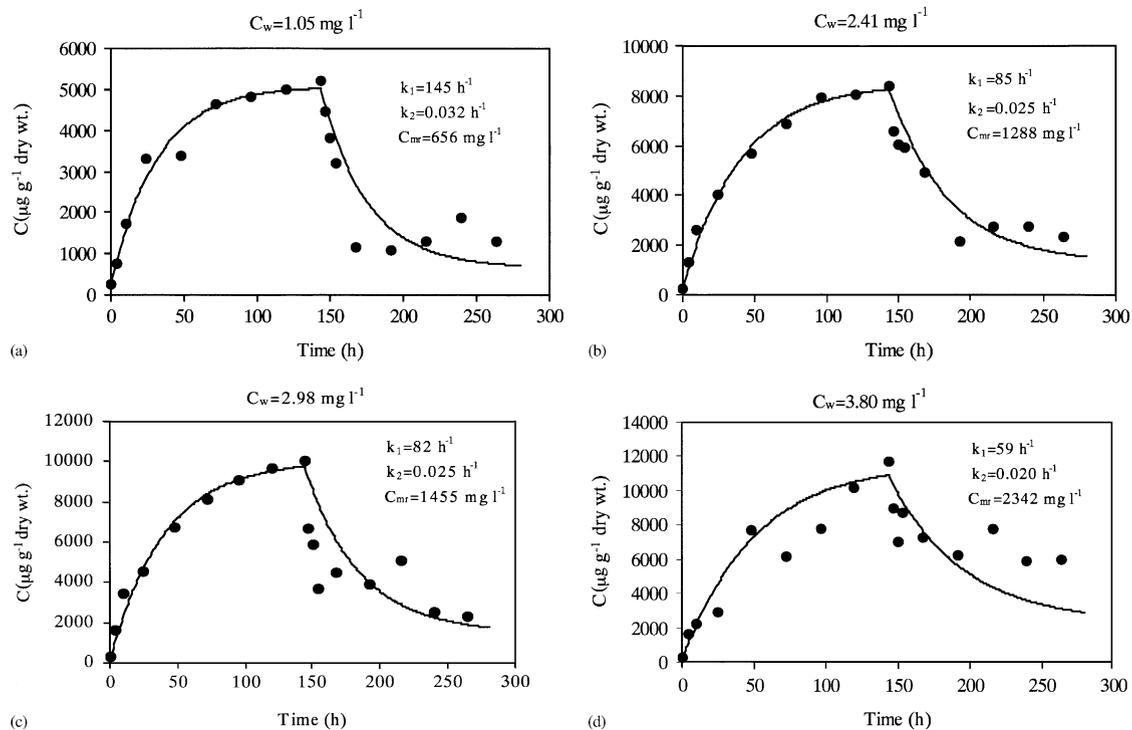


Fig. 3. Uptake and release of zinc by *Fontinalis antipyretica* for different metal concentrations in water: (a) 1.05 mg l^{-1} , (b) 2.41 mg l^{-1} , (c) 2.98 mg l^{-1} , (d) 3.80 mg l^{-1} (—, model; ●, experimental data).

$C_w \text{ (mg l}^{-1}\text{)}$	BCF	BEF
1.05	4531	0.87
2.41	3400	0.84
2.98	3280	0.85
3.80	2950	0.78

at equilibrium equal to $18\,876 \mu\text{g}$ per gram of moss (dry wt.), a value much higher than that found in this study using a 1.05 mg l^{-1} zinc solution ($5030 \mu\text{g}$ per gram of moss, dry wt.). A low soluble organic matter content in the water used in the experiments with copper may be responsible for the higher uptake capacity obtained for this metal. The BCF for Cu and Zn are 31 400 and 4531, respectively, which shows that *Fontinalis antipyretica* can accumulate Cu about seven times more than Zn. The observed higher capacity to accumulate Cu is also in agreement with the results found by Al-Asheh and Duvnjak [24]. The uptake rate, k_1 , for Cu (628 h^{-1}) is largely higher than for Zn (145 h^{-1}). Part of the

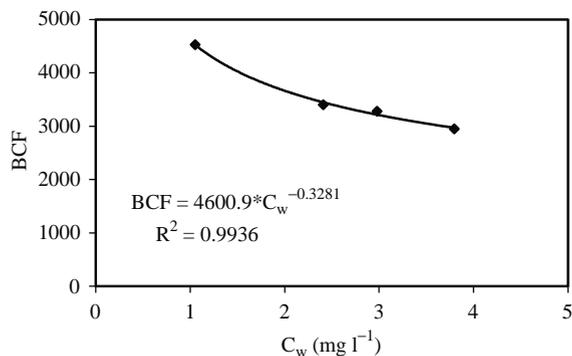


Fig. 4. Exponential relationship between the bioconcentration factor (BCF) and the zinc concentration in water (C_w).

difference can be attributed to the lower concentration of Cu in solution but anyway the accumulation of Cu in the plant is faster. On the contrary, the desorption rate, k_2 , for Cu is lower than for Zn, 0.020 and 0.032 h^{-1} , respectively, i.e., Cu is more strongly retained by the plant. When exposing previously contaminated mosses

to metal-free water, about 84% of Zn is released to water, whereas for Cu that value decreases to 60%.

5. Conclusions

A first-order mass transfer model was fitted to the experimental data obtained during the uptake and release stages of Zn by aquatic mosses. This model satisfactorily describes both periods, permitting to calculate the kinetic constants and equilibrium concentrations.

For aqueous solutions of zinc in the concentration range 1.05–3.80 mg l⁻¹, results show that the aquatic moss *Fontinalis antipyretica* retains, at equilibrium, the metal ion by a factor 4531–2950 (Zn concentration in the moss, µg g⁻¹, dry wt./Zn concentration in water, mg l⁻¹).

The metal uptake rate 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.

Exposing contaminated mosses to zinc-free water, plants only retain, at the end of the decontamination period, about 13–20% of the metal previously accumulated, depending on the amount of zinc retained by plants at the end of the uptake period.

Comparing Zn and Cu accumulation and release by the same moss species, it was observed that, for similar concentrations in water, Zn uptake is slower and the amount retained in the plant is lower, by a factor of about seven. On the other hand, Zn is more easily removed from the plant when contacting with clean water. So the performance of *Fontinalis antipyretica* as bioindicator for waters contaminated by zinc is not so good as for copper. Nevertheless this moss species may be used for monitoring aquatic systems where zinc is present as pollutant.

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