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Kinetics of bioaccumulation of heavy metals in *Odontesthes bonariensis* is explained by a single and common mechanism

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ABSTRACT

Fish are often considered a very sensitive indicator of heavy metal contamination in aquatic ecosystems; however, the detailed mechanisms of bioaccumulation remain unclear. Here, we study the bioaccumulation processes of three relevant heavy metals (Cd^{2+}, Cu^{2+} and Cr^{6+}) in *Odontesthes bonariensis* using mathematical modelling. We developed a general compartmental kinetic model that describes the transport of heavy metals between the surrounding water and the gills and liver of fish. The general model was reduced to a simple one still capable of reproducing previous experimental data and suggesting a common mechanism for the three metals. The modelling results indicate that bioaccumulation of Cd^{2+}, Cu^{2+}, and Cr^{6+} is described by a combination of a concentration-independent and saturable uptake kinetics in both organs with a unidirectional path of elimination from gills to liver to waterborne. Finally, the good agreement between the parameter values predicted by the model and previously published data suggests that our modelling approach may shed light on the mechanisms of heavy metal bioaccumulation in other species.

1. Introduction

Heavy metals are non-biodegradable, tend to rapidly accumulate in the environment and are able to reach toxic levels in short periods of time. Furthermore, their removal from contaminated areas is rather difficult and sometimes impossible. Fish are considered very sensitive indicators of heavy metal contamination in aquatic ecosystems, as they are vertebrates whose life cycle is completely aquatic. Although the bioaccumulation of heavy metals in fish has been well studied (Pritchard, 1993), the underlying mechanisms have not been completely elucidated so far (Couture and Pyle, 2011).

Odontesthes bonariensis (*O. bonariensis* (Valenciennes, 1835), also known as "pejerrey") is a fish of the southern sector of the River Plate basin which has been anthropically introduced into other water bodies around the world and its biology has been extensively investigated (Grosman, 2002). In a previous report, Carriquiriborde et al. experimentally studied the bioaccumulation of three heavy metals, Cd^{2+}, Cu^{2+} and Cr^{6+} in liver and gill of juvenile specimens of *O. bonariensis* (Carriquiriborde and Ronco, 2008). In that work, the cumulative kinetics of the three metals was separately analyzed for each tissue by assuming first-order kinetics and passive diffusion mechanisms of bioaccumulation. Although passive diffusion can correctly describe the transport of hydrophobic compounds across lipid bilayers, it is known that heavy metals are usually transported by ion channels or transporters allocated in the plasma membranes (Deb and Fukushima, 1999). On the other hand, this kind of channels and transporters shows a saturable kinetics behaviour. Thus, instead of a process in which the rate of metal bioaccumulation linearly depends on concentration (as with simple diffusion), saturable kinetics, or the combination of both, should be considered. Moreover, the kinetics of bioaccumulation of a heavy metal in a given tissue/organ could be non-independent of the transport and concentration of the same metal in another tissue/organ. Therefore, a model combining and connecting the transport processes between more than one tissue/organ should be envisaged. In this work, to gain a deeper insight on the mechanisms responsible of bioaccumulation in different tissues, we developed a mathematical model to study the temporal bioaccumulation of Cd^{2+}, Cu^{2+} and Cr^{6+} in *O. bonariensis*. The proposed model defines the fish organs (liver and gill) and the waterborne

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as interconnected compartments linked by a combination of linear and saturable processes. The model was able to successfully describe previous experimental results, and the parameter values are in good agreement with phenomenological coefficients reported for other species.

2. Methods

2.1. Mathematical model

The process of bioaccumulation of heavy metals in *O. bonariensis* was modelled by a system of first order differential equations describing the transport of the heavy metals between three compartments: the water surrounding the fish (e), the liver (l), and the gills (g) (see conceptual diagram in Fig. 1A). The three compartments are assumed to be homogeneous and the variable describing the state of each compartment is the concentration of the metal under study (C_i). The transport of metal between two compartments i and j is described by a flow J_{ij}. Hence, the rate of concentration change in gills (g) and liver (l) is simply the algebraic sum of the contributing flows, either sources or sinks of metal, and can be represented as:

$$\frac{dc_g}{dt} = J_{eg} - J_{ge} + J_{lg} - J_{gl} \quad (1)$$

$$\frac{dc_l}{dt} = J_{gl} - J_{lg} + J_{el} - J_{le} \quad (2)$$

where each flow (J) is described by this generic functional form:

$$J_{ij} = \frac{f_{ij}c_i}{s_{ij} + c_i} + b_{ij} \quad (3)$$

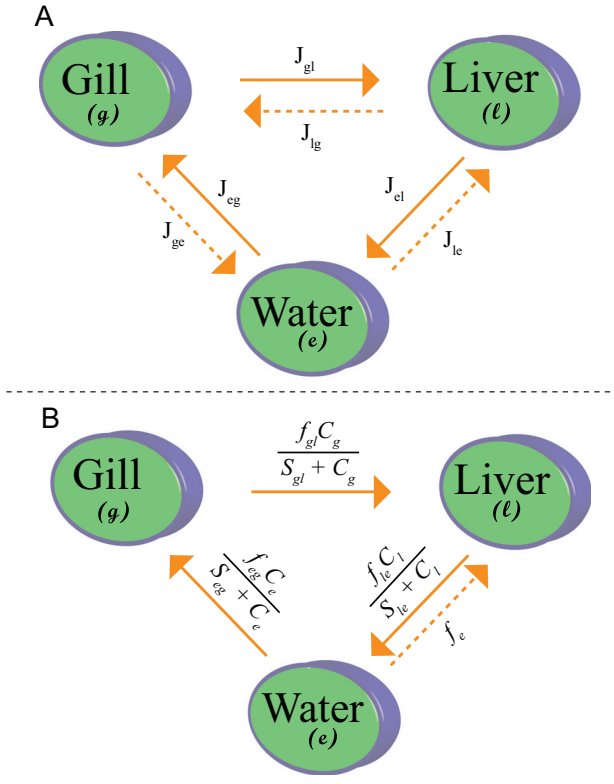

Fig. 1. Conceptual diagrams. (A) Scheme depicting the general mathematical model in which the metal is transported between organs and water by means of the fluxes J_{ij} (see main text). (B) Conceptual diagram of the mathematical model selected by fitting, including the final functions describing the flow between compartments.

Table 1
Detailed experimental concentration of heavy metals in waterborne (C_e) (Carriquiriborde and Ronco, 2008).

Metal	Concentration ($\mu g\,L^{-1}$)		
	Low	Medium	High
Cu^{2+}	10	50	100
Cr^{6+}	100	500	1000
Cd^{2+}	1	5	10

This flow is modelled as a Michaelis–Menten-like expression in which the parameter f_{ij} represents the maximum flow rate at infinite concentration for the internalization or elimination of a given metal; the parameter S_{ij} represents the inverse of the metal affinity of the compartment (organ or exterior); and the parameter b_{ij} indicates the concentration-independent flow (0 order flow rate) for the internalization or elimination of the metal.

The concentration of metals in the water surrounding the fish is modelled as a Heaviside function (i.e. a step function) multiplied by a constant determined from previous experimental data (Carriquiriborde and Ronco, 2008). The resulting model is given by a system of non-linear differential equations that were numerically solved and fitted to previous experimental data, as described in the next section.

2.2. Computational implementations

Model simulations were performed by integrating Eqs. (1)–(3) using a 4th order Runge–Kutta (RK) numerical scheme with an integration step of 0.1 day. This step size ensured the order of accuracy and the stability of the RK method. The procedure resulted in a prediction of the metal concentration time course in the two different tissues of the fish (liver and gill) at different waterborne metal concentrations. The model was globally fitted to previously reported experimental data on the bioaccumulation of Cu^{2+}, Cr^{6+} and Cd^{2+} in *O. bonariensis* (Carriquiriborde and Ronco, 2008). In the original experiments, juvenile *O. bonariensis* were exposed to three different concentrations (low, middle and high, see Table 1) of the heavy metal, and the concentration of metal in gills and liver was measured before (time 0) and after 2 and 16 days of exposure (Fig. 2, filled dots) (Carriquiriborde and Ronco, 2008).

Algebraically, the problem of fitting involves the exploration of the parameter space while minimizing a given fitness function, e.g. the residual sum of the squares of the differences between the measured concentration values in each organ and the values simulated by the model at each experimental concentration and time step. The standard errors of the parameters were calculated from the correlation matrix according to the procedure described by Sebber and Wild (Seber and Wild, 1989) using the Solver Statics Add-in (Billo, 2007).

3. Results

3.1. Fitting the model to experimental datasets

The proposed mathematical model was validated by fitting it to previous experimental data of bioaccumulation of 3 heavy metals (Cd^{2+}, Cu^{2+} and Cr^{6+}) in gills and liver of *O. bonariensis* (Carriquiriborde and Ronco, 2008). Initially, the model involved 18 parameters and was globally fitted to six experimental datasets for each metal; the best fitting values of the model parameters are shown in Table 2. Interestingly, the fitting procedure converged to a solution where 7 parameters were enough to accurately describe the experimental data (i.e. from the initial 18 parameters, only 7 resulted non zero). Furthermore, the structure of the model

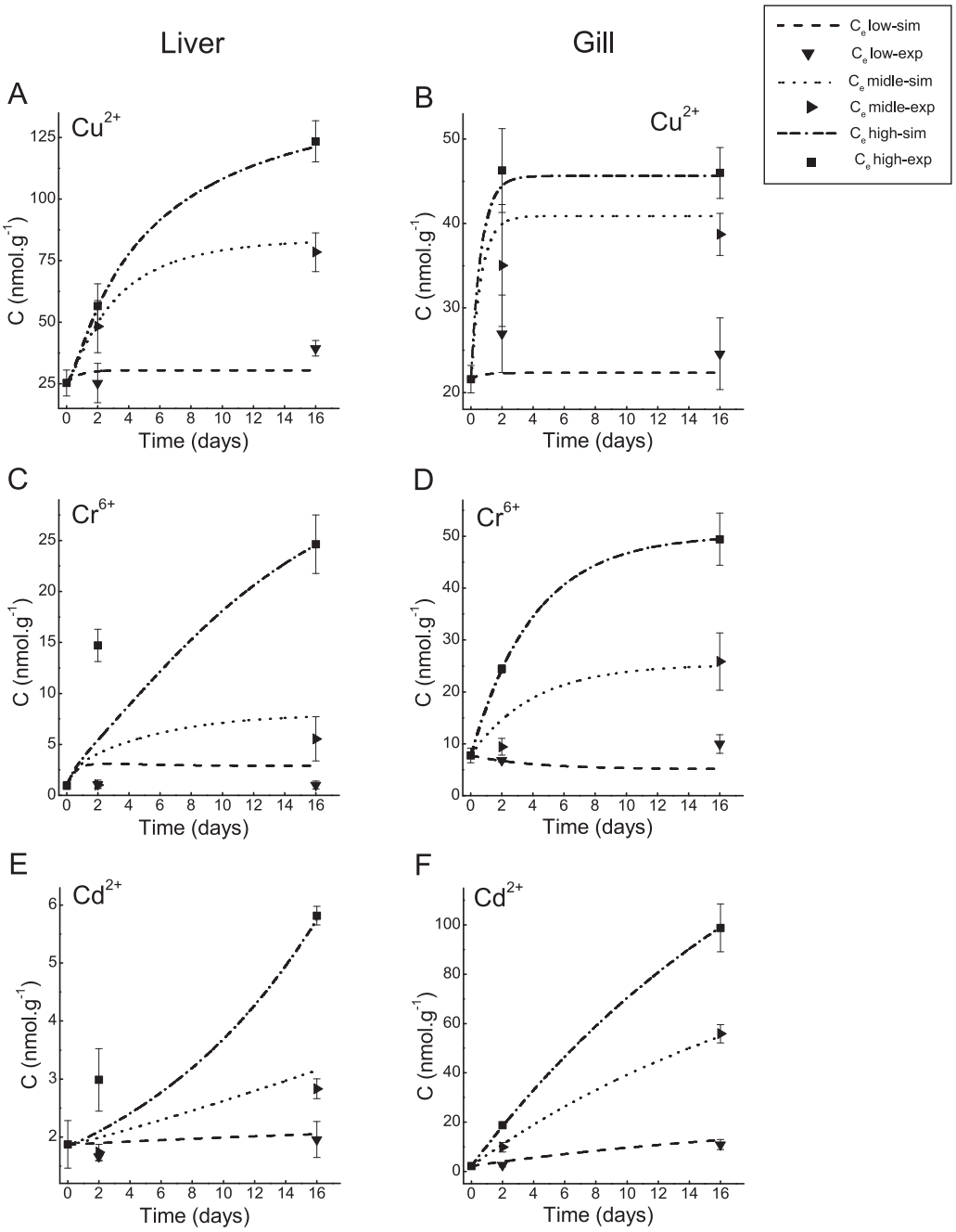

Fig. 2. Best fitting models for the bioaccumulation of Cu^{2+}, Cr^{6+} and Cd^{2+} in liver and gill of *O. bonariensis*. Experimental data (solid symbols) and simulated time courses (solid lines) for Cu^{2+} (A and B), Cr^{6+} (C and D) and Cd^{2+} (E and F) with the best fitting parameter values depicted in Table 2. Experimental waterborne concentration (C_e) is detailed in Table 1. Solid symbols and error bars represent the average and standard deviation of three independent experimental groups involving different fishes (Carriquiriborde and Ronco, 2008).

selected by the fitting was not affected by the metal under study and it is defined by (Fig. 1B):

$$\frac{dc_g}{dt} = \frac{f_{eg}c_e}{s_{eg}} - \frac{f_{gl}c_e}{s_{gl} + c_g} \quad (4)$$

$$\frac{dc_l}{dt} = f_{el} - \frac{f_{le}c_l}{s_{le} + c_l} + \frac{f_{gl}c_g}{s_{gl} + c_g} \quad (5)$$

Our results suggest that the uptake of metals by the fish occurs through the gills (parameters f_{eg}, S_{eg}) and the metals are then unidirectionally transported from the gills to the liver (parameters f_{gl} and S_{gl}), both processes being characterized by saturation. Bioaccumulation in the liver is described by a linear influx from the waterborne and from the gill (parameters f_{gl}, S_{gl} and f_{el}), as well as by a saturable efflux to the waterborne (parameters f_{le} and S_{le}).

Adding Eqs. (4) and (5), we have a description of the total bioaccumulation kinetics in the fish that takes the following form:

$$\frac{dc_g}{dt} + \frac{dc_l}{dt} = \frac{f_{eg}c_e}{s_{eg} + c_e} + \frac{f_{le}c_l}{s_{le} + c_l} \quad (6)$$

Eq. (6) shows that the metal would enter the fish through the gills and the liver under saturable and linear kinetics, respectively, while it would be eliminated only through the liver by means of a process involving saturation.

The fitting indicates that the metals tend to accumulate in the gill faster than in the liver (Fig. 2). On the other hand, the

Table 2
Best-fitting parameter values for the model. Parameters f_{eg} and f_{el} (μg^{-1} L day^{-1}) represent the maximum metal uptake through the gill and intestinal tract at infinite concentration. Analogously, f_{ge} and f_{le} (nmol g^{-1} day^{-1}) represent the maximum transfer from gill and liver to the exterior, while f_{gl} and f_{lg} (nmol g^{-1} day^{-1}) represent the maximum flow between liver and gill and vice versa. Parameters b_{eg} and b_{el} (μg^{-1} L day^{-1}) represent a constant flow uptake (independent of the metal concentration) through the gill and intestinal tract; b_{ge} and b_{le} (nmol g^{-1} day^{-1}) represent the maximum flow transfer from gill and liver to the exterior; and b_{gl} and b_{lg} (nmol g^{-1} day^{-1}) represent the transport between liver and gill. Parameter S_{eg} (μg L^{-1}) represents the inverse of the affinity of the gill for metal uptake from external water, while parameter S_{gl} (nmol g^{-1}) represents the inverse of the affinity of the liver for the metal coming from the gill; finally, S_{le} (nmol g^{-1}) represents the inverse of the affinity of the exterior metal (bulk water) coming from the liver.

Parameters	Metal		
	Cr^{6+}	Cd^{2+}	Cu^{2+}
f_{gl}	$(3.5 \pm 0.2)\ 10^3$	$(3.9 \pm 0.3)\ 10^3$	$(4.4 \pm 0.2)\ 10^6$
f_{lg}	–	–	–
f_{le}	20 ± 4	46 ± 1	$(3.0 \pm 0.2)\ 10^2$
f_{el}	5 ± 3	40.6 ± 0.3	$(2.2 \pm 0.4)\ 10^2$
f_{eg}	$(6.9 \pm 0.3)\ 10^2$	49 ± 1	77 ± 1
f_{ge}	–	–	–
S_{gl}	$(1.4 \pm 0.1)\ 10^4$	$(9.2 \pm 0.6)\ 10^4$	$(2.9 \pm 0.1)\ 10^6$
S_{lg}	–	–	–
S_{le}	7 ± 4	0.3 ± 0.1	5.5 ± 0.5
S_{el}	–	–	–
S_{eg}	$(5.5 \pm 0.2)\ 10^4$	47 ± 1	13 ± 2
S_{ge}	–	–	–
b_{gl}	–	–	–
b_{lg}	–	–	–
b_{le}	–	–	–
b_{el}	–	–	–
b_{eg}	–	–	–
b_{ge}	–	–	–

convergence to the steady state of Cd^{2+} seems to be slower than for the other two metals, independently of the organ (Fig. 2). The inverse of the affinity of the gills for the metals coming from the waterborne is given by the parameter S_{eg}. The best-fit values of this parameter indicate that the affinity of the gills for the metals coming from the waterborne is significantly lower for Cr^{6+} than for the other two metals (Table 2). Additionally, the best-fit values of S_{gl} (i.e. affinity of the liver for the metal coming from the gills) suggest that the affinity of the liver for Cr^{6+} coming from the gills is also lower than for the other two metals.

3.2. Simultaneous exploration of concentrations and exposure times

To evaluate the time courses of heavy metal bioaccumulation in O. *bonariensis* predicted by our model, additional simulations were performed with different external concentrations of each metal for longer exposure times (30 days). The simulations were carried out by fixing the parameters at their best-fit values (Table 2) and by exploring the metal exposure concentration in a sub-toxic range.

According to our simulations (Fig. 3A and B), Cu^{2+} rapidly reaches a plateau in the gills and the liver. The relationship between the metal exposure concentration (C_e) and the concentration accumulated after 30 days of exposure is nearly linear in the liver and clearly non-linear in the gills, showing a tendency to a plateau at high exposure concentrations. Moreover, the accumulated concentration of Cu^{2+} in the liver after 30 days of exposure to the highest sub-toxic concentration was three times higher than that obtained in the gills.

Although Cr^{6+} tends to a plateau after 30 days of exposure in the gill for all the tested exposure concentrations, the convergence to a plateau is slower in the liver with the higher exposure concentrations (Fig. 3C and D). In contrast with Cu^{2+}, the accumulated concentration of Cr^{6+} at 30 days increases linearly with the exposure concentration in the gill but grows exponentially in the liver. An additional observed difference with Cu^{2+} is that the accumulated concentration of Cr^{6+} in the liver after 30 days of exposure to the highest sub-toxic concentration is approximately the same than in the gill.

Finally, Cd^{2+} converges to a plateau at the slowest rate, especially in the liver (Fig. 3E and F). The maximum accumulated concentration of the metal in the gill at 30 days of exposure was almost 6 times higher than in the liver. As in the case of Cr^{6+}, the accumulated concentration of Cd^{2+} at 30 days increases linearly with the exposure concentration in the gill but grows exponentially in the liver.

3.3. Predicted steady-state bioaccumulation

The model selected by the fitting procedure shown in the previous section can be used to shed some light on the bioaccumulation of metals in each organ at the steady state. By solving Eqs. (4) and (5), and assuming that $dC_g/dt = dC_l/dt = 0$, the following general expression of the theoretical steady-state metal concentration in gill and liver can be obtained:

$$C_g^{ss} = \frac{s_{gl}}{(f_{gl}/f_{eg})(s_{eg}/c_e) - 1} \tag{7}$$

$$C_l^{ss} = \frac{s_{le}}{(f_{le})/(f_{el} + (f_{eg})/(1 + s_{eg}/c_e)) - 1} \tag{8}$$

The above equations were constrained by fixing the exposure concentration of each metal at its medium value and using the best-fitting values of the model parameters previously obtained (see Table 2). We observed that the relative bioaccumulation (C^{SS}/C^{exp}) of Cd^{2+} is markedly higher than that of Cu^{2+} and Cr^{6+} in the gill at the steady state (Fig. 4A), while the bioaccumulation of Cd^{2+} in the liver is comparable to that of Cu^{2+} but significantly higher than that of Cr^{6+} (Fig. 4A). Interestingly, although Cd^{2+} and Cr^{6+} bio-accumulate 10 and 30 times more in the gill than in the liver, for Cu^{2+} this ratio is inverted and there is 2 times more Cu^{2+} bioaccumulation in the liver than in the gill (Fig. 4A).

Finally, we calculated the time needed to achieve 75% of bioaccumulation of each metal at the steady state ($Time^{0.75\ SS}$) in both organs (Fig. 4B). Our results show that the global kinetics of the bioaccumulation of Cr^{6+} and Cu^{2+} until steady-state conditions are reached, are similar in both organs, while the bioaccumulation of Cd^{2+} was the slowest. In good agreement with our simulations (Fig. 3), the kinetics of bioaccumulation of the three metals in the liver were slower than in the gill.

4. Discussion

Bioaccumulation in aquatic organisms is commonly described by a simple mass transfer kinetic model. The fish is assumed to be a single homogenous compartment delimited by a permeable membrane in direct contact with the surrounding medium. This medium is considered as an infinite supply of the chemical under study at a given concentration (Barron et al., 1990; Newman, 1995; Rand, 1995). Although useful in a first approximation, by using these models it is not possible to elucidate the mechanisms responsible for the redistribution of the accumulated substance in the animal organs or tissues. On the other hand, although this kind of models have been used to describe bioaccumulation of metals (Luoma and Rainbow, 2005), they were clearly more successful describing the response to hydrophobic organic substances (Barron et al., 1990; Branson et al., 1975; Erickson and McKim, 1990; Feijtel et al., 1997; Holden, 1962; Kenaga, 1980; Krzeminski et al., 1977; Meylan et al., 1999; Neely et al., 1974; Veith et al., 1979; Yu et al., 2002). These models work well for neutral organic compounds,

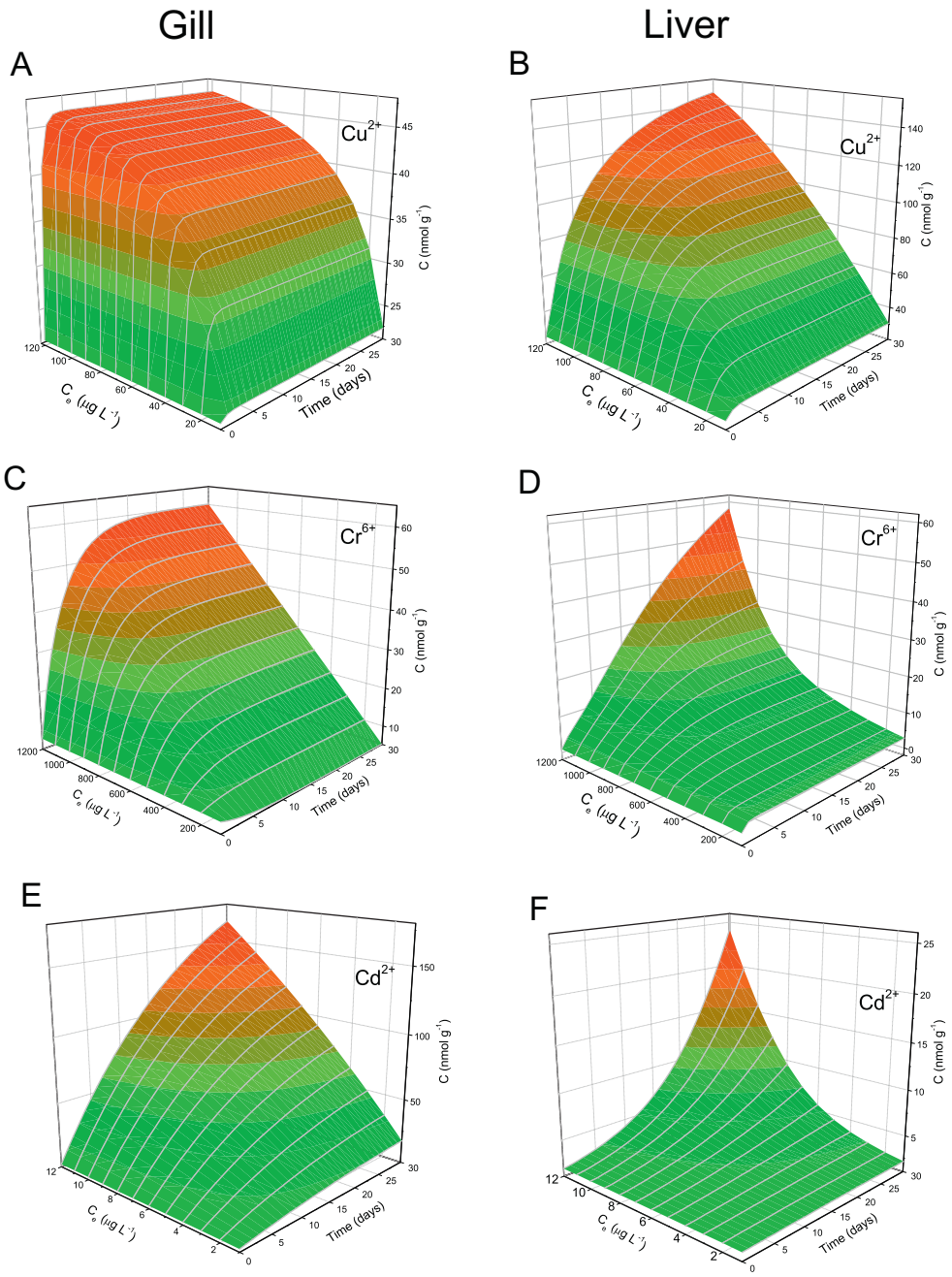

Fig. 3. Effect of concentration and exposure time on the bioaccumulation of heavy metals. Predicted bioaccumulation of Cu^{2+} (A and B), Cr^{6+} (C and D) and Cd^{2+} (E and F) in gills and liver (C) at different water concentrations (C_e) and exposure times.

since the uptake of lipophilic substances into biota occurs via simple passive diffusion, ruled by Fick's Law, across the lipid bilayer of plasma membranes (McKim, 1994). Nevertheless, rather than a simple diffusion process, the transport of the vast majority of metals is a physiological process which takes place via a number of specific routes, most of which are characterized by saturable transport kinetics (Kiss and Osipenko, 1994; McDonald and Wood, 1993; McKim, 1994; Newman, 1995; Simkiss and Taylor, 1989; Wood, 2001).

In this article, we put forward a mathematical model that was specifically developed to simultaneously study the bioaccumulation of heavy metals in two different organs by combining saturable and linear processes connecting both organs of the fish and the waterborne. In this way, we aimed to mimic the actual response of individual tissues and organs that can transport heavy metals both by active and passive processes and to reproduce the internal dynamics of the heavy metals in the animal by introducing an explicit connection (in both directions) between the organs and the surrounding water.

Our results indicate that bioaccumulation of Cu^{2+}, Cr^{6+} and Cd^{2+} in *O. bonariensis* can be described by a simple and common mechanism. Could this mechanism be more general? A first step to answer this question is to compare the model parameters values (Table 1) with phenomenological coefficients previously reported for other species. Given the scarce data regarding bioaccumulation of Cr^{+6} and Cd^{+2}, we centred our analysis on Cu^{+2}.

According to our fitting procedure, the parameter f_{eg}, related to the process of Cu^{+2} uptake by the gills, is equal to (nmol L g^{-1} d^{-1} µg^{-1}) 77.7 (Table 2), while the phenomenological cupper uptake via gills measured in *Oreochromis mossambicus*

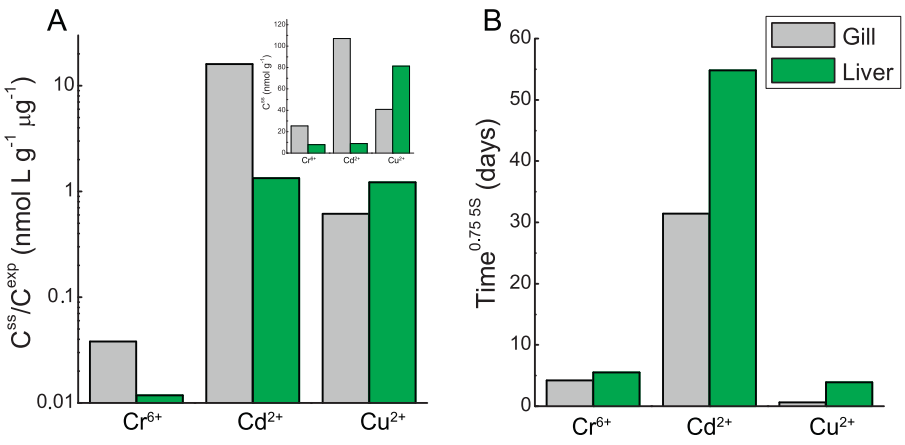

Fig. 4. Predicted steady-state bioaccumulation for Cu^{2+}, Cr^{6+} and Cd^{2+}. (A) The steady-state bioaccumulation of heavy metals in the liver and gill normalized by the exposure concentrations (C^{SS}/C^{exp}). The inset shows the absolute values of the metal concentrations at the steady state (C^{SS}). (B) The time required to achieve 75% bioaccumulation in both organs at the steady state ($Time^{0.75\ SS}$).

(commonly known as tilapia) and *Mytilus edulis* were estimated as (nmol L g^{-1} d^{-1} μg^{-1}) 2.79 and 67.9 respectively (Tsai et al., 2013; Sanchez-Marin et al., 2012). The parameter f_{el}, describing the rate of hepatic accumulation of Cu^{2+} is equal to (nmol L g^{-1} d^{-1} μg^{-1}) 222 (Table 2), whereas the experimental hepatic accumulation rate of *O. mossambicus* previously reported is equal to (nmol L g^{-1} d^{-1} μg^{-1}) 112.17 (Tsai et al., 2013) under exposure concentrations similar to those employed in our work. The model parameter S_{eg}, representing the inverse of the affinity of the gills for Cu^{+2}, is here calculated as ($\mu g\,L^{-1}$) 13.12. A study carried out in *Danio rerio* (Zebrafish) showed that the inverse of the affinity of the gills for Cu^{2+} can be estimated as ~3 $\mu g\,L^{-1}$ (Grosell, personal communication). Overall, the parameter values reported in this work describing the uptake and elimination of Cu^{+2} from gills and liver are in good agreement with the values of phenomenological coefficients previously found in the literature for other species, suggesting that the mechanism proposed here could be extrapolated to other organisms.

Our modelling results also show that heavy metals uptake is characterized by a saturation mechanism, suggesting a major role played by ion channels or transporters in metal bioaccumulation for both organs. Interestingly, there is strong evidence in the literature that a proportion of Cu^{2+} enters through the gills via an apical Na^+ channel in freshwater rainbow trout (Grosell and Wood, 2002; Laurén and McDonald, 1987a,b) and in *O. mossambicus* (Li et al., 1998). In addition, Cu^{2+} can bind covalently to SH-groups of Na^+/K^+-ATPase, modifying the conformational form of the protein and affecting the branchial transport of Na^+ (Grosell and Wood, 2002; Laurén and McDonald, 1987a,b; Li et al., 1998). In the case of Cd^{2+} uptake, it was previously reported that this metal enters via Ca^{2+} channels located on apical membranes in gills of *Salmo gairdneri* (Verbost et al., 1987, 1988, 1989). In agreement with this, it has been observed that waterborne Cd^{2+} has an inhibitory effect on the Ca^{2+} uptake of rainbow trout and brown trout (Reader and Morris, 1988; Reid and McDonald, 1988). Furthermore, inhibition of Cd^{2+} uptake by Ca^{2+} channel blockers in gills of freshwater clams and molluscs has also been reported (Holwerda et al., 1989; Roesijadi and Unger, 1993). Finally, and also in good agreement with our findings, it was reported that Cd^{2+} uptake along the gastrointestinal tract in rainbow trout (*Oncorhynchus mykiss*) would involve Ca^{2+} channels (Klinck and Wood, 2011).

To sum up, our mathematical modelling approach allowed us to propose a new common mechanism of heavy metal bioaccumulation in *O. bonariensis* that takes into account active processes in cellular transport combined with passive diffusion. Also, the good agreement between the parameter values found with our model and those experimentally obtained for other species suggest that the proposed model could be extended and successfully applied to describe in detail the mechanisms of bioaccumulation of other species.

5. Concluding remarks

This work studied the bioaccumulation processes of three heavy metals in fish by mathematical modelling. Using previously obtained experimental data involving the exposure of *O. bonariensis* to three heavy metals, Cd^{2+}, Cu^{2+} and Cr^{6+} (Carriquiriborde and Ronco, 2008), we proposed a kinetic model combining realistic, albeit simple, assumptions.

Our results showed that the bioaccumulation of these three metals in *O. bonariensis* can be described by a single and common mechanism in which: (i) uptake by the gills and elimination through the liver show saturation; (ii) a saturable transport from the gills to the liver is the only path of transport of metal from the gills; (iii) there is no metal transport from liver to gill; and (iv) the hepatic uptake (via the intestine, from the waterborne) follows a concentration-independent kinetics. The good agreement between our predicted parameter values and previously published data strongly suggests that our model could be applicable beyond the fish species studied here. Additional experimental work should be done to further confirm these modelling predictions.

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