Predicting aqueous copper and zinc accumulation in the Upside-down jellyfish, *Cassiopea maremetens* through the use of biokinetic models

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Abstract

Jellyfish have a demonstrated capability to accumulate metals within their tissues, but to date there have been no quantitative assessments of accumulation and retention rates and patterns. Bioconcentration patterns of copper and zinc in the Upside-down jellyfish *Cassiopea maremetens* were modelled over a 28 day study (14 days exposure followed by 14 days clearance). *C. maremetens* accumulated copper over 14 days with the maximum calculated copper concentrations at 33.78 $\mu g.g^{-1}$ dry weight and bioconcentrated to 99 times water concentrations. Zinc was also accumulated during the exposure period and retained for longer. The maximum theoretical zinc concentration was 125.1 $\mu g.g^{-1}$ dry weight with a kinetic bioconcentration factor of 104. The patterns of uptake and retention were different between the elements. The use of kinetic models provided adequate predictions of aqueous metal uptake and retention in *C. maremetens*. This species has the capacity to very rapidly absorb measurable metals from short-term water-metal exposure.

Keywords: Bioaccumulation, biokinetic model, *Cassiopea maremetens*, copper, jellyfish, zinc

1. Introduction

Metal pollution in marine and estuarine environments can affect biota directly through metal toxicity or indirectly through bioaccumulation and biomagnification within and up food webs. Because of the complexity of metal uptake, detoxification and excretion processes within organisms, it is difficult to directly predict all mechanisms and routes of bioaccumulation (Walker 1990). It is necessary though, to recognise the key chemical and biological processes affecting contaminant fluxes within organisms as well as the surrounding external medium (Walker 1990). The effects of metal bioaccumulation are an important aspect of understanding the cycling of pollutants within aquatic systems and their impacts on system health and biodiversity.

Field measures of accumulation make the implicit assumption of a system in equilibrium and compare body concentrations with ambient water concentrations to determine the bioconcentration factor (e.g. Benson & Summons 1981; Hanna & Muir 1990; Esslemont 2000). However, the actual mechanisms of uptake, accumulation and excretion are complex and dynamic making them difficult to predict from field measurements. As a result, research has focussed on predicting patterns of accumulation according to trophic pathways. This has led to the development of theoretical kinetic models which can be used to predict uptake and retention of metals in both terrestrial and aquatic species (eg Wang et al. 1996; Kahle & Zauke 2002; Luoma & Rainbow 2005).

Historically, there has been little work undertaken on the role of jellyfishes in biogeochemical cycling of elements in the marine environment despite them being conspicuous components of that system (Richardson et al. 2009). Field studies on the Great Barrier Reef have demonstrated that jellyfishes have the capacity to accumulate metals well above ambient concentrations, and show marked species, metal, temporal and spatial variations in accumulation (Templeman & Kingsford 2012).

Metal accumulation has been variable among elements in *Cassiopea* sp. with some elements actively regulated (e.g. lithium) while others (e.g. copper, manganese, zinc) have been accumulated up to two hundred times that of the ambient seawater concentrations (Templeman & Kingsford 2010; 2012). The Upside-down jellyfish (*Cassiopea* sp.) is a scyphozoan jellyfish possessing endosymbiotic dinoflagellate zooxanthellae and has the atypical behaviour of resting upside down in shallow tropical and sub-tropical estuaries and coastal marine waters. Recent work has shown that *Cassiopea* sp. are capable of remobilising pore water nutrients from the sediment as a feeding strategy for its endosymbiont *Symbiodinium* sp. (Jantzen et al. 2010).

While this research demonstrates that jellyfish including *Cassiopea* sp. accumulate metals above ambient seawater, there have been no studies quantifying uptake rates and retention. The objective of this study was to quantitatively determine bioconcentration of aqueous copper and zinc in the Upside-down Jellyfish (*Cassiopea maremetens*) and compare the kinetic bioconcentration factors (BCF_{kin}) among species. The study aimed to: 1) establish if biokinetic modelling could predict

bioconcentration patterns of aqueous copper and zinc in *C. maremetens*; and 2) quantify the bioconcentration capacity of *C. maremetens* for copper or zinc from the aqueous phase.

2. Materials and Methods

The control / dilution seawater for both culturing purposes and the experiment was collected adjacent to the Australian Institute of Marine Science wharf (AIMS) located approximately 50 km south of Townsville, Queensland. Water was filtered through a sand filter followed by a 20µm woven fibre cartridge filter before use and used as both control and dilution water. A 20µm mesh was selected as culturing processes indicated that the use of fine filtration (<20µm pore) tended to lead to growth inhibition in the jellyfish although the exact cause/s of this were not established.

All experimental equipment was cleaned with phosphate free detergent, rinsed in tap water and then soaked in 10 % AR grade nitric acid for a minimum of 12 hours. Acid soaked equipment was removed from the acid, rinsed three times with Milli-Q water and air dried in a Class 100 laminar flow unit. Two litre plastic containers calibrated to one litre flow through volume were used as the test chambers with all testing equipment equilibrated for 48 hours by pumping clean seawater through them prior to the commencement of the study. The pump setup comprised an Altivar 31H variable speed drive pump with Watson-Marlow multi-channel micro-cassette peristaltic pump (1.02 mm diameter). The pump was pre-calibrated to a flow rate of 3.5 $mL.min^{-1}$ which equated to approximately 5 litres / container / 24 hours.

Lighting was provided by an Aqualina dual fluorescent reflector containing 2 x Dual CA PL-L 96W 10000K fluorescent tubes on a 12:12hr cycle. The photosynthetic active radiation light intensity was 115 $\mu mol.m^2.s^{-1}$ and was measured using a Li-COR meter prior to test commencement. The light intensity exceeded the minimum of 50 $\mu mol.m^2.s^{-1}$ required for photosynthetic compensation (Welsh et al. 2009).

Copper and zinc were the selected metals as field studies had shown field bioaccumulation factors up to 200 times ambient water concentrations in both urban marine systems and on the Great Barrier Reef (Templeman & Kingsford 2010; 2012). Three pre-labelled 100 litre black polythene holding drums containing 80 litres of 20 µm filtered seawater were used as the holding containers for the control (no added metals), a nominal 15 $\mu g.L^{-1}$ copper treatment and a nominal 60 $\mu g.L^{-1}$ zinc treatment. These concentrations were based on previous work that established 96 hour acute toxicity (LC_{50}) for *C. maremetens* ephyra at 24.3 $\mu g.L^{-1}$ Cu and 1.84 $mg.L^{-1}$ Zn (Templeman & Kingsford *in prep*). These concentrations are also at the higher end of what can be expected in coastal waters and are within ranges measured in some industrialised estuaries (e.g. Matthiessen et al 1999, Luoma & Rainbow 2008).

Appropriately diluted stock solution (as either $CuCl_2$ or $ZnSO_4$) was added to the treatment drums to provide the nominal treatment solutions. Drums were refilled every 96 hours over the duration of the study and water quality measured every second day using a TPS WD-90 multi-parameter meter.

Approximately 130 medusae from in-house cultures approximately 8 weeks of age and with a mean size of 17 ± 2 mm were selected from a larger pool of animals and placed in clean 10 litre plastic aquaria containing 20 µm filtered seawater for 96 hours prior to the start of the experiment. Animals were fed with newly hatched *Artemia* sp. daily to ensure they were healthy and actively feeding.

The test was set up with 4 replicate chambers for each treatment and the Control, and 10 jellyfish randomly allocated to each replicate chamber. The maximum animal loading was 5 grams per litre at test commencement with a flow-through rate of 5 litres per 24 hours. One animal was randomly removed from each replicate chamber at each sampling point. Test chambers were covered using clean, clear semi-rigid plastic sheets to minimise evaporation and potential dust contamination.

Animals in each test container were fed every second day with approximately one millilitre of concentrated freshly hatched *Artemia* sp. Any uneaten food or debris that was not flushed from the containers via the flow-through apparatus was removed using a clean acid-washed pipette after 24 hours.

All tubing, test containers and holding tanks were replaced with clean equipment at the end of the uptake phase of the study (Day 14) to ensure no carryover of metals in the clearance phase of the study. No mortality occurred in any container over the duration of the study.

2.1 Analytical measurements

Duplicate water samples were taken every second day from each of the treatment and control tanks for analytical purposes. Water samples were acidified with 300 µL 20% Univar grade nitric acid and stored at 4 ^{0}C until analysed. Jellyfish were sampled on Day 0, 1, 2, 4, 8, 14 (Uptake Phase), 15, 16, 18, 21, 28 (Clearance Phase) of the study. At each sampling point, one animal from each replicate container was removed and placed in cleaned and acid washed 30 mL vials containing control seawater to remove any weakly adsorbed surface copper or zinc. The bell diameter of each animal at the extension of the pulse phase was measured to the nearest millimetre using a plastic ruler and accurately weighed to the nearest 0.01 mg using a Sartorius Genius ME analytical balance. Individuals were placed in pre-cleaned acid washed 10 mL vials and stored at -18^{0}C until digested. All samples were digested within four weeks of collection. Tissues were digested using the nitric acid / hydrogen peroxide method from Templeman & Kingsford (2010). Due to the small size of the animals, the final sample volume was 10 mL.

Water and digested tissue samples were analysed using a Varian 820-MS Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Al, As, Ba, Cu, Cd, Cr, Li, Mn, Pb, Sr and Zn and a Varian Liberty

Series II Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) for calcium, magnesium and iron. Control water was analysed for the full suite of elements while treatment concentrations were analysed for copper and zinc only. To determine the baseline concentration of elements in the jellyfish, a subset of digested tissue samples from the control treatment was analysed for the full suite of elements as above. The remaining control animals and the copper and zinc exposed animals were analysed for copper and zinc only.

Due to issues with signal suppression, it was necessary to dilute the water samples 1:10 (seawater:diluent) prior to analysis. Subsets of water samples were spiked with known concentrations of all elements for quality control purposes and to determine recoveries (78-126%).

Due to the lack of an appropriate standard reference material, subsets of digested jellyfish samples were spiked with known concentrations of all elements for quality control purposes and to determine recoveries in digested tissues (85-127%). Indium, gallium and yttrium were used as internal standards to correct for potential instrument drift and matrix effects. Digested tissue samples were diluted 1:2 (tissue:diluent) to minimise issues of signal suppression. Analytical data was checked to ensure signal strength exceeded three standard deviations for all analyses. Digestion blanks were included with all tissue digestions to ensure sample integrity. Digestion blanks had low levels of elements and tissue data was corrected for blank results before statistical analysis.

2.2 Data analysis

Variations in bell diameter were tested using a two-way ANOVA for independent data after testing for homogeneity using Bartlett's test. Comparisons between background tissue concentrations were analysed using a student's t-test. Analyses and data plotting were carried out using GraphPad Prism Version 6.02.

The time course experiment was assessed using the kinetic rate constant model which is also referred to as single compartment or two compartment models in some studies (Landrum et al. 1992; Kahle & Zauke 2002; Clason et al. 2004; Jung & Zauke 2008). In this model, the water–metal is considered the source compartment (compartment 1) and the animal or tissue as compartment two (Landrum et al. 1992; Clason & Zauke, 2000; Kahle & Zauke 2002).

In the model, C_A represents the mean metal concentration in the animal tissue ($\mu g.g^{-1}$); C_W the mean measured metal exposure during the uptake phase ($\mu g.L^{-1}$); k_M the growth rate (d^{-1}); k_V the adsorption / volatilisation constant (d^{-1}); k_U the rate constant for uptake ($L.g.d^{-1}$) and k_E the rate constant for clearance (d^{-1}) (Figure 1). As both copper and zinc are essential metals, a background concentration was present in the animals. This is defined as C_O and is the mean concentration in animal tissue at t = 0 ($\mu g.g^{-1}$). In this study, the growth rate (k_M) was considered insignificant as there was no significant change in size over the study period (two-way ANOVA $p>0.05$). The flow-through design with

constant water turnover compensated for potential surface adsorption / volatilisation of the metals (k_V). All tissue metal concentrations were measured as $\mu g.g^{-1}$ wet weight rather than dry weight due to the confounding presence of residual bound water of hydration remaining in jellyfish tissues after drying (Larson 1986; Arai 1997).

INSERT FIGURE 1 HERE

Figure 1: Diagrammatic representation of biokinetic models with water as the first compartment and animal as the second compartment.

The model parameters k_U and k_E were estimated simultaneously for the uptake and clearance phases ([Eq 1] and [Eq 2]), using nonlinear iterative least square methods in Excel 2007 with Solver add-in. In the rate constant model (or two compartment model) the uptake phase - $0 < t \leq t^*$, with t^* = end of uptake phase (days) was described by the equation:

$$C_A = C_O + C_W\, k_U/\, k_E\, (1-e^{-kE \cdot t}) \qquad \text{[Eq 1]}$$

and the clearance phase ($t > t^*$) was described by:

$$C_A = C_O + C_W\, k_U/\, k_E\, (e^{-k_E \cdot (t - t^*) - k_E \cdot t}) \qquad \text{[Eq 2]}$$

The kinetic BCF (BCF_{kin}) was then calculated using the equation:

$$BCF_{kin} = k_U/\, k_E \qquad \text{[Eq 3]}$$

while the biological half-life was calculated by:

$$t_{1/2} = \ln (2)/\, k_E \qquad \text{[Eq 4]}$$

and the theoretical maximum tissue concentration at equilibrium as:

$$C_{A(max)} = BCF_{kin} * C_W \qquad \text{[Eq 5]}$$

The kinetic bioconcentration factor ($BCF_{kin)}$ used in the rate constant model has the assumption of equilibrium between the tissue and water metal concentrations. The initial goodness of fit of the curves was calculated using: $R^2 = 1- (SS_{res}/SS_{tot})$ with Excel 2007 Solver add-in. A linear regression of observed versus predicted model was then performed in Statistica Version 10.0 to compare the model with the measured data to determine agreement between observed and modelled data (Clason et al. 2004).

3. Results

3.1 Baseline measurements

Tissue concentrations of Cu and Zn were measured in control and test animals over the 28 day study. The mean background tissue concentrations over the test duration were 0.104 $\mu g.g^{-1}$ wet weight (2.08 $\mu g.g^{-1}$ dry weight) for copper and 1.327 $\mu g.g^{-1}$ wet weight (26.54 $\mu g.g^{-1}$ dry weight) for zinc. These data were used as the copper and zinc C_O constant for the kinetic models. The measured mean water concentration of copper in the copper uptake phase was 17.1 ± 2.9 $\mu g.L^{-1}$ Cu, and mean water zinc concentration was 59.9 ± 1.9 $\mu g.L^{-1}$ Zn. The mean bell diameter of medusae at the start of the study was 16.6 ± 0.5 mm and 17.6 ± 0.4 mm at the end of the study, with no significant change in size among over the duration of the experiment (two-way ANOVA $p > 0.05$).

All tissue metal concentrations were measured as $\mu g.g^{-1}$ wet weight rather than dry weight due to the confounding presence of residual bound water of hydration remaining in jellyfish tissues after drying (Larson 1986; Arai 1997). However for comparative purposes with other studies, estimated 95% water content for *Cassiopea* sp. was used for calculations of dry weight concentrations (Templeman & Kingsford 2010).

The salinity in all solutions ranged from 32.7 – 33.1 ppt, pH 8.17 – 8.20, oxygen 90-95% saturation and temperature 24-27^{0}C.

3.2 Copper uptake and retention

Copper was very rapidly accumulated in the jellyfish tissues (Figure 2). Initial accumulation of copper was rapid with a 680% increase from background concentration after 24 hours of exposure. Accumulation continued to rapidly rise before reaching an asymptote after 7 days, indicating some level of copper regulation by this time (Figure 2). The rate constant model provided a good fit of the observed to expected data (R^2=0.931) with a calculated kinetic maximum tissue concentration $C_{A(max)}$ of 1.689 $\mu g.g^{-1}$ wet weight (33.78 $\mu g.g^{-1}$ dry weight) after 14 days and a kinetic bioconcentration factor (BCF_{kin}) of 99 (Figure 2). Upon transfer to clean seawater (clearance phase), copper was rapidly excreted with tissue copper concentrations close to background within 14 days (Figure 2) with a biological half-life ($t_{1/2}$) of 1.68 days (Table 1).

INSERT FIGURE 2 HERE

Figure 2: Copper bioconcentration and regulation in *C. maremetens*. Observed = Mean measured data ± SEM. R^2 indicates goodness of the model fit to the measured data.

3.3 Zinc uptake and retention

Zinc accumulation in the *C. maremetens* was slower and more linear than copper (Figure 3). Zinc accumulation did not reach saturation within the 14 days and was retained for longer. The mean maximum measured bioconcentration after 14 days exposure was 5.685 $\mu g.g^{-1}$ wet weight (113.70 $\mu g.g^{-1}$ dry weight) with a calculated $C_{A(max)}$ of 6.250 $\mu g.g^{-1}$ wet weight (125.00 $\mu g.g^{-1}$ dry weight) and model fit of R^2=0.720 (Figure 3; Table 1). Zinc clearance was also slower with a mean tissue concentration after 14 days of 3.166 $\mu g.g^{-1}$ wet weight (63.32 $\mu g.g^{-1}$ dry weight) which was approximately double the background zinc concentration (Figure 3). The kinetic BCF (BCF_{kin}) for zinc was 104.4 and biological half-life ($t_{1/2}$) was 9.11 days (Table 1).

INSERT FIGURE 3 HERE

Figure 3: Experimental zinc bioconcentration and excretion in *C. maremetens*. Observed = Mean measured data ± SEM; R^2 indicates goodness of the model fit to the measured data.

A comparison of the observed data to the model predictions can be assessed using a linear regression of goodness of fit. A slope value of '1' and a constant value of '0' indicates complete agreement between the model and observed data sets, and if the 95% confidence intervals bracket these values and the constant is not significantly different from 0 then the model can also be regarded as a good fit (Clason et al. 2004). In this study the slope for copper was 0.982 with the constant not significantly different from 0 indicating a very good fit of the data to model predictions, while for zinc the slope was 0.840 with a 95% confidence interval of 0.684-0.995 indicating a slightly less robust the goodness of fit (Table 1).

INSERT TABLE 1 HERE

4. Discussion

<u>Uptake</u>

C. maremetens was capable of rapidly accumulating aqueous copper and zinc. The uptake patterns were different between the metals and this was reflected in the respective model fits (Figures 2 & 3). In both cases, there was very good agreement between the observed and predicted values, although the model was a slightly better descriptor for copper than for zinc (0.931 cv 0.720).

Copper accumulation was very rapid increasing by almost 600% above background in the first twenty-four hours of exposure while zinc was more slowly absorbed (120% above background in 24

hours). Copper accumulation plateaued around day 7 indicating possible saturation and the potential for subsequent regulation (Figure 2). The mean copper accumulation after 7 and 14 days was 33.96 and 34.44 $\mu g.g^{-1}$ dry weight respectively. This result was comparable to accumulation patterns seen in the symbiotic anemone *Aiptasia pallida*, although there was a level of dose dependency in accumulation rates for *A. pallida* (Brock & Bielmyer 2013). Copper bioconcentration in this study was also greater than seen in another anthozoan, *Anemonia viridis*, with accumulation again very dependent on the exposure concentrations (Harland & Nganro 1990). This supports the theory there may be a threshold concentration required for accumulation in some species, but also reinforces the dependency of steady state tissue concentration on exposure conditions. For *C. maremetens* this also suggests they are capable of bioconcentrating at lower aqueous concentrations than many other species and are able to rapidly accumulate metals from the aqueous phase.

The respective uptake rates for copper and zinc in *C. maremetens* were much greater than generally reported for other species. As summarised in Kalman et al. (2014), reported uptake rates for water-borne zinc range from 0.026 for the capitellid annelid *Arenicola marina* (Casado-Martinez et al. 2009) to 1.131 for the mussel *Mytilis edulis* (Wang et al. 1996) compared to 7.942 in this study. Jellyfish have previously been reported to have very high water turnover or clearance rates up to 3.8 $l.h^{-1}$ (Riisgard & Madsen 2011). This can have a major influence on the uptake rate of water-borne metals.

Retention / Depuration

While the accumulation of copper was rapid, depuration was also rapid with a calculated half-life of 1.68 days (Table 1). The depuration rates seen in *C. maremetens* was similar to rates in *A. pallida* (Brock & Bielmyer 2013). The depuration rates here are much more rapid than has been seen in many other invertebrate studies (eg Kahle & Zauke 2002; Jung & Zauke 2008; Kalman et al. 2014) and suggests that some cnidarians including *C. maremetens* can be very efficient at detoxifying post-exposure. This also suggests that the most likely pattern of accumulation of copper in *C. maremetens* is excretion from a detoxified store (Rainbow 2002). This combined with the rapid water turnover can explain the rapid depuration capability in this species.

Retention of zinc was longer with a calculated half-life of 9.1 days (Table 1) but low compared with other studies (e.g. Xu & Pascoe 1993; Kahle & Zauke 2002). In a study of dietary uptake in jellyfish, the retention time ($t_{1/2}$) of zinc was 28-65 days for *Cassiopea andromeda* and 20-29 days for was *Aurelia aurita* suggesting the metal source is likely to be key to the retention time (Fowler et al. 2004).

Unlike copper, zinc uptake did not reach saturation during the study and may have been a carrier-mediated process as is seen in some other aquatic species (Wang & Fisher 1999). It is possible that there may also be other underlying processes of localised binding, storage and release of zinc that increases the complexity of the overall patterns of accumulation and retention.

BCF

The calculated copper and zinc BCF_{kin} for *C. maremetens* from this study were lower than reported for many other species (e.g. Kahle & Zauke 2002; Clason et al. 2004; Li et al. 2010). However, field studies have reported field BCF for copper up to 151 times seawater concentrations for *Cassiopea* sp. on the Great Barrier Reef (GBR) and up to 84 times ambient seawater concentrations from multiple other urban marine locations (Templeman & Kingsford 2010; 2012).

For zinc the field BCF on the GBR was 221 for *Cassiopea* sp. and 193 times ambient seawater concentrations for *Mastigias* sp. while in urban marine locations it ranged from 190-756 times ambient zinc (Templeman & Kingsford 2010; 2012). Aqueous copper and zinc concentrations were low in both studies suggesting *Cassiopea* sp. has the capacity to accumulate metals at low ambient seawater concentrations and/or that dietary uptake may be an equally important uptake route. Fowler et al. (2004) also demonstrated that food is a key uptake route for both bioaccumulation and retention of zinc in jellyfish (Fowler et al. 2004). Similarly studies with other species and metals (e.g. Luoma & Rainbow 2005; Jung & Zauke 2008) have demonstrated that the dietary uptake is a key component in quantifying metal bioaccumulation. Integrating the effects of metal accumulation from diet and sediment exposure are considered important next steps in fully evaluating model parameters and assessing the biomonitoring potential of this species.

Under short-term exposure conditions, *C. maremetens* may be considered a net accumulator of both copper and zinc rather than a regulator due to the high relative uptake rates, however, their utility for longer term biomonitoring is likely to be limited. The rapid uptake and excretion of metals suggests *C. maremetens* may be an efficient copper and zinc scavenger under environmental conditions and possibly have an important role in biogeochemical cycling of these metals. However, additional work will be required to validate this.

5. Conclusions

To date, there has been little investigation into the role of jellyfish in accumulating and/or cycling metals in marine environments. Limited work has suggested they are capable of metal accumulation but no information exists on rates and retention. This study demonstrated that *C. maremetens* was capable of concentrating aqueous copper and zinc to levels up to 100 times ambient water concentrations. *C. maremetens* was capable of concentrating copper at lower concentrations than many other species suggesting they are effective copper scavengers. For zinc, the clearance constant, maximum tissue concentration and half-life were comparable to other taxa, with accumulation patterns suggesting dietary zinc may be an important source of zinc to this species. Overall, the patterns of aqueous copper and zinc accumulation and clearance in *C. maremetens* were able to be predicted using kinetic models.

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Figure 1

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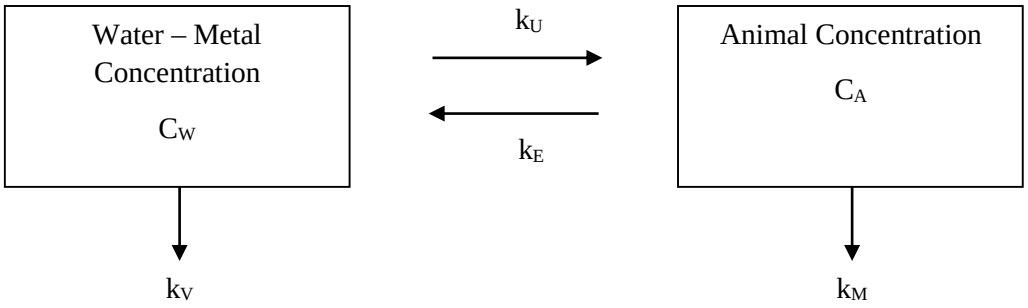

Figure 2
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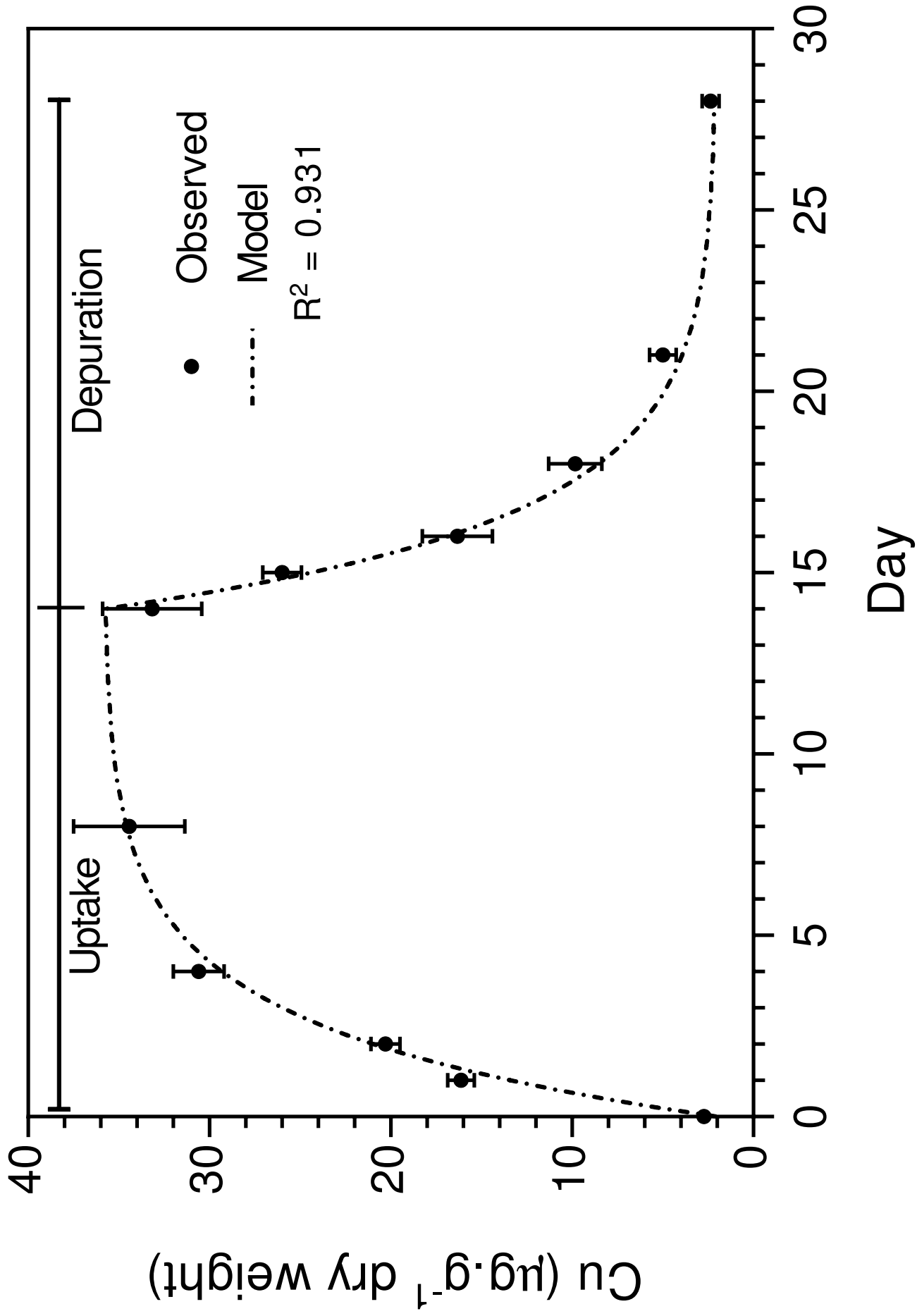

Figure 3
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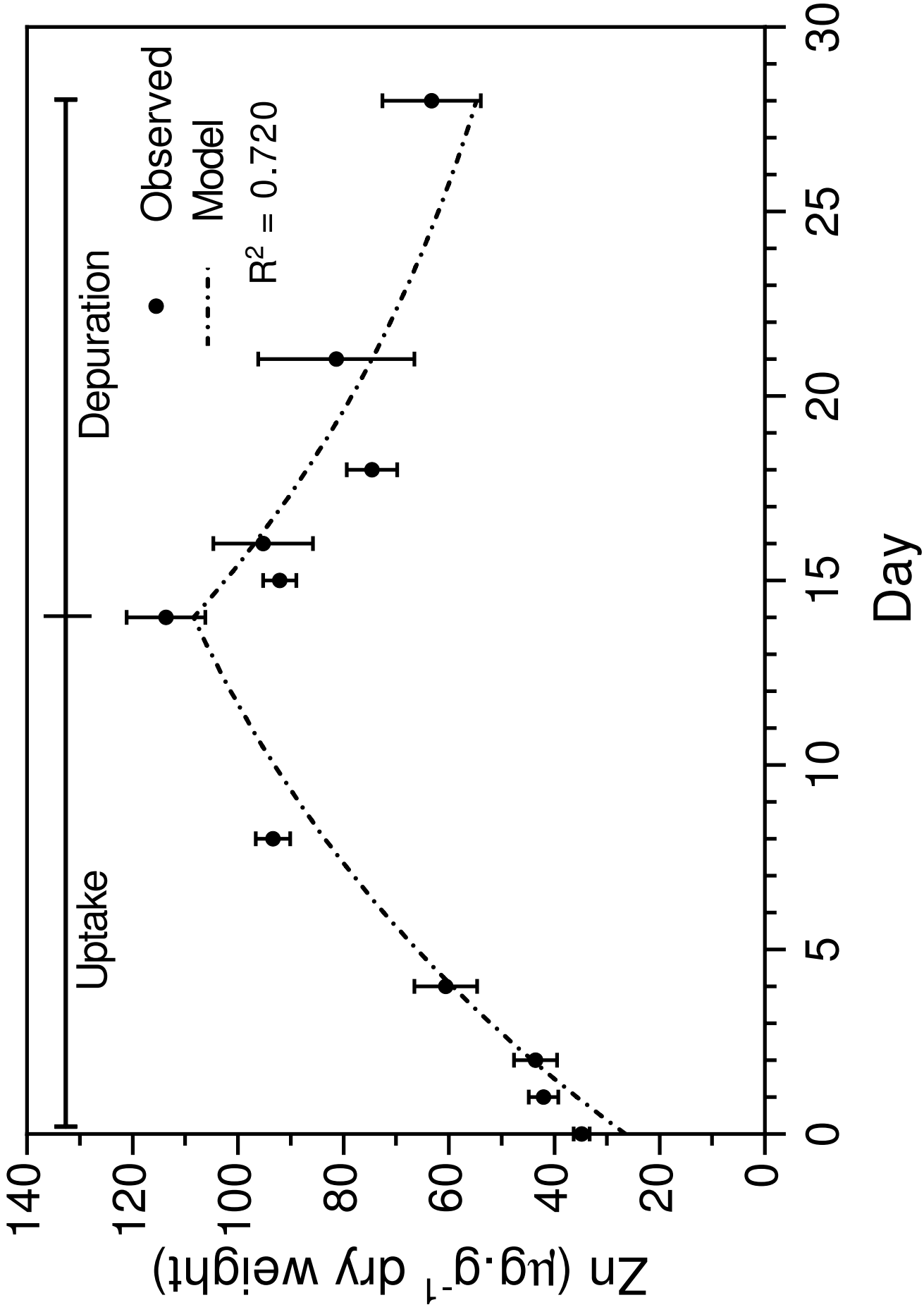

Table 1
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Table 1: Kinetic parameters and linear goodness of fit agreement for copper and zinc accumulation. k_U, k_E = uptake and clearance constants respectively; BCF_{kin} = kinetic bioconcentration factor; $C_{A(max)}$ = maximum tissue concentration at steady state (reported as $\mu g.g^{-1}$ dry weight) ; $t_{1/2}$ = biological half-life of the element (days). Slope (95% CI) = slope of linear regression and 95% confidence interval; C = the calculated constant of the linear regression;

Metal	**k_U** **($L.g.d^{-1}$)**	**k_E** **(d^{-1})**	**BCF_{kin}**	**$C_{A(max)}$** **($\mu g.g^{-1}$)**	**$t_{1/2}$** **(d^{-1})**	**R^2**	**Slope** **(95% CI)**	**C**
Cu	40.738	0.4114	99.01	33.78	1.68	0.931	0.982 (0.901-1.063)	-5.976
Zn	7.942	0.076	104.4	113.70	9.11	0.720	0.840 (0.684-0.995)	482.1