20th International Congress on Modelling and Simulation, Adelaide, Australia, 1–6 December 2013
www.mssanz.org.au/modsim2013

What is the Role of Sediment Resuspension in the Bioaccumulation of Heavy Metals in Oysters?

J.H. Lee [a], R.G. Richards [b], and G.F. Birch [a]

[a] *Environmental Geology Group, School of Geosciences, University of Sydney, Sydney, New South Wales, Australia*
[b] *Griffith Centre for Coastal Management, Griffith University, Gold Coast, Queensland, Australia*
Email: jungho.lee@sydney.edu.au

Abstract: The Sydney estuary (Port Jackson) is a highly modified waterway in which surficial sediments are extensively contaminated by a suite of chemicals including trace metals. These surficial sediments undergo resuspension into the water column on a daily basis due to both natural and anthropogenic processes which includes tides, currents, bioturbation, shipping, and dredging. As a result, sediment resuspension significantly increases the risk of trace metal contaminant exposure to marine biota. The status of trace metal contamination in surficial sediments, suspended particulate matter, and aquatic organisms has been studied in detail, and in all three media high concentrations have been detected, particularly for Cu, Pb, and Zn. However, a significant relationship linking these processes together has yet to be made and the effect that sediment-bound trace metals inflict on local fauna has not been documented previously.

The current study aims to identify the processes controlling bioaccumulation of trace metal contaminants in Sydney estuary using laboratory-based mesocosm experiments. The native Sydney rock oyster (*Saccostrea glomerata*) was used as a bioindicator species and was exposed to contaminated suspended sediment at a range of controlled concentrations and loads to mimic previously observed field conditions. The oysters were analysed for total bioaccumulated tissue metal concentrations, as well as changes in protein expression to identify probable early-onset bioindicators.

The results from the laboratory experiments will be used to parameterise a biogeochemical model to help explain the different mechanisms of trace metal bioaccumulation in Sydney estuary.

Keywords: *Heavy metals, sediment resuspension, oysters, bioaccumulation, proteomics*

1. INTRODUCTION

Recent research has investigated the status of contamination throughout the Sydney estuary, New South Wales (NSW). Of interest are the elevated concentrations of heavy metals that have been observed because of their ubiquity (Birch and Taylor, 1999). Contemporary metal contamination in sediments have been identified as originating from stormwater discharge and reclaimed-land leachates (Birch and Taylor, 1999), however research has so far yet to conclusively establish a correlative link between sediment bound metals and bioaccumulation in epifaunal suspension filter-feeders (e.g. oysters, mussels).

The contaminated bottom sediments could act as a mechanism for increased uptake and accumulation of particulate-bound metals through ingestion (i.e. feeding) of resuspended material (especially organic) and through influencing the biogeochemical conditions in the water column leading to changes in the availability of dissolved metal species (e.g. increased or decreased concentration of the highly bioavailable ionic forms such as Pb^{2+}, Zn^{2+}, Cu^{2+}, etc). Bioaccumulated contaminants can impair an organism's physiology by inducing chemical inactivation of key enzymes, competing against essential nutrients, or by disrupting important metabolic pathways (Babukutty and Chacko, 1995; Chapman et al., 1998).

Most recently, Apostolatos (2010) reported high enrichment of Cu, Pb and Zn in the soft tissue of native Sydney Rock oysters (*Saccostrea glomerata*) (SRO) across the Sydney estuary at average concentrations of 1480.50, 9.39 and 6299.70 $\mu g\ g^{-1}$ (dry weight), respectively. Median background concentrations in NSW oysters for Cu, Pb, and Zn are 170, 0.6, 2610 $\mu g\ g^{-1}$ (dry weight), respectively (Scanes and Roach, 1999).

Given this backdrop of evident metal contamination of SROs in Sydney estuary, the main aim of this study is to evaluate the processes controlling bioaccumulation of trace metals in biological tissues in this system. Strong emphasis is placed on better understanding the role of sediment resuspension in this bioaccumulation. The outcomes of this investigation into the role of sediment resuspension as a mechanism for metal bioaccumulation will then be used to inform a generalised bioaccumulation model of the experimental conditions. A conceptualisation of this bioaccumulation model is presented in this paper along with the algorithms that underpin the causal relationships between variables.

2. MESOCOSM EXPERIMENT

Mesocosm experiments were conducted in the aquarium facilities at the Sydney Institute of Marine Science (SIMS) (Chowder Bay, Sydney, NSW, Australia) to investigate sediment-bound metal uptake in estuarine filter-feeder animals. The main objective was to mimic previously observed field conditions in the Sydney estuary and to expose test SROs to these conditions over a two month period. Laboratory-based mesocosm experiments allowed concentrations of metals as well as the volume load of resuspended sediments to be purposely manipulated, and in addition eliminate variability in a number of other parameters such as pH, dissolved oxygen, temperature, and salinity.

The experiment involved exposing ten individual SRO specimens (aged eighteen month to two years old purchased from Aquaculture Enterprises Pty Ltd (Millingandi, NSW, Australia)) in 30 litre plastic tanks to a range of low to high metal contaminated sediments. Highly contaminated sediments were collected from Iron Cove (Birch and Taylor, 1999), and low contaminated sediments were collected from Bonnet Bay (Simpson et al. 2004). Equal volumes of both sediments were mixed to produce a third test sediment of medium metal concentrations.

A modified sediment entrainment or 'Shaker' device (Figure 1), which was first proposed by Tsai and Lick (1986), was custom built using wood and poly(methyl methacrylate). Each device was powered by a 240 volt AC motor which vertically oscillated three perforated grid paddles positioned inside each aquarium tank. Paddles agitated sediments in suspension to promote dietary ingestion by oyster specimens.

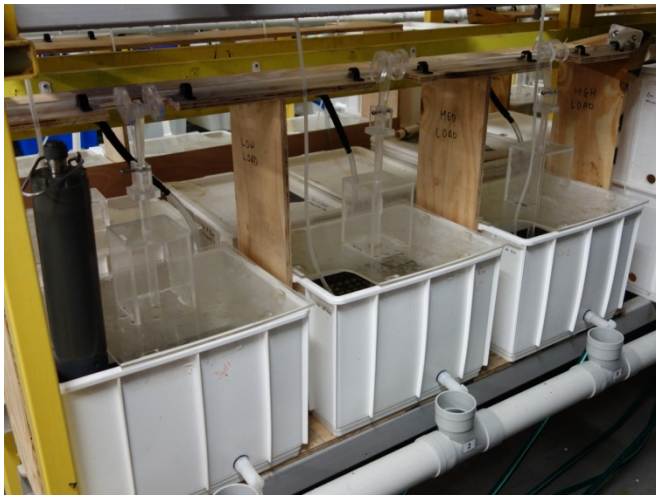

Figure 1. Modified sediment 'Shaker' device.

For each metal concentration, there existed a gradient of turbidities at 10, 20, and 40 Nephelometric Turbidity Units (NTU) reflecting those previously detected in waters of Sydney estuary, and sediment resuspension at these levels were maintained for a four hour period (Table 1). These values corresponded to 14.8, 30.4, and 61.6 mg/l total suspended solids, respectively (R^2=0.96). Three control tanks where sediments were not resuspended into the water column were included, as well as a fourth control tank containing no sediment. Water changes with fresh filtered seawater occurred daily over the two month period and were also sampled for dissolved metal concentrations.

Table 1. Mesocosm schematic design. Note each number refers to an aquarium tank containing ten oysters.

		Sediment Concentration									
		Low			Medium			High			No Sediment
Resuspension Load (mg/l)	14.8	1			4			7			
	30.4		2			5			8		
	61.6			3			6			9	
	No Resuspension	10			11			12			13

Upon conclusion of the experiment, SRO samples were measured, weighed, shucked, freeze-dried, and microwave digested in 70% HNO_3 at SIMS facilities. Metal concentrations for Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Ti, and Zn were analysed using ICP-AES at The University of Sydney. Sample analysis also included blanks which showed very minor contamination of samples for Al, Ca, and Zn. The oyster standard reference material NIST-1566b was included in analysis and showed poor recovery for Al, As, Ca, Co, Ni, and Pb, while certified reference values do not exist for Cr and Ti at present.

3. MODEL DESCRIPTION

A coupled kinetic-equilibrium model was developed to replicate the mesocosm experimental set-up (Figure 2). Cu and Zn were selected from the suite of metals in the model because speciation patterns are inherently species-specific, whilst Pb was excluded due to poor bioaccumulation as documented in the literature. Furthermore, calculation of the speciation calculations requires an iterative approach to finding the numerical solutions (i.e. the concentrations of the individual species) and therefore limiting the metals to Cu and Zn lowers the computational load of the model at each time step.

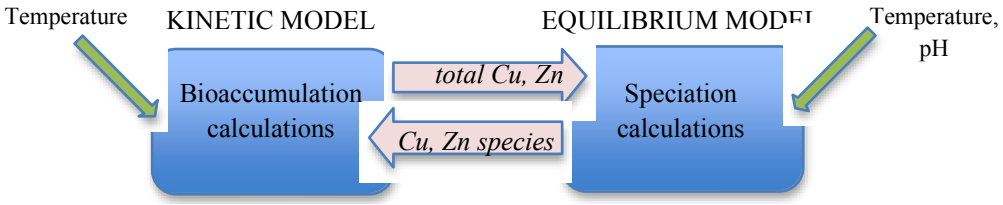

FIGURE 2. Conceptualisation of the coupled kinetic - equilibrium model.

3.1 Kinetic Modelling Of Bioaccumulation

The kinetic model was used to calculate the temporal dynamics of oyster uptake, accumulation and depuration of metals through the particulate (dietary) and dissolved (absorption) pathways in a single oyster:

$$\frac{dOYS_{Cu}}{dt} = (AE_{Cu} \times CR \times Cu_P) + (\alpha_{Cu} \times CR \times Cu_D) - (k_e \times OYS_{Cu}) \quad (1)$$

where OYS_{Cu} is the soft-tissue Cu concentration in the oyster (µg g^{-1} dry-weight basis); Cu_P and Cu_D are the respective particulate and dissolved concentrations of Cu in the water column (both µg l^{-1}); AE_{Cu} is the assimilation efficiency for Cu ingested by the oyster (no units); α_{Cu} is the absorption rate for Cu absorbed (no units); CR is the clearance rate (l g^{-1} d^{-1}); and k_e is the metal-specific efflux rate (d^{-1}). Note that it is assumed that $\alpha_{Cu} \times Cu_D$ was equivalent to the cupric ion concentration, $[Cu^{2+}]$ because it is acknowledged as the most bioavailable form of the dissolved Cu species (Richards et al., 2010). Values for these parameters are the same as described in Richards et al., (2010).

The concentrations of Cu (µg-Cu l^{-1}), particulate organic matter (POM; mg l^{-1}) and particulate inorganic matter (PIM; mg l^{-1}) in the water column are modelled using the following ODEs:

$$\frac{d[Cu(Aq)]}{dt} = -Settling_{rate} \times [Cu(Aq)_{PART}] \quad (2)$$

$$\frac{d[POM(Aq)]}{dt} = Settling_{rate} \times [POM(Aq)] \quad (3)$$

$$\frac{d[PIM(Aq)]}{dt} = Settling_{rate} \times [PIM(Aq)] \quad (4)$$

where $Cu(Aq)_{PART}$ is the concentration of particulate Cu in the water column (µg l^{-1}). At the start of the modelling, we assume that the concentration of sediment particulates in the water column is zero and the aqueous Cu concentration is equivalent to the 'background' concentration of the seawater that is used 0.23333 µg l^{-1}.

During the period where the mesocosm is agitated (4 hour period every day), we assume that no settling out of the particulates occurs and therefore settling rate is set to zero. During the times (20 hours per day) that the agitator is stopped, settling rate is activated to allow particulates to settle out of the water column.

3.2 Equilibrium Modelling Of Bioaccumulation

The equilibrium model imports the total concentrations of Cu(aqueous), POM and PIM and uses these to estimate the concentrations of each within the individual species using the method of principal components (van der Lee, 1998). This method requires that all speciation reactions (Table 3) are written in terms of a set of principal components (Cu^{+2}, H^+, POM^-, hydrous ferric oxide-hydroxyl (HFO_OH) [PIM], dissolved organic matter (DOM^-)) from which secondary species can be derived (refer to Richards et al., 2010). The concentrations of the chemical species are estimated using a modified Newton Raphson root finding method (van der Lee 1998). This requires an iterative process of minimising the error between the estimated

concentrations for total Cu (Cu_T – estimated using the principal components) and their known values, a process that continues until a specified tolerance in the error is achieved for all elements. The initial guess for each principal component was set at 1 x 10^{-7} mol l^{-1}. A polishing factor approach is used to ensure convergence and avoid divergence of the error (refer van der Lee 1998). pH is used to force the speciation model, constraining the effect of H^+ as a principal component.

3.3 Modelling Scenarios and Calibration

The model was run for 61 days (i.e. two months) at one hour time steps to replicate the mesocosm operating conditions. The model was set up to simulate the scenario where sediment from Iron Cove was used in the experiment under the three turbidity regimes (40, 20 and 10 NTU). The DOM (10, 50, 100, 250, 500 and 1000 nM) was adjusted to test the sensitivity of the model outputs (oyster soft-tissue and aqueous Cu speciation) to the parameter. This range of values for the Cu-complexing DOMs is representative of that observed in coastal seawater (e.g. Midorikawa and Tanoue, 1994).

4. RESULTS

4.1 Mesocosm Experimental Results

Oyster specimens in all treatments (including controls) were found to have bioaccumulated Cu, Pb, and Zn into tissues (Table 2) above background concentrations after the conclusion of the two month experiment. However no correlation could be found between sediment metal concentrations and/or turbidity to bioaccumulated metals in tissues. Average soft tissue weight decreased for a number of treatment tanks, but this was found to be negligible and did not impact the results of the experiment, especially when tissue metal concentrations were normalised to 1g dry weight to account for changes to biomass.

Table 2. Oyster tissue metal concentration results (background values in parentheses). Units are in μg g^{-1} (dry weight).

	Cu (33)				Zn (774)			
	Sediment				Sediment			
Resuspension Load (mg/l)	Low	Medium	High	No Sediment	Low	Medium	High	No Sediment
Low (14.8)	77	54	60		1593	1028	1107	
Medium (30.4)	50	60	72		1169	1339	1467	
High (61.6)	67	60	74		1489	1102	1106	
Control	53	74	61	91	1023	1589	1246	1730

4.2 Performance of the Model

Oysters in the model were unable to bioaccumulate Cu into tissues at turbidity = 10 NTU for all DOM concentrations and instead exhibited net loss of Cu from tissues, even at DOM = 0 nM (Figure 3). The model did not accurately capture the bioaccumulation process which occurred in the mesocosm experiment at 10 NTU. At turbidity = 20 NTU, net bioaccumulation of Cu into oyster tissue was seen (Figure 3) for DOM concentrations up to 250 nM. When DOM was set to 500 nM and 1000 nM, net bioaccumulation ceased and loss of Cu from tissues was observed in a similar manner to Figure 3. For turbidity = 40 NTU, tissue Cu bioaccumulation was seen for all DOM concentrations akin to Figure 3.

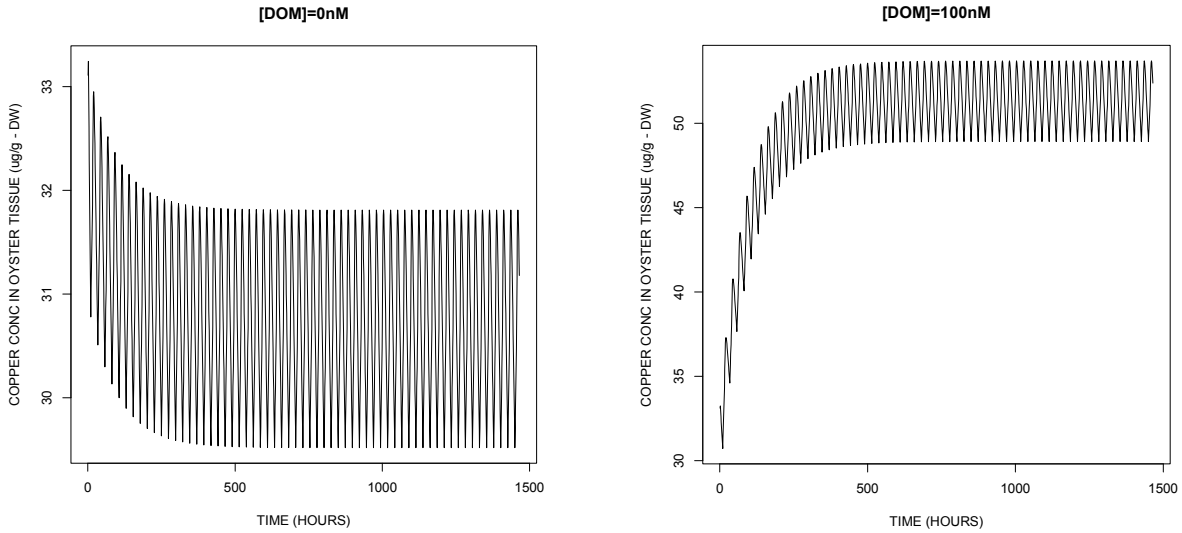

Figure 3. Decrease in Cu concentrations in oyster tissue at turbidity = 10 NTU and DOM = 0 nM, and bioaccumulation of Cu into oyster tissue at turbidity = 20 NTU and DOM = 100 nM

5. DISCUSSION

The results of the mesocosm experiment have shown uptake and bioaccumulation of metals Cu, Pb, and Zn into tissues for all treatment tanks, regardless of the presence of the 'Shaker' device due to the presence of metals in the tissues of control specimens. Moreover, metals were taken up into tissues even without the presence of contaminated sediment, as can be seen from the elevated concentrations in the 'No Sediment' tank. It can be seen that uptake and bioaccumulation of heavy metals is a complex process that is more than simply dietary ingestion of contaminated sediment. The uptake and bioaccumulation of contaminants by a marine organism can vary significantly depending on a wide range of variables that include growth stage, seasonal effect (including temperature-mediated changes in both physiological response and metal bioavailability), behavioural patterns, sexual condition, temperature, and length of contaminant exposure (Tessier and Campbell, 1987; Babukutty and Chacko, 1995; Richards and Chaloupka, 2009).

Another explanation for the observed bioaccumulation in the experiment includes the uptake of metals from the dissolved phase and will require analysis of seawater samples. The lack of a discernible trend or pattern between tissue bioaccumulation and sediment concentration and/or turbidity can be explained by a probable lack of bioavailability of sediment-bound metals and will require further laboratory analysis.

Poor bioavailability of metals can be attributed to the presence of DOMs which are ubiquitous in the marine environment. DOMs are known to bind and form stable complexes with dissolved trace metals including Cu, Cd, Ni, and Zn and ultimately sequester them (Vraspir and Butler, 2009). DOMs dominate the dissolved Cu speciation in seawater and are likely to be correlated to the concentration of particulate matter (Midorikawa and Tanoue, 1984). However, the model could not bioaccumulate Cu in tissues when DOM was set to zero, which demonstrates a constraint to the model. A plausible explanation is that sediment-bound Cu had adsorbed to the surface of Fe particulates such as HFO which is known to occur readily (Dzombak and Morel, 1990) and was not incorporated into the model. All of the low, medium, and high concentration sediments used in the mesocosm experiment contain high concentrations of Fe between 32,600 to 43,300 $\mu g\ g^{-1}$, therefore it is highly likely that Cu formed complexes with HFOs and reduced their bioavailability.

Physiological functions of oysters such as the clearance rate, assimilation rate, and efflux rate that was employed in the model could be wrong and may explain why the model did not accurately represent the bioaccumulation process. To illustrate, the assumed clearance rate for oysters in this model was set at 2 l/g/hr and might have been too low and studies in the literature have shown a range of different clearance rates. Moreover, the assumed clearance rate was for 1g of oyster tissue dry weight.

Finally, although the model may have been unable to precisely simulate the bioaccumulation of metals into tissues as observed by the mesocosm experiment, the modelling results should not be seen as a failure. Whilst all models are *in simulacra* and are simplifications of the processes that occur in reality, models posses their own unique complexities (e.g. the dynamic coupling of biokinetic processes and thermodynamic speciation is not easy or often undertaken). Furthermore, models can be seen as 'numerical playgrounds' used to formulate testable hypotheses. In this case, the unexpectedly low bioaccumulation levels of Cu in the test oysters, even those exposed to high Cu sediment concentrations and turbidities, provides an opportunity to explore 'what-if' scenarios that might have been playing out during the experiment. For example, the potential (yet unknown) role of DOMs and HFOs as regulators of Cu bioaccumulation can be explored with the model.

REFERENCES

Apostolatos, C. (2010). The use of the Sydney rock oyster (*Saccostrea glomerata*) and black mussel (*Mytilus galloprovincialis*) in determining the role of sediment in metal bioaccumulation in Sydney estuary, Australia. Unpubl. MSc thesis, School of Geosciences, Sydney University, Sydney.

Babukutty, Y. and J. Chacko (1995). Chemical partitioning and bioavailability of lead and nickel in an estuarine system. *Environmental Toxicology & Chemistry*, 14(3), 427–434.

Birch, G., and S. Taylor (1999). Source of heavy metals in sediments of the Port Jackson estuary, Australia. *The Science of the Total Environment*, 227, 123-138.

Chapman, P.M., F. Wang, C. Janssen, G. Persoone, & H.E. Allen (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Canadian Journal of Fisheries and Aquatic Sciences*, 55, 2221–2243.

Dzombak, D.A. and F.M.M. Morel (1990). Surface complexation modelling: hydrous ferric oxide. New York: Wiley.

Midorikawa, T. and E. Tanoue (1994). Detection of a strong ligand for copper in seawater and determination of its stability constant. *Analytica Chimica Acta*, 284, 605-619.

Richards, R.G. & M. Chaloupka (2009). Temperature-dependent bioaccumulation of copper in an estuarine oyster. *Science of the Total Environment*, 407, 5901-5906.

Richards, R.G., M. Chaloupka, and R. Tomlinson (2010). Towards an integrated ecosystem-based bioaccumulation and metal speciation model. *Ecosystems*, 13, 1303-1318.

Scanes, P.R. and A.C. Roach (1999). Determining natural `background' concentrations of trace metals in oysters from New South Wales, Australia. *Environmental Pollution* 105, 437-446.

Tsai, C.H. and W. Lick (1986). A portable device for measuring sediment resuspension. *Journal of Great Lakes Research*, 12, 314-321.

van der Lee, J., (1998). Thermodynamic and mathematical concepts of CHESS. Technical Report Nr LHM/RD/98/39, Ecole des Mines de Paris, Fontainebleau, France.

Vraspir, J.M., and A. Butler (2009). Chemistry of marine ligands and siderophores. *Annual Review of Marine Science*, 1, 43-63.