Research projects available in 2010
Environmental chemistry & toxicology

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ENVIRONMENTAL CHEM PROJECT 1 (Based at CSIRO-Menai + several weeks at UoW)

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Topic: Chemistry (sediments and toxicity)

Project: Finding a link between chemical and biological responses to contaminated sediments in marine ecosystems (focus on copper, zinc, cadmium)

Background

Human activities have resulted in widespread contamination of waterways by a range of inorganic substances, including numerous metals (described as trace or heavy metals), metalloids and non-metals.[1a] Unlike many organic contaminants, inorganic contaminants do not degrade over time into less toxic substances,[1b] but accumulate and persist in a range of chemical forms (species)[1c,2a] with varying biological availabilities[1d] and toxicities.[1e] Many metals have essential micronutrient functions (although some do not) and organisms have evolved to take up these elements at typical background concentrations. Consequently, many organisms find it difficult to adequately regulate body burdens (total amount in their tissues) of some elements when they are present in the environment at high concentrations.[1b] Contamination by toxic elements may therefore have persistent adverse or, sometimes, tragic consequences for both local human societies (e.g. mercury at Minamata and cadmium induced 'itai-itai' disease, both in Japan)[1a] and ecosystems (e.g. copper in Macquarie Harbour, Australia[3] and tributyltin in coastal waters off Brittany, France[4]). As one third of the World's population and 80% of Australians live within 50 km of the coast, contamination of coastal and estuarine waterways is virtually ubiquitous near major coastal urban, and industrial centres.[1f]

Inorganic contaminants in the water column often adsorb onto suspended material (e.g. sediment particles), or are taken up by organisms that produce waste material or organic detritus when they die, much of which settles onto the sediment surface.[1f] In estuaries, nearly all of this material settles out, meaning that sediments within estuaries typically have higher contaminant concentrations than other coastal waters. These materials and associated inorganic contaminants can be resuspended, consumed, or buried by the deposition of additional material.[1f] In addition, within the sediment, elemental species undergo a range of characteristic and complex processes, some processes making them more soluble. These processes may lead to chemical remobilisation into the water column, and accumulation within benthic organisms and the associated food webs, including animals consumed by humans, or eventual burial within the sediment.[1f] Coastal sediments therefore act both as long-term sinks, but potentially also as a shorter-term source of inorganic contaminants.

Sediment contamination can be determined by biological and chemical approaches. Chemical techniques rely on total amounts, chemical species, and sometimes partitioning (dissolved or bound), however these results do not confirm the toxicity of the contaminant. Biological techniques usually measure the total amount in the organisms' body or tissues, or place them in to contaminated samples and determine their ability to survive or reproduce, however these tests are expensive, time consuming and require high numbers of individuals. A robust relationship between chemical measures and biological toxicity of metals has not yet been determined.

The Project

This project applies a novel approach to investigate the correlation between chemical and biological testing, applying new two-dimensional *in-situ* sampling devices (chemical) with established biological methods (toxicity) for assessing the toxicity of contaminated marine sediments. The research will utilise two approaches: (i) whole sediment toxicity bioassays to directly determine the toxicity of the metal contaminated sediments to a sediment ingesting marine organism; and (ii) diffusive gradients in thin films (DGT, a time integrated *in-situ* monitoring technique) analysed by inductively coupled plasma–mass spectrometry (ICP-AES or MS) to identify the solubility (biological availability) of the metals in the same sediment.

No prior knowledge in sampling, sediment handling, toxicology or instrument use is required. Training is provided.

Skills obtained: sediment sampling, amphipod culturing (little marine critters!), sample handling and analyses (including porewaters and extractable metals), metal extraction, sample digestion & analysis (ICP-AES or MS).

Supervision: The University supervisor will be Dr Dianne Jolley, and the **CSIRO supervisor will be Dr Stuart Simpson**. The sediment and organism aspects will be carried out at CSIRO Land and Water Lucas Heights (most of the time), and the DGT preparation and sectioning will be conducted in Chemistry at UoW.

References

1. Luoma, SN & Rainbow, PS (2008). *Metal* Cambridge University Press: a) Preface, 1-5; b) 9-11, Chapter 7; c) 83-91; d) Chapter 7; e) Chapter 9; f) 63, Chapter 6; f) Chapter 13. [and the references therein].
2. Stumm, & Morgan, (1996). John Wiley & Sons, NY: a) 258; b) 473-479; c) 903-906.

ENVIRONMENTAL CHEM PROJECT 2 (Based at UoW)
Dr Dianne Jolley Tel: 4221 3516 Email: djolley@uow.edu.au

Topic: Chemistry (sediments and toxicity)

Project: Finding a link between chemical and biological responses to contaminated sediments in marine ecosystems (focus on Arsenic and selenium)

Background (same as above)

Human activities have resulted in widespread contamination of waterways by a range of inorganic substances, including numerous metals (described as trace or heavy metals), metalloids and non-metals.[1a] Unlike many organic contaminants, inorganic contaminants do not degrade over time into less toxic substances,[1b] but accumulate and persist in a range of chemical forms (species)[1c,2a] with varying biological availabilities[1d] and toxicities.[1e] Many metals have essential micronutrient functions (although some do not) and organisms have evolved to take up these elements at typical background concentrations. Consequently, many organisms find it difficult to adequately regulate body burdens (total amount in their tissues) of some elements when they are present in the environment at high concentrations.[1b] Contamination by toxic elements may therefore have persistent adverse or, sometimes, tragic consequences for both local human societies (e.g. mercury at Minamata and cadmium induced 'itai-itai' disease, both in Japan)[1a] and ecosystems (e.g. copper in Macquarie Harbour, Australia[3] and tributyltin in coastal waters off Brittany, France[4]). As one third of the World's population and 80% of Australians live within 50 km of the coast, contamination of coastal and estuarine waterways is virtually ubiquitous near major coastal urban, and industrial centres.[1f]

Inorganic contaminants in the water column often adsorb onto suspended material (e.g. sediment particles), or are taken up by organisms that produce waste material or organic detritus when they die, much of which settles onto the sediment surface.[1f] In estuaries, nearly all of this material settles out, meaning that sediments within estuaries typically have higher contaminant concentrations than other coastal waters. These materials and associated inorganic contaminants can be resuspended, consumed, or buried by the deposition of additional material.[1f] In addition, within the sediment, elemental species undergo a range of characteristic and complex processes, some processes making them more soluble. These processes may lead to chemical remobilisation into the water column, and accumulation within benthic organisms and the associated food webs, including animals consumed by humans, or eventual burial within the sediment.[1f] Coastal sediments therefore act both as long-term sinks, but potentially also as a shorter-term source of inorganic contaminants.

Sediment contamination can be determined by biological and chemical approaches. Chemical techniques rely on total amounts, chemical species, and sometimes partitioning (dissolved or bound), however these results do not confirm the toxicity of the contaminant. Biological techniques usually measure the total amount in the organisms' body or tissues, or place them in to contaminated samples and determine their ability to survive or reproduce, however these tests are expensive, time consuming and require high numbers of individuals. A robust relationship between chemical measures and biological toxicity of metals has not yet been determined.

The Project (same as above but using a different DGT resin, and freshly collected little bivalve mussels)

This project applies a novel approach to investigate the correlation between chemical and biological testing, applying new two-dimensional *in-situ* sampling devices (chemical) with established biological methods (toxicity) for assessing the toxicity of contaminated marine sediments. The research will utilise two approaches: (i) whole sediment toxicity bioassays to directly determine the toxicity of the metal contaminated sediments to a sediment ingesting marine organism; and (ii) diffusive gradients in thin films (DGT, a time integrated *in-situ* monitoring technique) analysed by inductively coupled plasma–mass spectrometry (ICP-AES or MS) to identify the solubility (biological availability) of the metals in the same sediment.

No prior knowledge in sampling, sediment handling, toxicology or instruments assumed: Training is provided.

Skills obtained: sediment sampling, maintaining bivalves in a lab, sample handling and analyses (including porewaters and extractable metals), metal extraction, sample digestion and metal analysis (ICP-AES or MS).

Supervision: The University supervisor will be Dr Dianne Jolley.

References

1. Luoma, SN & Rainbow, PS (2008). *Metal* Cambridge University Press: a) Preface, 1-5; b) 9-11, Chapter 7; c) 83-91; d) Chapter 7; e) Chapter 9; f) 63, Chapter 6; f) Chapter 13. [and the references therein].
2. Stumm, & Morgan, (1996). John Wiley & Sons, NY: a) 258; b) 473-479; c) 903-906.

ENVIRONMENTAL CHEM PROJECT 3 (Based mainly at CSIRO-Menai (ANSTO site)

Dr Dianne Jolley Tel: 4221 3516 Email: djolley@uow.edu.au

Supervisory team: Drs Olivia Campana, Stuart Simpson (CSIRO) and Dianne Jolley (UOW)

Topic: Chemistry (sediments and toxicity)

Project: Ecotoxicological responses and exposure pathways of gastropods (mud snails) inhabiting contaminated sediments.

This research proposes to use laboratory-based experiments to determine ecotoxicological responses and exposure pathways of gastropods inhabiting metals contaminated sediments. Chemical analyses will be used to identify exposure pathways and determine effects thresholds. We will investigate three species of gastropod (*Tatea huonensis*, *Assiminea sp*., *Cassidula zonata*) chosen because of their feeding behaviour and ecological relevance. In general, the research will reveal the different sensitivity of each species to the metal contamination. Particularly, the impact of the sediment contamination on the behavioural activity of the organisms and bioaccumulation-toxicity relationships will be determined in order to relate sublethal endpoints with whole-body metal concentration and metal toxicity. The student will also develop skills in animal husbandry, ecotoxicity bioassays and sediment chemistry.

Why investigate organism responses to sediment contaminants?

Sediments are extremely important to the food web and serve as the ultimate repository of most of the contaminants entering aquatic systems. Contaminated sediments often become a source of contaminants to overlying waters. For this reason, it is appropriate that regulatory attention addresses the ecological risks that the sediment contaminants might pose to ecosystems.

Metals are a ubiquitous class of contaminants in aquatic sediments and their concentrations are often elevated above background levels in sediments that have been affected by human activity. The last decade has seen an exponential growth in our understanding of contaminants in aquatic sediments; however our ability to accurately assess the risk posed by metal-contaminated sediments remains inadequate and this often results in unnecessarily high assessment costs to industries and time for regulators.

While thresholds for acute toxic effects of metals are reasonably well understood, there is a poor understanding of the sublethal and chronic effects of metals in sediments. Furthermore, while chronic effects have been considered for some classes of organisms, e.g. amphipods, for some classes of organisms, e.g. gastropods (mud snails), there has been much less research into effects.

No prior knowledge in sampling, sediment handling, toxicology or instrument use is required. Training is provided.

Skills obtained: sediment sampling, gastropod culturing (little marine critters!), sample handling and analyses (including porewaters and extractable metals), metal extraction, digestion & sample analysis (ICP-AES or MS).

Supervision: The University supervisor will be Dr Dianne Jolley, and the **CSIRO supervisor will be Drs Olivia Campana and Stuart Simpson**. The project will be based solely at CSIRO Land and Water, Menai.

References.

- Kitching RL, Chapman HF, Hughes JM. 1987. Levels of activity as indicators of sublethal impacts of copper contamination and salinity reduction in the intertidal gastropod, *Polinices incei.* Marine Environmental Research 23:79-87.
- Borgmann U.2000. Methods for assessing the toxicological significance of metals in aquatic ecosystems: bio-accumulation-toxicity relationship, water concentration and sediment spiking approaches. Aquat.Ecosys.Health Manag. 3:227-289.
- Orvain F, Sauriau PG. 2002. Environmental and behavioural factors affecting activity in the intertidal gastropod *Hydrobia ulvae*. J.Exp.Mar.Biol. 272:191-216.

TOXICOLOGY AND CHEMISTRY PROJECT 4 (Based at UoW)

Dr Dianne Jolley Tel: 4221 3516 Email: djolley@uow.edu.au

Topic: Chemistry / Toxicology

Project: Understanding mechanisms of metal toxicity: Identifying the intracellular fate of toxic metals

Prior knowledge: No prior knowledge in toxicology or instrument use is required - training is provided.

Project summary. This research proposes to apply advanced techniques in subcellular fractionation to unravel the intracellular distribution and fate of toxic metals in microalgae. We will investigate three species of algae (*Phaeodactylum tricornutum*, *Tetraselmis sp*., and *Dunaliella tertiolecta*) chosen because of their differing sensitivities to metals. The research will reveal the "physiological" fate of metals within algae using a combination of flow cytometry (to count cells), sonication (to disrupt cell membranes), ultracentrifugation (to separate subcellular fractions) and enzymatic essays (cytochrome c oxidase and citrate synthase oxidase) to confirm the success of the separation. The student will also develop skills in cell culturing and toxicity bioassays.

Why investigate metal stress in marine micro-algae? Microalgae are easy to culture, are not biohazards and require no ethics clearance! In addition they are key primary producers in aquatic ecosystems, as they form the base of the food web. It is widely recognised that the toxicity of metals to organisms depends on (i) the metals' chemical speciation, and (ii) the competitive binding of the metal to the critical receptor site(s) of the organism (whether that be within a specific compartment within the cell, or on the cell surface). Critical biological binding sites for metals are referred to as "biotic ligands". Understanding the nature of the biotic ligand(s) has become more critical, as recently developed biotic ligand models are increasingly being used by industry and environmental discharge regulators to predict metal bioavailability and toxicity in aquatic systems (and hence formulate discharge licences). This project aims to generate fundamental knowledge about the cellular distribution of copper and selenium in marine microalgae, and changes in cellular distribution at different metal exposure concentrations.

Previous work in our group demonstrated a clear link between copper exposure and cell stress in marine microalgae (Figure 1A). The cell stress has been measured as decreased cell division, increased levels of Reactive Oxygen Species, changes in mitochondrial membrane potential and the proportion of cells at different stages of the cell cycle. There is also a clear relationship between copper exposure and the number of acidic compartments within the cells (Figure 1B & C). However, there are significant differences in these cellular responses between algal species, and we hypothesis that these differences will be explained by the way that the algae compartmentalise the metals within the cells.

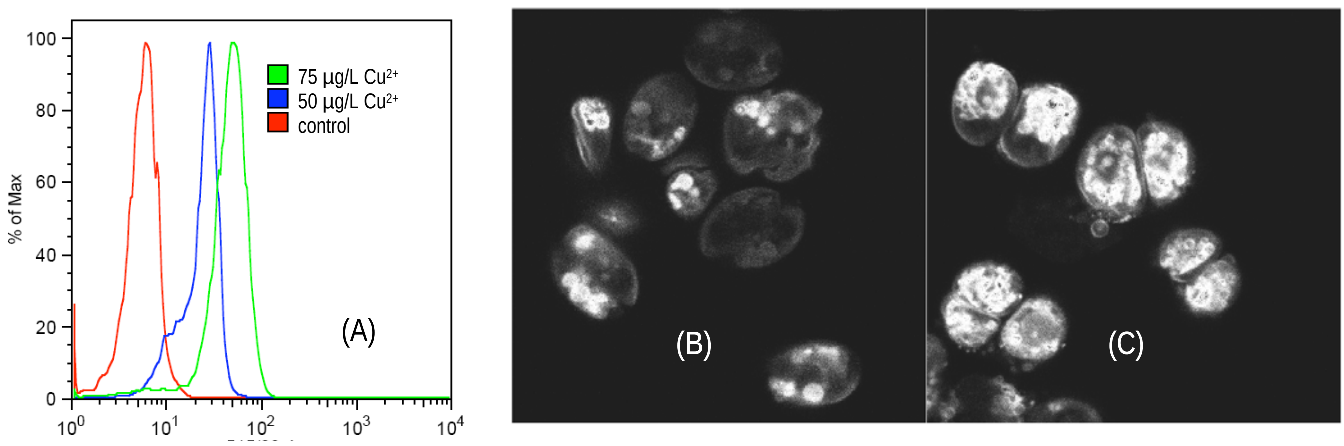

Figure 1. *Tetraselmis sp*. cells exposed to dissolved copper for 72 h. (A) An increase in reactive oxygen species (ROS) with increasing copper exposure, measured as an increase in fluorescence of a ROS-sensitive probe by flow cytometry. (1B & 1C) Cells exposed to LysoSensor Green reveal increased acidic compartments with increasing copper exposure [B= no Cu^{2+}; C= 75 µg/L Cu^{2+} exposed cells].

Supervisors: Dr Dianne Jolley (Chemistry) and Prof Mark Wilson (Biological Sciences)

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