REBECCA ZITOUN

A Delicate Balance between Copper Necessity and Toxicity

Recent human activities, such as urbanisation, industrialisation and agricultural intensification, have produced a concerning increase in the concentrations of trace metals in the aquatic environment.[1] While metals such as copper are essential micro-nutrients to aqueous organisms, they become toxicants when surpassing a critical concentration threshold in the aquatic environment.[2] The copper concentration of many natural water masses and tissue of aquatic organisms have been found to exceed essential levels. These elevated levels of copper lead to sub-lethal or toxic effects on adults or, more crucially, their larval stages,[3] drastically impacting the diversity, health, structure and functioning of affected ecosystems. The detection, monitoring and assessment of copper concentrations are therefore key to the integrity of aquatic environments and are becoming increasingly important as a result of legislation and increasing public awareness.

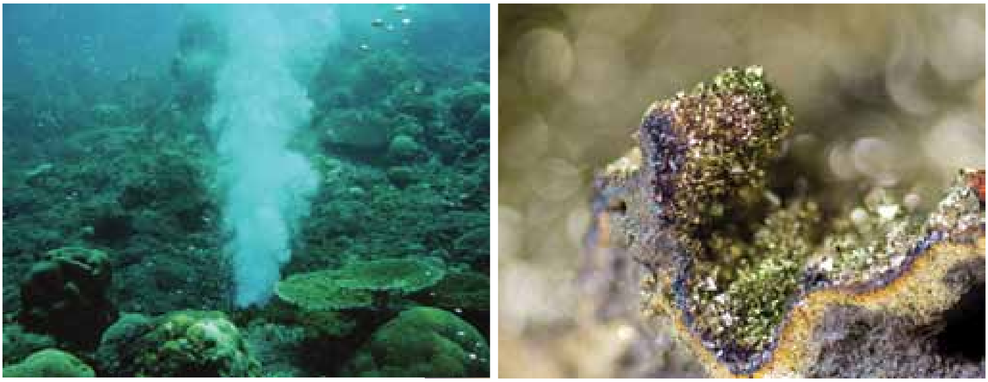

Figure 1. (L) Shallow marine hydrothermal vent – diffuse venting and gas bubbling;[4] (R) Deep-sea hydrothermal vent – chimney wall of a black smoker with a zonation of metal sulfide minerals.
Photographs: Nico Fröhberg (taken during the SO253 sampling campaign in 2017).

The bioavailability of copper to aquatic biota, with respect to both toxicity and necessity, is dependent on the chemical speciation (i.e., the range of chemical forms a metal is able to assume) and the concentration of the total dissolved copper in the water column.[5] The most bioavailable form of total dissolved copper is considered to be the inorganic free, hydrated copper-ion.[6] The level of free copper-ions in the water column is strongly mediated by (1) the presence of organic copper-complexing ligands, which alleviate metal limitation and reduce toxicity to affected organisms,[7] and (2) geochemical conditions such as pH, temperature and salinity.[8] For instance, low pH (i.e., ocean acidification) increases the free copper-ion concentration in the water column and can thus potentially shift an aquatic system in a short amount of time from a healthy to a toxic state. All in all, the final fate of copper has to be determined on a site-by-site basis, as varying local conditions prevent the generalisation of copper speciation and associated copper toxicity over regions.

Although copper contamination of the environment has been investigated intensively since the 1980s, research on copper speciation and associated biogeochemical processes in aquatic ecosystems is scarce, owing to time-consuming analysis techniques and difficulties in characterising the complex organic copper-binding ligand pool. So far, only a few organic ligands, such as thiols, humic acids, fulvic acids and other low molecular weight compounds,[9] passively or actively produced by some microorganisms, have been studied, despite their importance on copper-cycling and bioavailability. The chemical identity of these ligands is still largely unknown, which impedes our ability to understand the copper inventory of aquatic systems as well as the uptake and bioavailability of copper to aqueous biota.

The purpose of my research is to investigate copper speciation, fluxes and cycling in different aquatic environments to improve environmental risk assessments of copper in freshwater, estuarine and marine systems, as well as to establish unifying projections for our future oceans. Constraining the sources, sinks and overall quality of organic copper-binding ligands in aquatic systems is important for understanding the biogeochemical cycling of copper, as well as for clarifying the implications for economically and ecologically important species.

To improve and expand our current knowledge of copper speciation, I (1) participated in four research cruises to study estuaries (Amazon and Kongo river), coastal (Hauraki Gulf in New Zealand and Angola Basin in Africa) and open ocean systems (Hauraki Gulf); (2) analysed freshwater samples from New Zealand cave systems; (3) investigated shallow (White Island/Whakaari, New Zealand) and deep marine hydrothermal vents (Kermadec Island arc, New Zealand) (Figure 1) to evaluate their importance as sources of copper and other trace metals in the ocean, as well as to describe organic and inorganic complexation mechanisms within these extreme environments; and (4) conducted single species bioassays with mussel embryos to investigate the effect of changing geochemical conditions on copper speciation and toxicity.

Collecting uncontaminated water samples for trace metal analysis is difficult, as metals are ubiquitous constituents of the natural environment.[10] Trace metal studies therefore require strict trace metal clean procedures during sample collection, handling and analysis to avoid sample contamination. This includes the cleaning of labware and sample bottles with acid prior to usage, as well as the requirement for the sampling personnel to wear clean gloves and protective clothing when handling equipment or samples. Working on a metal ship adds another challenge to trace metal work, as samples can be easily contaminated and affected by rain, dust and the ship itself. Special sampling equipment must be used to eliminate these contamination risks. On board, the available equipment consists of a trace metal clean rosette (an aluminum frame covered with a plastic coating, Figure 2), Go-Flo bottles with few metal parts (internally totally metal-free), and a Class 100 trace metal clean container in which the air is fully HEPA-filtered to remove all airborne particles.

The container is used to process the obtained water samples, i.e., pressure filtration (0.2 μm), in a clean environment before freezing (-20°C) the samples for subsequent analysis in the home laboratory at the Trace Metal Centre of the University of Otago. We employ an electrochemical approach using competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-AdCSV) for copper-speciation analysis.[11] This is a well-established analytical method that allows for the determination of water ligand concentrations and metal binding strengths, but provides no information about the chemical structure of the ligands. Copper titration data is then individually fitted using the ProMCC software to obtain specific copper speciation parameters.[12] Measurements of total dissolved copper measurements are made using a High Resolution-Inductively Coupled Plasma-Mass Spectrometer (HR-ICP-MS),[13] or a Quadrupole Inductively Coupled Plasma-Mass Spectrometer (q-ICP-MS).

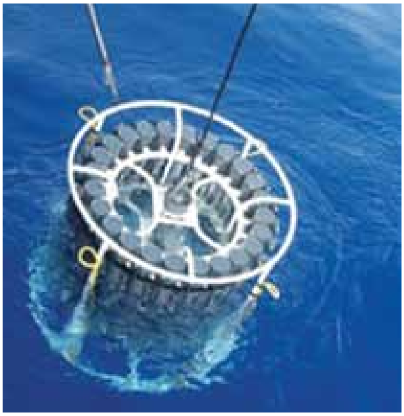

Figure 2. (L) German Research Vessel *Sonne*; (R) Trace metal clean rossette to collect trace metal clean water samples. Photographs: Nico Fröhberg (taken during the SO253 sampling campaign in 2017).

The results from the samples collected during the research expeditions and laboratory experiments will improve current knowledge of physical, biological and chemical processes that influence the concentration and bioavailability of copper and other trace metals in the aquatic environment.

Rebecca Zitoun is a PhD student with Sylvia Sander (Chemistry Department), and Russell Frew (Chemistry Department) and Abigail Smith (Marine Science Department) at the University of Otago.

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EMILY BRAIN

Natural Laboratories

> *Hydrothermal vents are great study sites to investigate metal stress to biota in an un-manipulated 'natural laboratory.' Hydrothermal systems along volcanic island arcs have mostly shallow water depths and strong magmatic input into their fluids and hydrothermal plumes. As these plumes often reach up into the productive photic zone, they discharge large quantities of material into the surface water layers. These systems are thus crucial to improve current knowledge regarding the global elemental budget of micro-nutrients and toxic elements in the ocean, as well as help scientists to clarify chemical and biological processes of metals in a natural multi-stressor environment. Shallow hydrothermal vents also provide a unique opportunity to investigate the impacts of ocean acidification and rising temperatures on metal-cycling and the effects of metals on aquatic organisms. (See Figure 1, p93.)*
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> Rebecca Zitoun

These "natural laboratories" were of particular interest to me. A visit to Rebecca's lab space in Dunedin, complete with lab-coated scientists and chemical smells, highlighted the difficulties of applying the scientific research to real-world scenarios. Nature has ways of reacting and adapting to harmful environments, and these naturally occurring labs give chemists a chance to observe these effects without disturbing native species. Marine organisms typically need a certain level of copper ions in their environment to function, but what happens when that level passes the threshold between healthy and toxic?

An artist's workshop can be a lot like a laboratory. We start with an idea, a theory. Then we combine materials, mix chemicals, run tests, record the results, and then test again until we either get the result we want or find something we could never have predicted. These pieces are both laboratory and experiment, combining copper and silver to create curious hydrothermal environments where biota might struggle or thrive.

Emily Brain is an Australian-born Jewellery and Metalsmithing graduate from the Dunedin School of Art.

Figure 1. Emily Brain, *Where's your lab?*, 2018, silver, copper. Photograph: Pam McKinlay.

Figure 2. Emily Brain, *Where's your lab?*, 2018, silver, copper. Photograph: Pam McKinlay.