An integrated investigation of anthropogenic selenium contamination in Lake Macquarie, NSW

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Abstract: Lake Macquarie, an estuarine lake on Australia's eastern seaboard, has been subject for almost a century to anthropogenic contamination by heavy metals and metalloids, including selenium, arising from industrial sources including a lead-zinc smelter, coal-fired power stations and sewage works. Selenium tissue concentrations in fish from the Lake have recently been reported at up to twelve times those recommended for human consumption. This paper describes an integrated investigation into selenium contamination in the Lake over a four-year period. One key component of these investigations is the ability of indigenous micro-organisms to reduce selenium in estuarine sediments from higher to lower oxidation states, therefore immobilising or volatilising it, and the use of this in the treatment of industrial wastewaters or potentially the in-situ bioremediation of contaminated sediments. A second key component is a study of the mechanisms controlling sediment-stored selenium concentrations and the extent to which they can be influenced by bioturbative organisms. Integrated into these two broad areas and described here are a number of related investigations into: bioavailability of sediment-bound selenium; computer modelling of selenium biogeochemistry; representation of heavy metal data in a GIS format; and determination of the specific historical contributions of a power station to selenium loads in the Lake. Through this integrated approach we have built on synergies between the separate components of our research program to add to our understanding of the nature of selenium biogeochemistry in the Lake and to propose potential bioremediation strategies for both the Lake and contaminated industrial wastewaters or surface water.

Keywords: selenium; Lake Macquarie; bioturbation; bioremediation

INTRODUCTION

Lake Macquarie, N.S.W., is an estuarine lake on the Australian east coast, approximately 85 km north of Sydney (Fig.1). The Lake has been subject to input of heavy metals and metalloids from anthropogenic sources since 1897, when a lead-zinc smelter opened at Boolaroo to the north of the Lake (ref.1). The subsequent operation of three coal-fired power stations, sewage treatment plants, coal mines, washeries and other industries (ref.2) have also contributed to the heavy metal load of the Lake.

Extending approximately 22 km in a north-south direction (Cockle Creek to Chain Valley Bay), Lake Macquarie has a maximum width of about 10 km (Dora Creek to Galgabba Point). The Lake has a maximum depth of approximately 11 m, although the Lake floor is generally flat with an average depth of 8 m (ref.3). Extremely irregular in outline, the Lake has a foreshore of approximately 166 km comprising a number of embayments and promontories and a total area of approximately 125 km², making it the largest estuary in NSW (ref.4). The Lake is linked with the Pacific Ocean via a tidal channel approximately 3.5 km long and 0.2-0.4 km wide at Swansea. Cockle Creek in the north, Dora Creek in the west and Pallamanaba (Wyee) Creek in the south are the main natural fluvial inputs into the Lake (refs.5,6), accounting for almost 70% of the catchment area (ref.6), with other smaller creeks flowing only intermittently. Flow in these main creeks is saline to brackish in their lower reaches except during heavy rainfall periods.
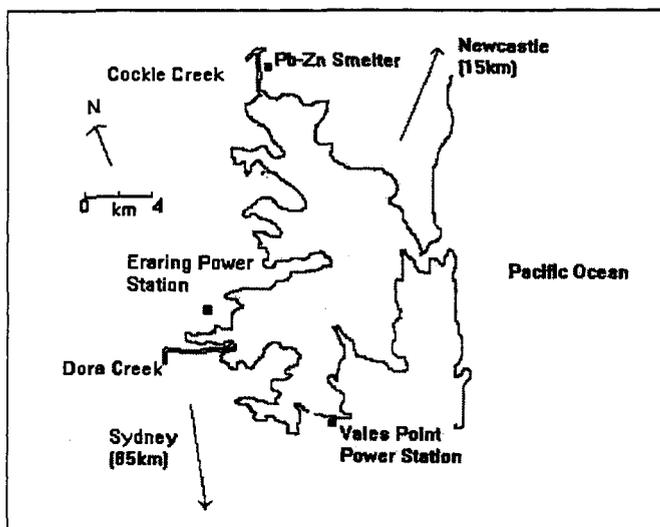
The Lake Macquarie catchment occupies an area of approximately 622 km^2 and consists of the shallow depression filled by the Lake, separated from the ocean by the entrance channel and sand-bars at Swansea and surrounded by low hills on the remaining three sides (ref.6).

Contamination of fish from Lake Macquarie with selenium tissue levels up to twelve times those recommended for human consumption by the Australian National Health and Medical Research Council (NHMRC) was identified in 1994 (ref.7). Bans on commercial and recreational fishing in the Lake have recently been considered (ref.8), with potentially devastating implications for the fishing and tourism industries in the area in the event that they are implemented.

Fig.1. Heavy Metals Sources and Location of Lake Macquarie

Selenium is a metalloid which is similar to sulfur in its chemical properties and, like sulfur, occurs in a number of oxidation states in elemental and combined forms, those typically found in nature being II-, 0, IV+ and VI+ (ref.9). Essential for human and animal nutrition (refs.10,11,12), elevated levels of selenium in either water or an animal's diet can result in acute toxicity, pathological changes in tissue, impaired reproduction (including mortality of embryos and developmental abnormalities of young) and chronic poisoning of adult animals (ref.13). Selenium contamination of aquatic systems has been demonstrated internationally to lead to bioaccumulation of selenium in plants as well as insects, birds, mammals, reptiles and amphibians at levels which may lead to toxicosis of the animals and those which prey on them (refs.13,14,15).

Studies on heavy metal contamination of Lake Macquarie to date (refs.4,16,17,18,19) have focused mainly on metal contaminants other than selenium, with only limited data (refs.3,16) previously available on selenium in the aquatic ecosystems of Lake Macquarie. Based on documented experiences internationally of the environmental and ecological consequences of elevated selenium levels, potential risks are posed to the ecosystem of Lake Macquarie by elevated levels of selenium, as well as to the terrestrial animals and birds which may feed upon aquatic organisms. Potential health risks are also posed to humans in eating selenium-contaminated fish and shellfish from the Lake (refs.7,17). Considering this, the large population living in Lake Macquarie Shire and the importance of the Lake for both commercial and recreational fishing (ref.6), a research program to address the issue of selenium contamination of Lake Macquarie was established at the Department of Chemical Engineering, University of Sydney in 1992 as discussed in this paper. The aim of this program was to study and add to the understanding of the complex biogeochemistry of selenium in Lake Macquarie and to investigate potential management and remediation strategies for both the Lake and other selenium-impacted sites worldwide, as well as identify strategies for treatment of selenium-contaminated waste streams before they enter the environment.

OVERVIEW OF THE INVESTIGATION

The biogeochemical cycling of selenium in an estuarine such as Lake Macquarie is complex, and is controlled by factors including Eh and pH, ligand complexing ability, solubility of selenium-containing minerals, sedimentation, sediment characteristics, microbially mediated reactions and physical reworking of sediments by biota. A conceptual model of Se cycling in wetlands systems has been proposed (ref.20) and has been adapted to the specific estuarine environment of Lake Macquarie (Fig.2).

The integrated investigation into selenium contamination in Lake Macquarie reported here is focused specifically on sediments and the sediment-water interface. The sediments in Lake Macquarie are important

in the cycling of selenium as they act as both a source and a sink for selenium: the Se-contaminated fish previously documented (ref.7) were species which foraged in the bottom muds of the Lake, into which selenium would have deposited from airborne and fluvial sources. The concentration of selenium in contaminated sediments of the Lake is typically much higher than in the overlying water column.

The two broad components of this investigation were, firstly, to understand and quantify the role of micro-organisms in both the sediment and water column in transforming selenium between oxidation states; and
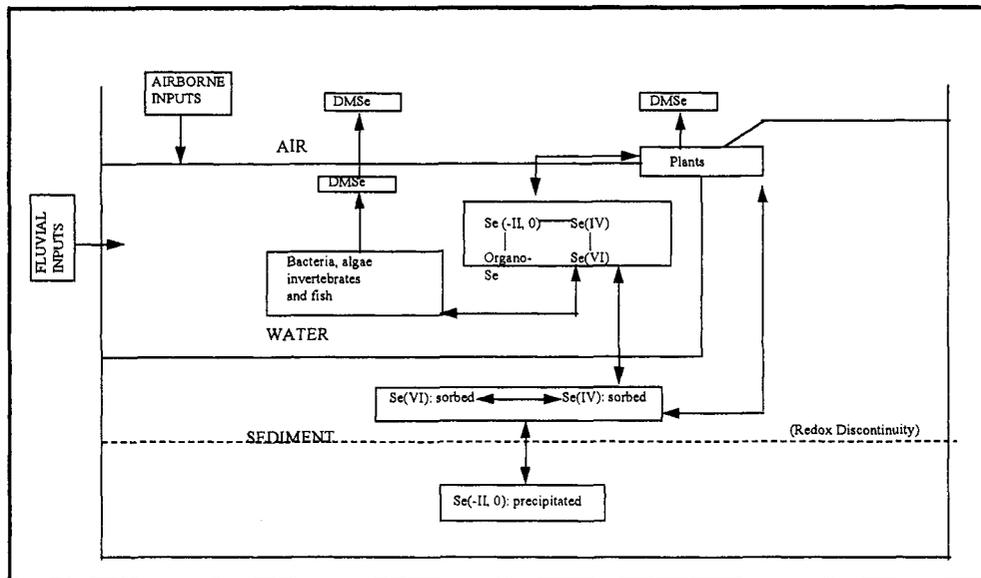

Fig.2 . Conceptual Model of Biogeochemical Cycling of Selenium in Lake Macquarie (ref.20)

secondly, to understand the mechanisms controlling sediment-stored selenium concentrations and to determine the specific impact of bioturbative organisms on selenium mobility.

Associated with these were a number of smaller projects investigating in detail specific component of these broad areas. These were the determination of the bioavailability of sediment-bound selenium in the Lake; computer modelling of selenium biogeochemistry in the Lake; presentating data concerning selenium and heavy metal concentrations in the environmental compartments of the Lake in a Geographical Information System (GIS) format; and the determination of the contribution of a specific power station ash dam to selenium loads in the Lake.

MICROBIAL TRANSFORMATIONS OF SELENIUM IN LAKE MACQUARIE

Microbially mediated transformations of selenium between its four main naturally occurring oxidation states have been extensively documented (refs.21,22,23,24,25), with the bioremediation of selenium-contaminated sediments and soils in the Kesterson Reservoir, USA, using indigenous micro-organisms having recently been shown to be a potentially effective remediation strategy (ref.26) for such sediments. In this instance, micro-organisms converted selenium present in the contaminated sediments from the VI+ or IV+ oxidation states into volatile selenium (Se(II-)), which is then dispersed into the atmosphere where it is diluted to extremely low and non-toxic concentrations.

Selenium reduction by ash-dam isolates

Initial investigations into the role that micro-organisms play in the biogeochemical cycling of selenium in Lake Macquarie were concentrated on power station ash dams around the Lake, areas where the spent ash slurries from the operation of the power stations are impounded. Selenium is a trace element typically found in coals due to its analogous association with and ability to substitute for sulfur. Six bacterial species were identified
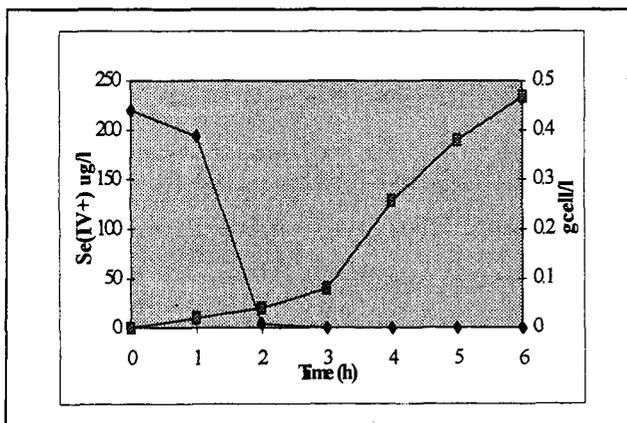
Fig.3. Selenium Reduction by *Shewanella putrefaciens* (adapted from Riadi, Barford and Linkson, 1994)

in water and sediment samples from the ash dams which could reduce selenium from selenite (Se(IV+)) to elemental (Se(0)) selenium (ref.27). Of these, one organism, *Shewanella putrefaciens*, was found (ref.29) to have a specific uptake rate (Q_{Se}) for selenium of 2474 gSe $(gcell)^{-1}h^{-1}$ and effected a 100% reduction of added selenium (approximately $200gl^{-1}$) in just over 3 hours (Fig. 3). This organism was subsequently used in a bioreactor where cells of the organism were immobilised onto a calcium alginate carrier and then packed into a continuous flow immobilised cell reactor (ref.27). At an initial selenium concentration of approximately 30 mgl^{-1}(ppm) in the liquid stream pumped through the reactor, selenium in the outflowing liquid was found to be approximately 3 mgl^{-1}(ppm), a reduction of approximately 90%.

Subsequently, sediment and water samples were collected in 1994 from two sites in Lake Macquarie. Surficial sediment samples were serially diluted in 0.9% saline diluent to a dilution of 1:1000. 200 l aliquots of the diluted samples were transferred to plastic petri dishes containing nutrient agar (CM3, Oxoid, Melbourne) and $100mgl^{-1}$ of either selenite (Se(IV+)), added as Na_2SeO_3 (GPR Grade, BDH Chemicals, VIC), or selenate (Se(VI+)), added as Na_2SeO_4 (AnalaR, BDH, VIC). Cultures were incubated at 28 C for up to 5 days, with individual, distinct colonies picked and plated onto media containing either selenate or selenite, as appropriate. This was then repeated on two subsequent occasions, with seven distinct organisms isolated. These bacterial isolates were identified by the NSW Department of Agriculture using gas chromatography analysis of the fatty acid composition of the organisms and calculation of similarity indices, comparing these against a database of known bacterial compositions. Two organisms highly matched were identified as *Bacillus brevis* and *Bacillus sphaericus* and four organisms were less highly matched and were tentatively identified as *Bacillus mycoides, Shewanella putrefaciens, Cellulomonas biazotea or Bacillus* sp. and *Pseuodomonas* sp. One isolate (30-8-5-A) recorded no match against any library organisms and may therefore be indicative of a newly discovered organism.
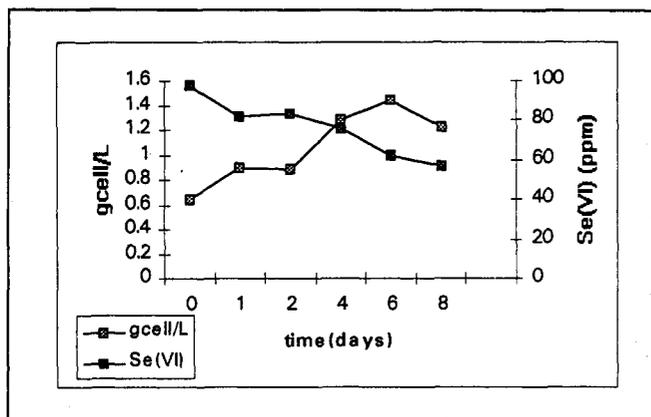

Fig.4 Selenate reduction by *Bacillus brevis* (ref.30)

Quantitative growth of the organisms on Se(IV+) and Se(VI+) media was studied (ref.30) by growing the organisms in nutrient broth (CM1, Oxoid, Melbourne) under microaerophilic (oxygen-limited) conditions in a reciprocating water bath at 80 o.p.m. Se(IV+) was added aseptically to the broth to produce levels of 1, 10 and $100mgl^{-1}$ for one set of studies. In studies of Se(VI+), only a level of $100mgl^{-1}$ was used. Controls contained organisms and broth but no selenium. Organisms were incubated over a period of up to 10 days and were sampled at least daily for cell dry weight determination (by filtration through a 0.45 μm membrane filter and subsequent

drying of the membrane filters) and analysis of selenium levels and speciation in the filtered broth. The method (ref.31) was used to convert Se in samples to the IV+ oxidation state, and hydride generation atomic absorption spectroscopy using a SpectrAA 20 system (Varian, Melbourne) with a VGA-76 vapour generation accessory was used to determine selenium levels and species in the samples (ref.32).

All seven isolates were shown in the initial isolation studies to be able to transform Se(IV+) at levels of up to 100 mgl^{-1} to elemental Se, as evidenced by a red precipitate formed in the media and identified as such in previous studies by X-ray diffraction (Model 1130, Philips, The Netherlands). Additionally, all seven isolates grew on media containing up to 100 mgl^{-1} Se(VI+), although no precipitate was evidenced in these instances.

All isolates were found to effect a 100% reduction of Se(IV+) to elemental Se in the qualitative studies at 1, 10 and 100 mgl^{-1}. *S. putrefaciens, B. brevis* and *C. biazotea* all reduced 100% of added Se(IV+) to elemental Se within 90 hours. Specific rates of selenite reduction (Q_{Se}) were found to be 3040, 1680 and 1834 gSe(IV+).(gcells)$^{-1}$(h)$^{-1}$respectively.

Utilisation of Se(VI+) by six isolates (*Bacillus sp.* failed to grow) at the 100 mgl^{-1} level confirmed initial quantitative analyses that no red precipitate, indicative of elemental selenium, was formed. Mass balances on total Se, Se(IV+), Se(VI+) and Se (0+II-) fractions showed that for four of the isolates, total Se was conserved, Se(VI+) decreased and Se(0+II-) increased, indicative of the production of non-volatile organo-selenium compounds by the organisms. For the remaining two organisms (*B. brevis* and 30-8-5-A), total selenium and Se(VI+) decreased with no concomitant increase in Se(0+II-) levels, and it was hypothesised that these organisms were methylating selenium to volatile, organic forms which escaped from the system.

Minimum inhibitory concentrations and optimisation of carbon-source utilisation

To investigate the suitability of selenium-reducing indigenous micro-organisms in industrial applications, the minimum inhibitory concentrations (MIC) of selenate and selenite, that is the concentrations at which no microbial growth occurs, were determined according to the method of Brock and Madigan (ref.33). Mixed bacterial cultures isolated from lake sediments with the ability to tolerate selenium levels of 10 mgl-1 as selenate or selenite were used in these studies. Using nutrient broth as a growth media, isolates were introduced to flasks containing the media and selenium as either selenate or selenite at 1, 10, 100, 500 and 1000 mgl^{-1}. The MIC for selenite was found to be 50 mgl^{-1}, while for selenate, growth of all six mixed cultures studied was maintained at a concentration of 1000 mgl^{-1}. Given that the maximum sediment selenium concentrations reported for Lake Macquarie are 14 mgkg^{-1} these results indicate that the mixed cultures of micro-organisms isolated from the Lake may be suitable for consideration as a potential remediation option for highly contaminated lake sediments, as well as for the treatment of aqueous industrial waste streams containing either selenate or selenite below these MIC levels.

The selenium reduction studies described previously were conducted using nutrient broth agar as the source of metabolites and carbon required for microbial growth. However, the use of such media would be prohibitively expensive in large-scale industrial applications. Hence the basal salts medium of Maiers *et al.* (ref.23), containing essential mineral salts as well as a carbon source, was utilised as a growth medium to determine the suitability as carbon sources for large-scale bioreactors of a number of inexpensive carbon sources commonly available on an industrial scale (namely ethanol, acetate, lactate and casein hydrolysate). Casein hydrolysate produced the strongest growth in the cultures studied, and a basal salts medium containing 2g.l^{-1} casein hydrolysate is currently being studied with the mixed cultures and with added selenium in another series of experiments to determine its suitability for large-scale bioreactors.

MECHANISMS CONTROLLING SEDIMENT POREWATER SELENIUM CONCENTRATIONS

An additional suite of experiments have been performed in this Department to examine whether contaminants in the sediments of Lake Macquarie and similarly impacted ecosystems, whose bioavailability is strongly suppressed by certain environmental factors, may become bioavailable under certain conditions (ref.34). Dredging, global warming, chemical spills, changes to estuarine hydrology via the building of power stations and breakwaters can all influence sediment chemistry, and these effects can be complicated by the sediment ecosystem's response to the changes. Bioturbative processes in sediments may be enhanced or diminished by a range of these and other influences such as the natural variation in animal populations, and in turn may affect the mobility of sediment contaminants. Bioturbation is a natural process encompassing the feeding, burrowing, breathing and other activities of certain small organisms, and include two main sub-processes which concern

the environmental chemist: (1) Fluid bioturbation, being the enhanced exchange of water between the sediment and the overlying water; and (2) Particulate bioturbation, being the mixing of the sediment profile.

Profiling of pH and redox potential due to bioturbation

Sediments collected from a relatively pristine area of Lake Macquarie (Nord's Wharf) were screened through a 2mm mesh to remove large inhomogeneities, mixed and placed in 1.4 l containers. To allow the normal chemical profiles associated with early diagenesis to become established before the addition of animals (ref.25), these were kept for 12 days in oxygenated marine aquaria at about 20°C. The eunicid polychaete *Marphysa sanguinea* was obtained from commercial suppliers and the bivalvian molluscs *Notospisula trigonella*, *Sanguinolaria donacoides* and *Tellina deltoidalis* were obtained from local sediments and added at densities of 66, 660, 133 and 200 m^{-2}. respectively. An animal-free blank was kept.

Despite difficulties encountered with redox determinations (refs.36,37), the most serious of these relating to the lack of equilibrium of and between different redox couples in a natural system (ref.38) (the result of which is to preclude the use of measured E_h values with precision), redox potential is still widely used as a simple and rapid technique for examining the oxidation state of water and sediment (ref.20) and was utilised in the studies described here.

Microelectrodes capable of determining pH and redox potential were inserted in sediment cores using a micromanipulator constructed within the Department, with an Orion 611 meter was used to measure the electrode potential of the platinum electrode compared with a 90-02 double-junction Ag/AgCl reference electrode supplied by Orion. The pH determinations were performed using a Diamond General 757 electrode coupled to a Hanna HI8417 meter. The micro-size pH bulb is hidden in the bevelled tip of a 1.25 mm diameter needle, shielding it from damage which might otherwise result from insertion into sediments.
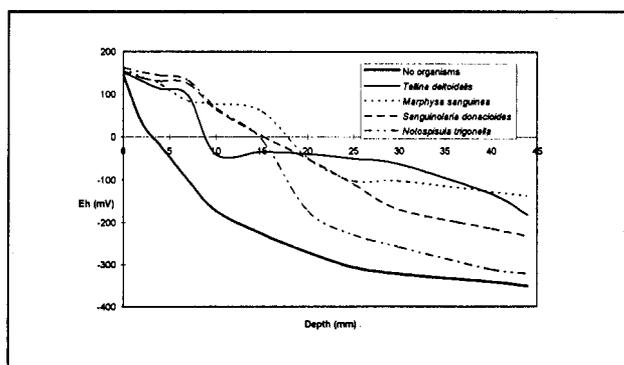

Fig.5 Sediment Redoc Potential as a Result of Bioturbation (ref.34)

Measurements of redox profiles revealing bioturbative disturbance and the deepening of the oxidised zone (here, with $E_h > 0$ mV) by approximately a centimetre is shown in Fig.5. Below this the redox potential measured is higher than in the fauna-free profiles. The presence of *M. sanguinea* and *T. deltoidalis* has raised the redox potential by 220 mV to -130 mV at 4 cm into the sediment. The burrows of *M. sanguinea*, which burrows extensively and deeply in its search for food, constitute enhanced pathways for the molecular diffusion of oxidised forms of elements into the sediment. On examination of the sediment core it was found that the first few millimetres of sediment immediately adjacent to the burrows was lighter in colour - an indication of the oxidation of the sulfur and iron components of the sediment. The burrows extended from the surface down to the bottom of the experimental vessels, a distance of 17 cm. The mechanisms by which *T. deltoidalis* deepens the oxidised layer relate to its preferred position 1 to 5 cm under the surface. From this position it is safer from epifaunal or pelagic predators. The tunnels it clears between its shell and the surface allow it to draw on the surface water and return it, but this feeding mechanism would also increase the exchange of water between the sediment and the overlying estuary by means of increased surface area and pumping. *T. deltoidalis* appeared to move little after initially burrowing into the sediment. The influence of *N. trigonella* is shallower, reflecting its relatively surficial habitat (0-3 cm into the sediment) and filter-feeding characteristics. Depending on the feeding mechanisms and mobility of the organisms, bioturbation causes a degree of deepening of the oxidised conditions in the sediment, impacting upon the speciation and therefore mobility of selenium and other sediment contaminants.

Contaminant remobilisation from sediment

Contaminant remobilisation from a selenium-spiked plug of sediment from Lake Macquarie was studied, with the spiked plug produced by incubating sieved sediment (<63 m fraction) in a reducing environment in the presence of selenite for 10 days, with reduction occurring by natural consumption of oxygen by sediment micro-organisms, enhanced by nitrogen sparging. Sequential extraction was performed according to the method of Wallmann *et al.* (ref.38). The sediment thus produced had 85-95% of the selenium present bound in Tessier's "organic fraction" (ref.39) and the remainder in the iron-manganese oxyhydroxide fraction.

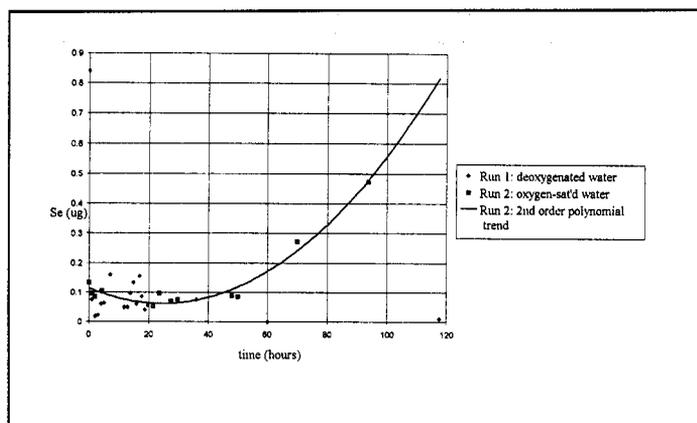The effect of exposure of a sediment plug to oxygenated porewater on the eluant selenium concentration was examined by first exposing the plug to deoxygenated seawater for five days, after which total selenium concentration was determined. The same plug was then exposed for five days to seawater saturated with oxygen As can be seen in Fig.6, after an initial peak of 120 mgl^{-1}, the porewater concentration dropped to 10 mgl^{-1} for the deoxygenated experiment. The initial peak can be explained in terms of the effluent selenium oxyanions from sediment at the bottom of the plug desorbing oxygen from the container. After 36 hours of exposure to oxygenated water, the eluant selenium concentration changed from being relatively steady at a low level (<5 mgl^{-1}) to a rising trend, reaching a maximum of 60 mgl^{-1} before the end of the experiment. Thus, when sediment with immobilised selenium contamination is exposed to oxygenated porewater, enhanced bioavailability of the contaminant can result. It was determined that the loss of selenium from the sediment had reduced the concentration of selenium in both Tessier's "oxide" and "organic" fractions. When considered in view of the deepening of the oxidised conditions in the sediment due to bioturbation previously demonstrated, this experiment predicts that exposure of contaminated reduced sediment to oxidising porewater arising from bioturbation resulted in mobilisation of selenium from the strongly binding "organic fraction" of the sediment.

Fig.6 Eluted selenium from a sediment plug (ref.34)

Thermodynamic predictions

The likely significance of the deepening of the redox profiles as shown in Fig.5 was investigated using StabCal, October 1995 revision, a comprehensive thermodynamic modelling package developed by Hsin-Husiung Huang of Montana Tech. Although the package allows only equilibrium calculations without the inclusion of kinetics, it nonetheless generates data useful for understanding the trends which can be expected in the long term due to changes in redox conditions. Simulations of equilibrium chemistry were performed for different concentrations of selenium in seawater at different pHs and temperatures. Figure 7a shows a typical result for 20 μgg^{-1} selenium at pH 7.5 and 25C. Simulation temperatures ranged from 5 to 25 degrees, and were found to be relatively unimportant in controlling selenium behaviour. Inspection of the data supporting Fig.7a shows that approximately 100% of soluble selenium is in the II- oxidation state below an E_h of 0 mV. This is significant because of the diminished mobility of selenium in the selenide form due to precipitation reactions. Our simulations predict low concentrations of soluble selenium between 0.12 and -0.12 mV due to the insolubility of elemental selenium (Fig.7b). Below this range the dominant oxidation state becomes II- but these ions are rapidly taken up by cations like Zn^{2+} (in preference to Fe^{3+} at the given concentrations) and insolubly bound into the sediment. At higher redox potentials, soluble selenite becomes the dominant selenium species present and the solubility exceeds the concentration of aqueous selenium (indicated by the blank square in the top right-hand corner of Fig.7b). At pH 7.5, selenous (Se(IV)) ions predominate at 12 μgg^{-1} with hydrogen selenite at 7.6 μgg^{-1}. Above 0.5 V, selenate replaces selenite as the dominant form. Since the $pK_{a,1}$
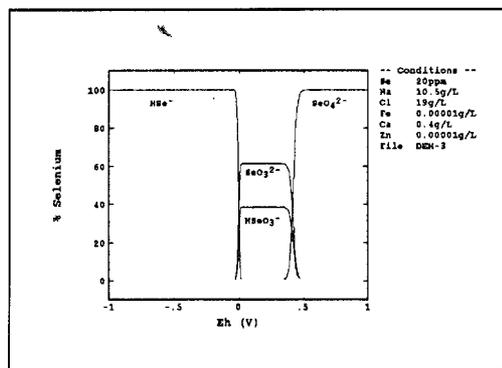
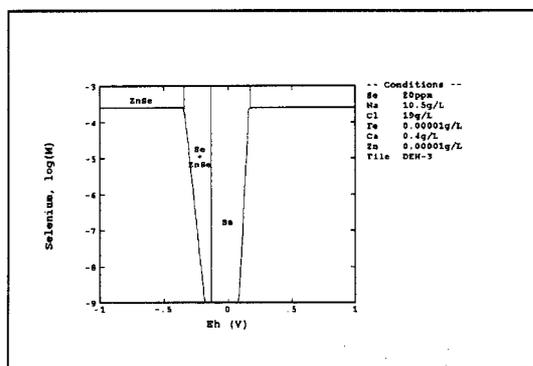Fig.7a Aqueous speciation of selenium at pH 7.5

Fig. 7b. Solubility of selenium in seawater at pH 7.5

value of selenous acid is 2.62 and $pK_{a,1}$ for selenic acid is less than zero, fully protonated selenium oxyanions are a relatively minor part of the total soluble selenium.

Figures 7a and 7b show that changes caused by processes such as bioturbation and dredging could result in remobilisation of selenium from contaminated estuarine sediments. Bioavailable oxyanions would be remobilised in selenium-contaminated sediments such as those found in Lake Macquarie if they were exposed to oxidising porewater. If mass transfer were not limited by chemical means (eg ref.40) this selenium could enter the water column.

OTHER ASPECTS OF SELENIUM BIOGEOCHEMISTRY IN LAKE MACQUARIE

Bioavailability of selenium in lake macquarie sediments

Total concentrations of heavy metals and metalloids do not provide a true indication of the risks posed to humans and the environment by heavy metal contaminants in soils and sediments as they include chemically inert forms or forms which are unable to be assimilated by biota (ref.17). Sequential extraction of sediment fractions to which metals and metalloids bind is a methodology which has been shown to be useful (refs.17,41) for determining the proportion of the total contaminant load available for uptake by organisms.

Two sequential extraction procedures, a modified Tessier extraction (refs.41,42) and that of the European Community Bureau of Reference (BCR) (ref.43), were recently applied to selenium in Lake Macquarie sediments (ref.44) to characterise selenium distribution within the different fractions of Lake Macquarie sediment. Total selenium concentrations from five sites within the Lake (including the vicinity of the power station at Vales Point and in Cockle Bay into which the lead-zinc smelter discharges via Cockle Creek) were then calculated as the sum of the selenium concentrations found within the various sediment fractions.

In this study, selenium was found by both the modified Tessier and BCR extractions to be predominantly bound to organic matter. For the modified Tessier procedure, and depending upon both inter- and intra-site variations, selenium bound to organic matter, expressed as a percentage of total selenium recovered, ranged from 70-100% in the top 8 cm of sediment samples. Selenium present in the exchangeable fraction varied from 0-28%, and from 0-18% in the carbonate-bound fraction (Fig. 8a). No selenium was recovered from the Fe-Mn oxides fraction using the Tessier procedure. Residual selenium concentrations were not determined, as it had previously been demonstrated (refs.45,46) that selenium concentrations in the residual fraction of a Tessier extraction scheme were insignificant in a study of selenium fractionation in sediments from a salt marsh in the USA. Total selenium concentrations, calculated as the sum of selenium obtained from the four fractions, were up to 1.94 +/- 0.14 μgg^{-1} off Bennet Park in the north-east of the Lake. Values of 1.75 +/- 0.03μgg^{-1} and 1.75 +/- 0.08 μgg^{-1} were recorded for samples from Cockle Bay and Vales Point (in the vicinity of the power station) respectively.

The original Tessier method has been criticised (ref.43) for readsorption during extraction, and the dependence of concentrations recovered on solid to solution ration has been noted (ref.47). While attempts have been made

to address some of these issues within the context of the Tessier extraction procedure, the BCR method (ref.43) represents a recent attempt to standardise extraction procedures to facilitate comparison of data, as well as to reduce the impact of readsorption and the effect of different solution to solid ratios. This method identifies four fractions instead of the five identified by the Tessier method: Exchangeable and Carbonatic; Fe-Mn Oxides; Organic Matter; and a Residual Phase. Here, the separate Exchangeable and Carbonatic fractions of the Tessier scheme are combined into a single, composite fraction.

Using the BCR method, selenium in oxic surficial Lake Macquarie sediments was found (ref.44) to be almost exclusively contained in the organic-fraction in the samples from Cockle Bay. The residual phase is sometimes left out of sequential extractions due to safety concerns and the limited environmental significance of contaminants requiring hydrofluoric acid exposure to become bioavailable. At Vales Point, 65.1% of Se recovered was associated with the organic matter fraction, with 26.1% associated with the exchangeable and carbonate fractions and 8.8% with the Fe-Mn oxide fraction (Fig.8a). Total selenium concentrations obtained from addition of selenium in the BCR fractions, again with the exception of the residual, varied from 0.46 +/- 0.02μgg^{-1} at Bennet Park to 2.07 +/- 0.04 μgg^{-1} in Cockle Bay.
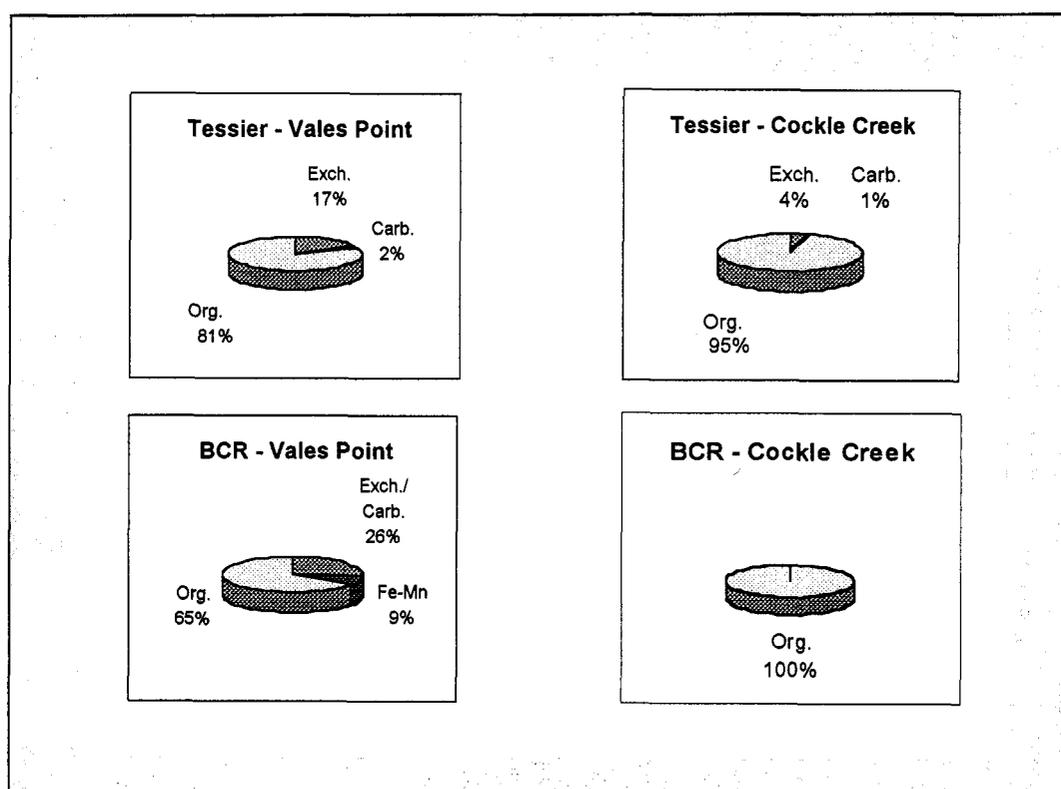

Fig. 8a. Selenium distribution between geochemical phases in lake macquarie sediments by Tessier and BCR methods.

(ref.44)

The overall findings that selenium in the sediments of Lake Macquarie was predominantly present in the organic fraction were supported by additional research carried out in our laboratories. This was based on a modified Tessier extraction performed on sediment obtained from Cockle Bay, conducted under a nitrogen atmosphere, and using deoxygenated and oxygen-saturated water regimes. In all instances, approximately 80-100% of selenium was found to be bound to organic matter in the sediment. Through the use of a flow-through sediment cell, charged with sediment from Cockle Bay, changes in selenium mobility in Lake Macquarie were examined under different redox potentials. It was concluded that the mobility of selenium in Lake Macquarie is favoured by oxidising conditions (where selenium typically exists as soluble and mobile selenite and selenate oxyanions) and is hindered when exposed to reducing conditions (where selenium exists as insoluble selenides) (Fig.8b).

While the majority of sediment-bound selenium was found by these studies and those reported (ref.44) to be bound to organic matter and therefore unavailable directly to aquatic organisms, up to 26% of selenium was bound in bioavailable forms. Additionally, ingestion of sediment particles by bottom-feeding organisms may also expose the selenium bound to the sediment to the harsh conditions inside the organism's gut, where it may be transformed to more available forms. While knowledge of the bioavailable fraction of selenium and heavy metals in sediments is important for regulatory bodies in determining acceptable limits of contaminants, changes in external conditions or operations such as dredging can impact on and increase the availability of heavy metals and metalloids to aquatic organisms.
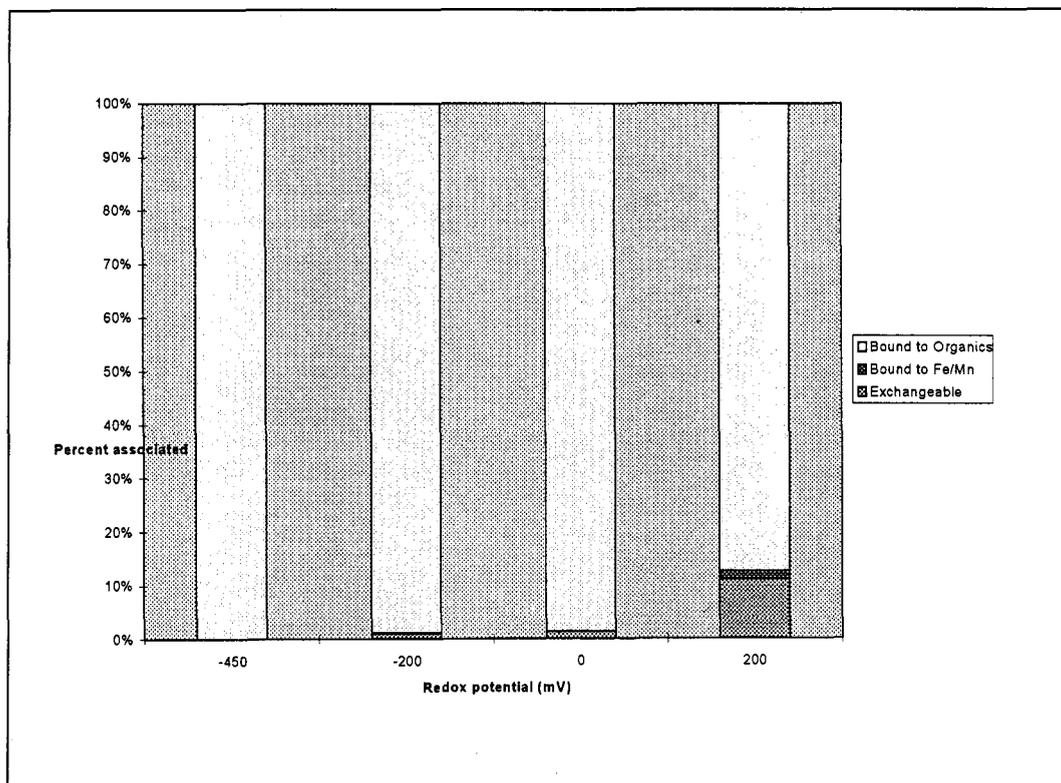
Modelling of selenium biogeochemistry

Fig. 8b. Relative fractionation of selenium in sediment under varying Eh conditions

Thermodynamic approaches to determining the speciation of selenium in environmental samples do not typically consider transformations of selenium by micro-organisms, which lead to kinetically stabilised species instead of thermodynamically predicted species (ref.20). Attempts to model selenium biogeochemistry therefore need to be cognisant of these limitations and alternative approaches are required. Using a conceptual model of Lake Macquarie (Fig.2) the Lake can be divided into four separate compartments: the water column; sediment; plants; and a compartment containing fish, algae, micro-organisms and invertebrates. For the sake of simplicity in modelling the Lake, only the water column and sediment components of the model have been considered to date.

SPEEDUP, a flowsheeting package commonly used in the chemical industry to model chemical processes (ref.48), has been used in an attempt to model selenium cycling in Lake Macquarie. Here, the Lake has been modelled with the assumption that it comprises two Continuously Stirred Tank Reactors (CSTR), one (termed Solid) representing the sediment and the other (called Liquid) the water column (Fig.9). Each CSTR is fed six different Se species: Se^{VI+}, Se^{IV+}, Se^{II-}, Se^0, Se^{Vol} and Se^{Org}, representing the four oxidation states of Se, volatile Se and organic Se, respectively. A mixer unit receives combined fluvial and atmospheric inputs into the Lake, and recycled, resuspended or remobilised selenium from the sediment column CSTR. The mixer outputs to
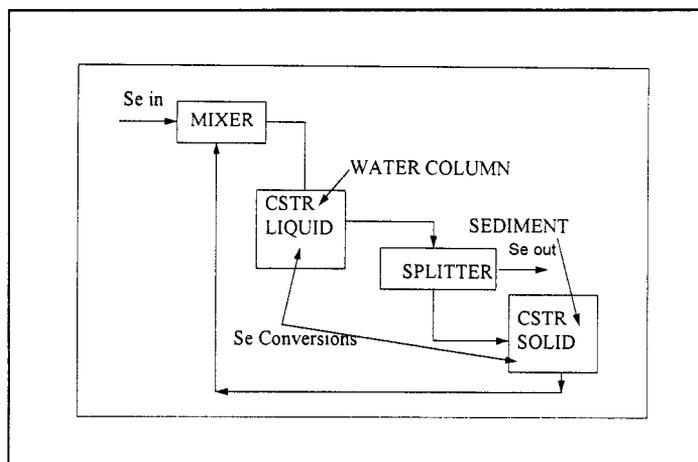
Fig. 9. Computer model of selenium cycling in Lake Macquarie

the first CSTR (water column), which in turn outputs to a splitter unit, where selenium either leaves as volatile selenium or deposits in the sediment (the second CSTR).

Liquid CSTR results from the model show the level of organic Se rising, simulating bioaccumulation within the Lake. Volatilisation from the splitter results in loss of Se from the system. In the Solid CSTR, concentrations of all species except for Se^{II-} fall, which is indicative of conversion of selenium to the selenide species in this CSTR.

While only a first step in the development of a comprehensive mathematical model of selenium biogeochemistry in Lake Macquarie, this modular approach to the modelling of chemical processes in the environment using SPEEDUP represents a powerful yet flexible means of predicting concentrations of species in the environment. The package readily allows for the inclusion of additional modules to represent, for example, plants and biota, as data concerning the rates of selenium transformations within these compartments and their interactions with existing compartments become available. An extensive database of temporally discrete selenium concentrations in sediment, water, plants and organisms in the Lake has been collected and will be used as input to the model.

To better handle changes in Eh, pH and dissolved oxygen concentrations in the Lake and to assess their impact on selenium cycling and speciation, future research will also seek to incorporate the USEPA thermodynamic modelling package MINTEQ (ref.49) or similar into the SPEEDUP model.

Selenium concentrations in a creek subject to ash dam overflows

Of three coal-fired power stations established on the perimeter of Lake Macquarie, two - at Vales point in the far south and Eraring in the south-west of the Lake - are still operational today. Selenium contributions to the Lake in aqueous discharges from these power stations have recently been quantified (ref.8) as $400kg.y^{-1}$. The Vales Point power station stores combustion fly ash in a former wetlands area, Mannering Lake. Overflows from the ash dam would, until recently, routinely flow from the ash dam into Wyee Creek, one of the main fluvial inputs into Lake Macquarie, with the distance from where the discharge from the ash dams joins the creek to the creek mouth into Lake Macquarie being approximately 7 kms. A closed circuit flyash pumping system has recently been introduced at the Vales Point power station, with the result that discharges from the ash dam will now only occur during heavy rain or when the pumping circuit is off-line due to maintenance or breakdown. Nonetheless, in addition to reports of selenium contamination of fish from Lake Macquarie proper (ref.7), fish from Wyee Creek and Mannering Bay, both in the vicinity of the Vales Point power station, have also recently been found to have from five to fourteen times the NHMRC guideline concentration of selenium (ref.50).

To investigate the historical contribution the Vales Point ash dam has made to selenium concentrations in Wyee Creek, and to determine whether this selenium is entering the water column of Lake Macquarie, ten sites at approximately 700 m intervals along the length of Wyee Creek were studied. Duplicate sediment core samples of up to 0.45 m depth were collected at each site and returned to the laboratory on ice in the upright position to ensure that structural integrity of the core was maintained. Frozen until required, cores were thawed and sectioned into 5 cm intervals under a nitrogen atmosphere to minimise speciation changes. Sections were then wet-sieved to obtain the gochemically significant <63m fraction for analysis. For selenium analysis, this fraction was dried at 60°C for 24 hours, then digested at 80°C in concentrated HNO_3 and H_2SO_4 at a ratio of 9:4 for 1 hour. The digest was then filtered through an acid-resistant 0.45 μm membrane filter and split into three

subsamples for analysis of selenium speciation. Conversion of selenium to the (IV+) oxidation state required for analysis and hydride generation atomic absorption spectroscopy using a SpectrAA 20 system were performed as previously described.

Preliminary results from these analyses indicate that in some locations in Wyee Creek, selenium concentrations in the sediments are up to two orders of magnitude greater than those reported in the literature (ref.17) for lake sediment, with potential implications for the health of the attendant ecosystem and humans who eat fish and shellfish from the creek.

Gis representation of Lake Macquarie heavy metals status

Data on the heavy metal concentrations in sediments, water column, pore-waters and biota in Lake Macquarie are widely scattered throughout journal articles, environmental reports, environmental impact statements, conference proceedings and undergraduate and postgraduate theses, many of which are difficult to obtain. In an attempt to produce a centralised and "user-friendly" source of this data, information relating to the heavy metal status of the Lake, as well as peripheral data such as tonnages of coal used in the power stations, has been collated. A map of Lake Macquarie was digitised into the GIS program MAPINFO (MapInfo Corporation, USA), and heavy metal and selenium data on both spatial and temporal bases was used as the data source for the (GIS) representation of the Lake. The map in MAPINFO allows the user "point-and-click" access to data on heavy metals in the Lake, associating clickable points on the map with available data on heavy metal concentrations in environmental compartments and the temporal variations in these for the corresponding region of the Lake. While much information has been obtained, considerable data remain to be collected and fed into the source files for the MAPINFO map. It is envisaged that this GIS representation of Lake Macquarie will provide a useful resource for both researchers and policy-makers in assessing what is known and remains to be determined regarding selenium and other heavy metal concentrations in the Lake.

CONCLUSIONS

Elevated levels of heavy metals, including the metalloid selenium, in the sediments, water column and biota of Lake Macquarie are directly attributable to anthropogenic industrial discharges occurring over the past century (ref.17). Remobilisation of metals to the overlying waters may occur from deeper sediments contaminated with heavy metals in equilibrium with the pore-waters. It has been shown in these investigations that bioturbation causes a degree of deepening of the oxidised conditions in the sediment. Simulations of the solubility of selenium in estuarine porewaters described here predict a mobilisation of this anthropogenic contaminant under these conditions. Preliminary experiments involving exposure of contaminated reduced sediment to oxidising porewater resulted in such a mobilisation from the strongly binding "organic fraction" of the sediment. It is anticipated that additional work under way in this area to determine the speciation of the mobilised selenium and the magnitude of losses from contaminated sediments due to natural oxidative processes will lead to a better understanding of the management issues associated with selenium contaminated sediments.

Remediation of contaminated sediments, especially those in the worst sections where contaminated sediments extend to depths of 50 cm over an area of 3 km^2, may need to be considered as a short-term management tool for the Lake to address the issue of already contaminated sediments. Reducing selenium inputs to the Lake will help address future contamination, with one potential option identified from this research program being the use of micro-organisms to reduce selenium from the selenate and selenite forms to either elemental selenium in the sediment or volatile forms. In addition, *in-situ* bioremediation of selenium-contaminated sediments and soils in the Kesterson Reservoir, San Joaquin Valley, CA, USA using indigenous micro-organisms has been shown to be a potentially effective remediation strategy (ref.26) and may prove a suitable longer term solution for highly contaminated parts of the Lake.

The excessive levels of selenium found in Wyee Creek, into which discharges from the Vales Point ash dam are directed, raises serious concerns about the contamination of biota in the region. Fish from Wyee Creek and Mannering Bay, both areas in the vicinity of the Vales Point power station and into which Wyee Creek discharges, have been found to have from five to fourteen times the NHMRC guideline concentration of selenium (ref.50). While it is impossible to make a firm conclusion based on the available data as to the origin

of selenium contamination in these fish, as details of their lifestyles and movements within the Lake are poorly known, it is a plausible hypothesis to suggest that selenium contamination of fish from the vicinity of the Vales Point power station is due to selenium contamination of sediments by a combination of fly-ash deposition and overflows from ash-dams, and that these bottom-feeding fishes ingest contaminated sediment while foraging in the bottom sediments for food. Potentially contaminated fish caught from Wyee Creek may therefore pose a potential health risk to humans and other animals.

Through these investigations, and the coupled investigations into broader areas of selenium biogeochemistry in Lake Macquarie described here, including computer modelling of selenium biogeochemistry and representation of heavy metal data in a GIS format, we have built on synergies between the separate components of our research to add to our understanding of the nature of selenium biogeochemistry in the Lake, to propose potential bioremediation strategies for both the Lake and contaminated industrial wastewaters, and to identify areas of specific concern for further investigation.

In conclusion, whilst elevated levels of heavy metals in Lake Macquarie, especially in the north, are of concern due to their potential environmental impact, selenium contamination of the sediments and biota in the Lake is already an issue, with consumption of fish from Lake Macquarie posing a potential health risk to humans. While no data are currently available on the impact of selenium on birds and other wildlife that feed in the Lake, the ecological devastation wreaked by selenium teratogenicity on the avian population of Kesterson Reservoir (ref.14) serves as a stark reminder of the impact of uncontrolled anthropogenic redistribution of selenium, and of the need for integrated research programs such as the one described here to understand the problem and work towards a solution.

ACKNOWLEDGMENTS

REFERENCES

1. SPCC (1993). Environmental Audit of Lake Macquarie. State Pollution Control Commission, NSW.
2. Batley, G.E. (1991). Current Heavy Metal Status of Lake Macquarie. In Lake Macquarie An Environmental Reappraisal, eds Whitehead, J.H. *et al.*, University of Newcastle, Newcastle, pp55-66.
3. Maunsell & Partners (1974), "Lake Macquarie Foreshore Study", Report for the Department of Public Works, NSW.
4. Roy, P.S. and Crawford, E.A. (1984). Heavy Metals in a Contaminated Australian Estuary - Dispersion and Accumulation Trend. Estuar., *Coast. Shelf Sci.*, 19, 341-358.
5. WCIC (1973). Water Resources of the Hawkesbury Valley Including Tuggerah Lakes and Lake Macquarie Systems". Report No. 25, Water Conservation and Irrigation Commission NSW.
6. TUNRA-DATEX (1977). Lake Macquarie Municipal Council - Strategic Planning Report. Lake Macquarie Municipal Council, Newcastle.
7. Roberts, B. (1994). The Accumulation and Distribution of Selenium in Fish from Lake Macquarie, NSW. B Sc. (Honours) Thesis, University of Canberra.
8. Woodford, J. (1995), Toxic risk in eating lake fish, Sydney Morning Herald, September 13, p1.
9. Kudryavtsev, A.A. (1974). The Chemistry & Technology of Selenium and Tellurium. Translated and revised by Elkin, E.M. Collet's (Publishers), London.
10. Schwarz, K. and Foltz, C.M. (1957). Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.*, 79, 3292.

11.   Guang-Qi (1987), In Selenium in Biology and Medicine, Part A., ed Combs Jr., C.G., Spallholz, J.E., Levander, O.A. and Oldfield, J.E.. Van Nostrand Reinhold, New York., pp9-31.

12.   Toyoda, H, Himeno, S. and Imura, N. (1989). The Regulation of Glutathione Peroxidase Gene Expression; Implication for Species Difference and the Effect of Dietary Selenium Manipulation. In Selenium in Biology and Medicine, ed Wendel, A., Springer-Verlag, Berlin.

13.   Ohlendorf, H.M. (1989). In Selenium in Agriculture and the Environment, ed Jacobs, L. M. Soil Science Society of America, Inc. and American Society of Agronomy, Inc., p. 133.

14.   Ohlendorf, H.M. and Hothem, R.L. (1995). Agricultural Drainwater Effects on Wildlife in Central California. In Handbook of Ecotoxicity, eds Hoffman, D.J. *et al.* CRC Press, Boca Raton, Ch26.

15.   Nriagu, J.O. and Wong, H.K. (1983). Selenium Pollution of lakes near the smelters of Sudbury, Ontario. *Nature*, 301, 55-57.

16.   Scanes, P. (1993). Trace Metal Uptake in cockles Anadara trapezium from Lake Macquarie, New South Wales. Mar. *Eco. Prog. Ser.*, 102, 135-142.

17.   Batley, G.E. (1987). Heavy Metal Speciation in Waters, Sediments and Biota from Lake Macquarie, New South Wales. *Aust. J. Mar. Freshw. Res.*, 38, 591-606.

18.   Furner, I.. (1979). Lead, Cadmium, Zinc and Copper in Organisms of Lake Macquarie, B.Sc. Thesis, Department of Geography, University of Newcastle.

19.   Crawford, E.A., Roy, P.S., Brooks, K. Zamberlain, A., Scott, T., MacKay, N.J. and Chvojka, R. (1976). Heavy Metals in bottom sediments and fish from Lake Macquarie. Geological Survey of NSW report GS 1976/283.

20.   Masscheleyn, P.H. and Patrick Jr., W.H. (1993). Biogeochemical Processes Affecting Selenium Cycling in Wetlands. *Environ. Toxicol. Chem.*, 12, 2235-2243.

21.   Fleming, R.W. and Alexander, M. (1973) Dimethylselenide and Dimethyltelluride Formation by a Strain of Penicillium., *Appl. Microbiol.*24(3), 424-429.

22.   Doran, J.W. and Alexander, M. (1977). Microbial Transformations of Selenium. *Appl. Environ. Micro* 33(1), 21-37.

23.   Maiers, D.T., Wichlacz, P.L., Thompson, D.L and Bruhn, D.F. (1988). Selenate Reduction by Bacteria from a Selenium-Rich Environment. *Appl. Env. Micro.*, 54 (10), 2591-2593.

24.   Doran, J.W. (1992). Micro-organisms and the Biological Cycling of Selenium. In *Advances in Microbial Ecology*, Volume 6, ed Marshall, K.C., pp1-32.

25.   Steinberg, N.A., Switzer Blum, J., Hochstein, L. and Oremland, R.S. (1992). Nitrate is a Preferred Electron Acceptor for Growth of Freshwater Selenate-Respiring Bacteria. *Appl. Environ. Microbiol.* 58(1), 426-428.

26.   Thompson-Eagle, E.T. and Frankenberger Jr., W.T. (1992). Bioremediation of Soils Contaminated with Selenium. *Adv. Soil Sci.*, 17, 261-310.

27.   Riadi, L. (1994). Biological removal of Selenium Oxyanions from a Selenium Contaminated Site. Ph.D. Thesis, Department of Chemical Engineering, University of Sydney.

28.   Riadi, L. and Barford, J.P. (1995). Bioremediation of Process Waters Contaminated with Selenium, Process Safety and Environmental Protection, 73(B2), 155-159.

29.   Riadi, L., Barford, J.P. and Linkson, P.B. (1994). Bioremediation of Selenite Using Free and Immobilised Cells. Proceedings, 3rd Asia-Pacific Biochemical Engineering Conference.

30.   Carroll, B.I., Barford, J.P., Gotham, K. and Reso, M. (1996) Selenium transformation and metabolism in bacterial isolates from Lake Macquarie, NSW. Proceedings, 23rd Hydrology and Water Resources Symposium, Hobart, Tasmania, 21-24 May. Institution of Engineers, Australia, pp731-732

31.   Fio, J.L. and Fujii, R. (1990). Selenium Speciation Methods and Application to Soil Saturation Extracts from San Joaquin Valley, California. Soil Sci. Soc. Am. J., 54, 363-369.

32.   Norheim, G. (1988). Determination of selenium in biological material using automated wet digestion and an automated hydride generation atomic absorption system. Varian Instruments at Work, AA-86.

33.   Brock, T.D. and Madigan, M.T. (1991). Biology of Micro-organisms. 6th Ed, Prentice Hall Int Inc, USA, p430, 1991.

34.   Peters, G.M., Barford J.P., Reible D.D., Gomes V. (1996). Redox Effects in Selenium Behaviour in Estuarine Porewaters. (accepted for publication) Proceedings, Chemeca 96, 30th September - 2nd October.

35.   Minnett A (1994). "Development of in-situ measurements of sediment porewaters". Honours thesis, University of Wollongong.

36.   Runnells D.D. and Lindberg R.D. (1990). Selenium in aqueous solutions: the impossibility of obtaining a meaningful E_h using a platinum electrode. *Geology*, 18, 212-215.

37.   Whitfield M. 1971. "Ion selective electrodes for the analysis of natural waters". Handbook 2, Aust. Mar. Sci. Assoc., Sydney

38.   Wallmann K., Kerten M., Gruber J., Förstner U (1993). Artefacts in the determination of trace metal binding forms in anoxic sediments by sequential extraction. *Int. J. Environ. Anal. Chem.*, 51, 1-4. 187-200.

39.   Lindberg R.D. and Runnells D.D. (1984). Ground water redox reactions: an analysis of equilibrium state applied to E_h measurement. Science, 225, pp925-927.

40.   Manning A.B. and Burau R.G. (1995) Selenium immobilisation in evaporation pond sediments by in situ precipitation of ferric oxyhydroxide. Environ. Sci. Technol., 29, 2639-2646.

41.   Tessier, A., Campbell, P.G.C. and Bisson, M. (1979). Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Anal. Chem.*, 51(7), 844-851.

42.   Orsini, L. and Bermond, A. (1993). Application of a Sequential Extraction Procedure to Calcareous Soil Samples. *Inter. J. Environ. Anal. Chem.*, 51, 97-108.

43.   Whalley, C. and Grant, A. (1993). Assessment of the Phase Selectivity of the European Community Bureau of Reference (BCR) Sequential Extraction Procedure for Metals in Sediment. *Anal. Chim. Acta*, 291, 287-295.

44.   Carroll, B.I., Nobbs, D.M. and Smith, G. (1996) Characterisation of Selenium Bioavailability by Two Sequential Extraction Methods in Sediments from an Estuarine Lake Subject to Anthropogenic Selenium Input. Proceedings, Asia Pacific Conference on Sustainable Energy and Environmental Technology, 19-21 June, Singapore. World Scientific Publishing Company, Singapore, pp72-79

45.   EPRI (1986). Speciation of Selenium and Arsenic in Natural Waters and Sediments, Volume 1: Selenium Speciation. EPRI Report #EA-4641. Electric Power Research Institute, Palo Alto, Ca, USA, 1986.

46.   EPRI (1994). Chemical Attenuation Reactions of Selenium. Report Summary, Project RP2485-03. Electric Power Research Institute, Palo Alto, Ca, USA.

47.   Ure, A.M., Quevauviller, Ph and Muntau, H. (1993). Speciation of Heavy Metals in Soils and Sediments. *Int. J. Environ. Anal. Chem.*, 51, 1-4, 135-151.

48.   Anonymous (1994). SPEEDUP User Manual Volume I, Release 5.4. Aspen Technology Inc, Cambridge, Massachusetts, USA.

49.   Anonymous (1991). MINTEQ Documentation version 3.11. Center for Exposure Assessment Modeling, USEPA, Athens, GA, USA.

50.   Lake Macquarie City Council (1995). Lake Macquarie Estuary Process Study, Volume I - Report. Prepared by Australian Water and Coastal Studies Pty. Ltd. and JH&ES Laxton, Pty. Ltd. for Lake Macquarie City Council.