BIOGEOCHEMISTRY AT LAKE TYRRELL

Or, what we found when we fell in the mud, except for Sara, who pulled us out

Sue Welch[1], Sara Beavis[2] & Peter Somerville[2]

[1]CRC LEME, Department of Earth and Marine Sciences and Research School of Earth Sciences,
Australian National University, ACT, 0200
[2]CRC LEME, Centre for Resource and Environmental Studies, Australian National University, ACT, 0200
[3]CRC LEME, School for Resources Environment and Society, Australian National University, ACT, 0200

INTRODUCTION

The purpose of this study was to characterize biologically and abiotically mediated sulfur cycling in an inland acidic sulfidic environment. Lake Tyrrell, in western Victoria, is an extremely acidic saline sulfate-rich environment. It is one of three adjacent saline groundwater discharge lakes (Tyrrell, Timboran and Wahpool) situated in the southern part of the Murray Basin. The geology, geochemistry and hydrology of this system have been described previously (see, for example, Macumber 1983, Macumber 1992, Long *et al*. 1992, Lyons *et al*. 1992 and Hines *et al*. 1992). The Murray Basin is a closed groundwater basin. Regional groundwater flows toward the centre of the Basin and discharge features include many salt lakes (salinas) scattered throughout the landscape. Lake Tyrell, ca. 160 km^2 in area, is the largest lake in the Basin to receive regional groundwater discharge. The Late Tertiary marine transgression resulted in the reworking and deposition of sands (Parilla Sands, average thickness ca. 60-70 m which act as the major aquifer) across the western Basin. The Parilla Sands are underlain by the Geera Clay, a ca. 160 m thick marine clay deposited during an earlier marine transgression which acts as an effective basal aquitard, and are overlain by the Blanchetown Clay, ca. 20 m thick, which was deposited in a slowly subsiding trough on the eastern side of the Tyrell Fault (trending north-south) during a marine transgression in the Early Pleistocene. The fault caused a 30 m displacement of the Parilla Sand to the west of Lake Tyrrell (Macumber 1992).

Hydrology is driven by groundwater flows between the Parilla Sands and the three lakes in the Tyrrell region (Tyrrell, Timboran, Wahpool). Groundwater in the aquifer has pH values generally < 4, but as low as 2.8 in springs discharging from the aquifer. Acidic groundwater is restricted to the upper parts of the aquifer, occurring at depths of 25 to 30 m below the water table (Macumber 1992). Reflux brines are located directly beneath the three lakes in the Tyrrell region. The brines had their origins as lake waters that were later refluxed into the groundwater systems to form discrete groundwater brine bodies. The reflux brines are ca. 5 times more saline than the regional groundwaters and are higher pH, anoxic and in some cases sulfidic (Macumber 1983). However, the presence of a large body of salt brine under Lake Tyrrell and only a thin halite evaporite crust within the lake implies that a through-flow of less saline shallow groundwater operated previously. The development of a closed system has occurred within the last 32,000 years (Macumber 1992). This change would account for the change in average groundwater salinities of 30,000 mg l^{-1} from this time, when the depth of the Lake was ca. 13 m, to 300,000 mg l^{-1} today.

A previous study (Hines *et al.* 1992) on biogeochemical cycling on this system focused on two sites on the western side of the lake. Results from Hines *et al*. (1992) showed that the pore water collected from near surface sediments (< 30 cm deep) was extremely acidic, pH ca. 3 to 4. In general, concentration of Fe and Mn increased away from the shore, suggesting either reduction of Fe and Mn or dissolution of reduced Fe and Mn minerals. Nitrate concentration was high and variable, 10s to 100s of µM, suggesting degradation of organic material and oxidation of reduced nitrogen species. Heterotrophic bacterial populations can mediate formation of these compounds. However, DOC was low, < 10 ppm, and generally decreased to below detection away from the shore, which would limit microbial activity. Although sulfide was detected, experiments showed no evidence of sulfate reduction. Hines *et al*. (1992) suggested that the reduced species that were detected at this site were produced at some remote location up-gradient from the discharge zone and that the geochemical zonation was the result of mixing of the anoxic waters of the reflux brines with the less saline oxygenated shallow groundwater.

METHODS

Sediment samples were collected from along several transects from locations along the eastern shore of Lake Tyrrell in Aug-Sep 2004 (Figure 1). Transects ran perpendicular to the shore, with the spacing of the sampling pits separated by ca. 3 to 10 m. The length of transects was variable, and ended when we found no obvious sulfidic layer in the top ca. 10 cm of the sediment. The different soil horizons were described in the field and subsamples of each horizon were collected and placed in sealed plastic bags to limit oxidation and

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evaporation. Selected samples were analysed for pH and EC with a standard 1:5 soil:water extract. Several samples were analysed for mineralogy by XRD. Selected samples from these sites were examined by a combination of light and epifluorescence microscopy, confocal scanning laser microscopy, and scanning electron microscopy (at EMU, RSBS, ANU) to determine the physical relationship between the microbial populations and mineral surfaces.

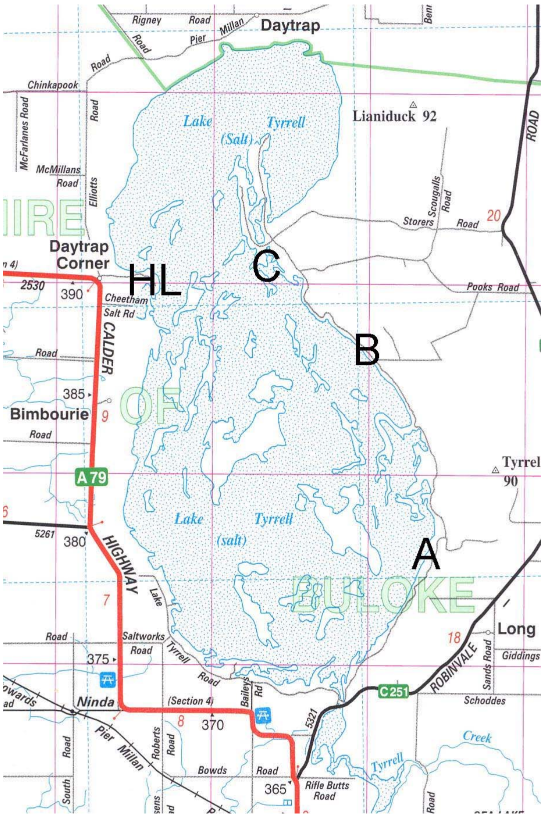

Figure 1: Map of Lake Tyrrell (from Lyons *et al*. 1992). Letters represent locations of study sites from previous workers (e.g., Lyons *et al.* 1992 (HL), Hines *et al*. 1992 (HL), Macumber 1992 and references therein). Sediment samples were collected from 3 locations from shallow pits along several transects along the eastern side of the lake (A, B, C).

RESULTS AND DISCUSSION

Although this a very harsh environment, the preliminary survey of the sediments and surface of the lake bed showed evidence of active microbial communities and biogeochemical cycling of iron and sulfur species. The surface sediments of the lake comprise sands and clays sourced off-site and transported via aeolian and surface erosional processes. A halite-gypsum crust, that can include algal mats, covers these sediments up to depths of 60 cm (Long *et al*. 1992). The surface of the dry salty lakebed at our sites was covered by a patchy, thin, desiccated, reddish brown organic film. Analysis of this material by SEM showed what appeared to be filamentous cyanobacteria, diatom tests, micron-sized microbial cells and extracellular polymer draped over halite crystals, suggesting that a very active microbial mat grows at the surface water interface of the lake during floods.

The sulphidic sediments occur as a well-defined near-surface continuous lens, of variable width, along the lake margin (Figure 2). These sediments, which may be massive or finely interbedded lamellae, are thixotropic black loams, which at some sites are underlain by sands or clays that include either iron or sulphidic mottles. Fine lamellae of interbedded sulphidic sediments and grey clays in some localities provide evidence of episodic deposition along the lake margins. Further evidence of rapid erosion and deposition is provided through historical aerial photography and the burial of a fence line that extends from the southeastern margin of the lake. This suggests that the sulphidic materials are of very recent origin. The surface sediments are underlain by the Late Pleistocene to Holocene Tyrrell Beds, which are ca. 5 m thick (Macumber 1992).

Perhaps the most striking evidence for microbial activity was observed in the mounded salt crusts a few meters from the shoreline (what we found when we fell in the mud). These mounds were a few metres in size, and were elevated a few centimetres above the adjacent lakebed surface. The surface of the mounds was noticeably damp and covered in a rough crust of pinkish salts. These features appeared to represent areas where saline groundwater was discharging to the surface of the lakebed along the edges of the lake. When the surface of the mound was disturbed, we observed a bright green layer, a few millimetres thick, a few millimetres below the surface of the salt. This green layer was nearly ubiquitous below the salt crusts, but was not found in the surface layers of the adjacent sediment.

The pinkish salt crusts were examined by SEM, light, and epifluorescence microscopy. Halite and gypsum crystals were readily identified based on their morphology in both light and electron microscopy. This was confirmed by EDS analysis. Analysis of the salts by light microscopy showed of a rusty coloured solid mineral phase, presumably goethite, which could account for the pinkish colour of the surface salt crust. However, epifluorescence and electron microscopy also showed the existence of micron-sized microbial cells associated with the salt. It is not yet known if these organisms are salt-tolerant microbes that were transported and deposited when the water evaporated, or if these organisms are halophilic bacteria or archaea that grow *in situ*. The halophilic archaea commonly have a beta-carotene-like photosensitive pigment called bacteriorhodopsin that is used to convert light energy into a proton gradient pump across the cell membrane

(Ohren 2000, 2002). This pigment is responsible for the pinkish colour that is often associated with hypersaline environments, and could in part be responsible for the colour of the salt crusts found here.

The green layer was examined by SEM, light, epifluorescence and confocal scanning laser microscopy (CSLM). Although the colour of the salts indicated that there must be an abundant photosynthetic community present, analysis of the material in the SEM initially showed sparse evidence for microbial life. The mineralogy of the salt crusts was dominated by gypsum and halite (Figure 3a). Filamentous cyanobacteria were observed on some surfaces or where crystals were broken (Figure 3a, b). However, further analysis of this sample by light and epifluorescence microscopy showed that cyanobacteria were abundant (Figure 4). Three-dimensional imaging with the CSLM showed that at least some of the cyanobacteria were truly endolithic (Figure 4b). Similar endolithic microbial communities have been described in other environments such as the salt ponds at Guerrero Negro and the Dry Valleys of Antarctica (Spear *et al*. 2003, de la Torre 2003). Clear salt crystals can act as light pipes to allow sunlight to be transmitted into the sediments. Living within salt crystals also protects the cells from extremes of desiccation and UV degradation. In addition to the cyanobacteria, micron-sized cells were observed closely associated with the cyanobacterial assemblages. These are presumably halophilic heterotrophic bacteria or Archaea that uses the cyanobacterial extracellular polysaccharides as an energy source.

Although previous work on the more acidic western side of the lake suggested that the metals sulfides that formed were the results of redox reactions that occurred at some distance up gradient from the discharge zone, we propose that the reduced Fe and S species were formed *in situ* on the eastern side of the lake. Phototrophic microbial communities on the surface of the sediments and within the evaporite crusts provide a source of organic material for the heterotrophic sulfate reducers. The finely interbedded sulfide layers, grey clays and salts in some localities provide evidence of episodic deposition, suggesting that the phototrophic communities are periodically buried beneath the photic zone, and are degraded by anaerobic microbial communities.

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