SURVEY AND DESCRIPTION OF SULFIDIC MATERIALS IN WETLANDS OF THE LOWER RIVER MURRAY FLOODPLAINS: IMPLICATIONS FOR FLOODPLAIN SALINITY MANAGEMENT

S. Lamontagne, W.S. Hicks, R.W. Fitzpatrick and S. Rogers

CRC LEME OPEN FILE REPORT 165

August 2004

CRCLEME

CSIRO Land and Water Technical Report 28/04

SURVEY AND DESCRIPTION OF SULFIDIC MATERIALS IN WETLANDS OF THE LOWER RIVER MURRAY FLOODPLAINS: IMPLICATIONS FOR FLOODPLAIN SALINITY MANAGEMENT

S. Lamontagne, W.S. Hicks, R.W. Fitzpatrick and S. Rogers

CRC LEME OPEN FILE REPORT 165

August 2004

CSIRO Land and Water Technical Report 28/04

© CRC LEME 2004

Addresses and affiliations of authors

S. Lamontagne
Cooperative Research Centre for Landscape
Environments and Mineral Exploration
c/- CSIRO Land and Water
Private Mail Bag 2,
Glen Osmond 5064
South Australia

W.S. Hicks
Cooperative Research Centre for Landscape
Environments and Mineral Exploration
c/- CSIRO Land and Water
GPO Box 1666,
Canberra 2601
ACT

R.W. Fitzpatrick
Cooperative Research Centre for Landscape
Environments and Mineral Exploration
c/- CSIRO Land and Water
Private Mail Bag 2,
Glen Osmond 5064
South Australia

S. Rogers
Cooperative Research Centre for Landscape
Environments and Mineral Exploration
c/- CSIRO Land and Water
Private Mail Bag 2,
Glen Osmond 5064
South Australia

Photography
RW Fitzpatrick, WS Hicks & S Lamontagne
© 2004 CSIRO

Table of Contents

Summary

This study was undertaken to determine whether environmentally significant deposits of sulfidic materials are present in Lower River Murray floodplains. Sulfidic materials are soils and sediments enriched in sulfide minerals, such as pyrite (FeS_2) and monosulfides (FeS). These materials tend to accumulate in environments where elevated SO_4^{2-} concentrations, a high availability of labile carbon and anoxic conditions favour high rates of sulfate reduction. It was suspected that wetlands of Lower River Murray floodplains would be at risk of accumulating sulfidic materials because current conditions (that is river regulation and salinisation) should have promoted the conditions favourable to high rates of sulfate reduction. Sulfidic materials are usually stable as long as they remain undisturbed. However, when they are exposed to oxygen (through drainage or resuspension in the water column) they pose a number of environmental risks, including deoxygenation of the water column, acidification and the generation of noxious smells. Whether or not sulfidic materials occur in Lower River Murray floodplains is important on a management point of view because many recently proposed floodplain salinity remediation initiatives could result in exposing sulfidic materials to the atmosphere.

Eight wetlands in the Riverland region of South Australia and one wetland near Buronga in New South Wales were surveyed for the presence of sulfidic materials. These wetlands were selected to represent a range in salinity and water regime manipulation, from freshwater wetlands with near natural wetting and drying cycles to hypersaline evaporation basins. The survey was exploratory with limited sampling (one to three sites) within each wetland. Within a wetland, specific sampling locations were chosen based on observed site conditions such as different phases of the wetting and drying cycle or changes in the wetland morphology. The presence and the characterisation of the sulfidic materials at each site was achieved through a range in chemical, mineralogical and microbiological analyses.

The survey showed that sulfidic materials are widespread in Lower River Murray floodplains and that the conditions for their formation are ubiquitous, with sufficient sulfate, iron and carbon available. The limiting factor in their formation appeared to be labile carbon. Although the conditions for formation existed, significant accumulation seemed to occur only when flooded conditions are maintained for significant periods (years to decades). Seasonal wetting and drying may prevent accumulation by destroying the sulfides as the wetland dries and conditions become oxidising.

A preliminary assessment of the environmental risks associated with sulfidic materials was also made. In general, acidification did not appear to be a major risk because wetlands with a high sulfide content also tended to have significant acid neutralising capacities (i.e., had high carbonate concentrations in their sediments). However, two wetlands had potential acid sulfate soil conditions (i.e., are at risk of acidification) and one (Bottle Bend Lagoon, NSW) had severely acidified (pH < 3) during a recent draw down event. The aesthetic risk (noxious smells) was widespread in Riverland disposal basins (including the Loveday, Berri and Ramco basins) as assessed by the response of the local communities to the recent drying of some of these basins. We could not define the deoxygenation risk because there is presently no agreed method to assess it. However, anecdotal evidence suggest that deoxygenation events have occurred in River Murray wetlands when sulfidic sediments have been disturbed during managed wetland wetting/drying operations. The factors that could contribute to the deoxygenation risk would include the suspended sediment load, sediment sulfide concentration, the form of sulfide present, water column residence time, the reaction rate of the sulfides, and the critical dissolved oxygen levels for the target organisms. It is important to note that a good acid neutralising potential (i.e., low acidification risk) has no bearing on the deoxygenation or aesthetic risks.

The issue of sulfidic materials in the Lower River Murray has some similarities and differences relative to the problem of acid sulfate soils (ASS) in coastal environments. We found the field measurements and tests used in coastal ASS to be directly transferable, as were the laboratory methods for sulfur species determination. However, the routine manometric method for soil carbonate has a detection limit that is too high in comparison with the trigger value for reduced sulfur. The major difference between the two environments may be that acidification is the main risk in coastal environments whereas it is not in the floodplain context. Thus, the guidelines used to trigger management action in the coastal ASS context may not be suitable for the floodplain one. This would be especially true for the deoxygenation risk, which currently does not have proper assessment guidelines.

The recommendations arising from this study include:

- Complete a survey of the habitats suspected to have accumulated significant sulfidic material deposits in the Lower Murray;
- Further define the regional-scale factors contributing to the acidification risk;
- Determine the rates at which sulfidic materials are formed or are oxidised under different salinity and water level management conditions;
- Assess the spatial variability in the distribution of sulfidic materials in representative wetlands;
- Identify the compounds responsible for the noxious smell problems and the optimal conditions under which these are produced and, conversely, minimised;
- Define the mass-balance for S and alkalinity during wetting-drying cycles in wetlands;
- Understand the role of sulfidic materials and of anoxic groundwater in causing wetland acidification and deoxygenation;
- Determine if monosulfides form a significant component of the reduced S pool in Riverland wetlands;
- Educate the management groups whose actions may impact the hydrology of River Murray wetlands about the risks associated with disturbing sulfidic materials.

Acknowledgements

Introduction

It is estimated that 25% of the Lower River Murray floodplains area are impacted by salinity, with this proportion potentially increasing to 50% by 2050 (RMCWMB 2003). Salinity is threatening the health of many ecosystems in these floodplains, including several Ramsar-listed wetlands and large tracts of Redgum (*Eucalyptus camaldulensis)* forests. There are several causes for the salinisation of the floodplains, including a decreased flooding frequency and an increased input of saline groundwater (induced by mallee clearing and irrigation mounds). Salinisation is also caused by increased waterlogging, for example through raised weir pool levels and the disposal of excess irrigation water on the floodplains (Jolly 1996). Improving the health of salinised floodplains will be a significant challenge for the managers of the River Murray in the next decades (RMCWMB 2003).

While the issue of salinity in the River Murray is now well recognised, the changes in biogeochemical cycles that will accompany waterlogging and increased salinity in this system have not received as much attention. While some of the ions that contribute to salinity are relatively unreactive in the environment (such as Na^+ and Cl^-), others have complex cycles. In particular, the increase availability of sulfate (SO_4^{2-}) in saline environments can significantly affect the cycle of carbon and of key nutrients such as phosphorus (Caraco et al. 1989; Waite 1997). Under the right conditions, environments rich in sulfate can also accumulate deposits of sulfidic materials, which can be a hazard. Understanding whether environmentally significant deposits of sulfidic material are present in this system is important for current and proposed management actions to mitigate floodplain salinity. If not managed properly, the environmental costs of disturbing sulfidic materials during salinity remediation actions may offset some of the environmental benefits.

Sulfidic materials have long been recognised as a significant environmental problem for large sections of the Australian coastline (National Working Party on Acid Sulfate Soils 1999). It is now recognized that similar sulfidic materials can develop inland, for example in areas impacted by dryland salinity (Fitzpatrick et al. 1996; George 2002). Notably, some of the drainage networks aimed at mitigating dryland salinity in Western Australia are plagued by very low pHs (George 2002).
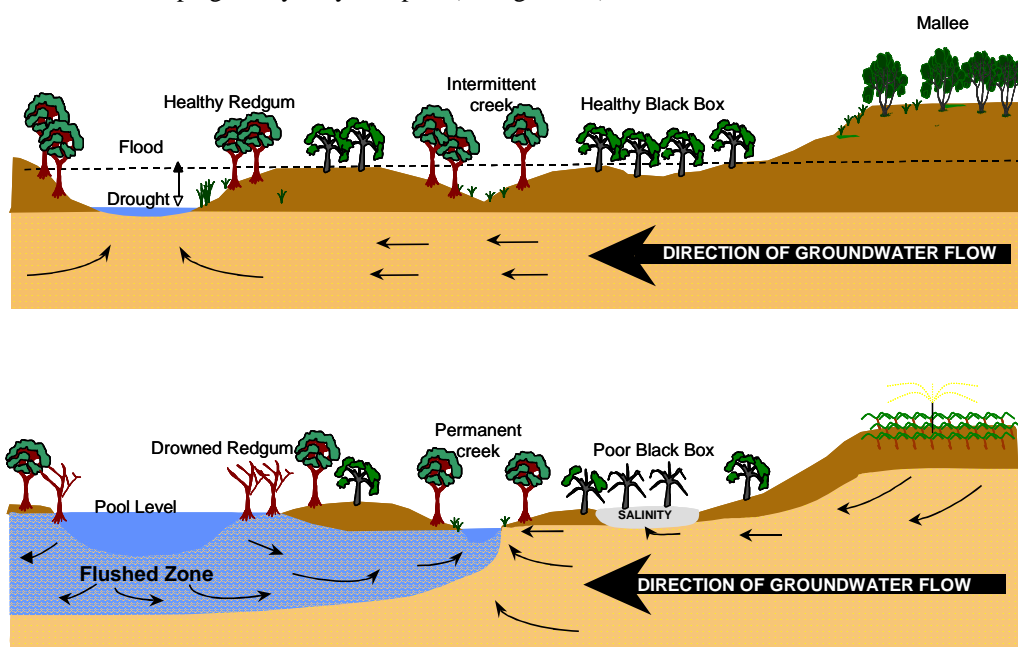
Figure 1: *Change in groundwater - surface water interactions before (top) and after (bottom) European settlement in River Murray floodplains (I. Jolly, CSIRO L&W, pers. comm.). A detailed review of irrigation and regulation impacts on River Murray floodplains is presented in Jolly (1996). The botanical names for species and vegetation associations referred to in the diagram are as follows: Black Box – Eucalyptus largiflorens; Mallee – E. gracilis, dumosa, santalifolia; Redgum – E. camaldulensis.*

What are sulfidic materials?

Sulfidic materials are mostly accumulations of iron sulfide minerals, one of the end products of the process of *sulfate reduction*. Sulfur occurs in the environment in several oxidation states, that is sulfates (+6), elemental sulfur (0) and sulfides (–2). Several organic and mineral forms of reduced sulfur occur in wetland sediments but two forms of iron sulfide minerals are of special interest on an environmental point of view: monosulfides (FeS) and pyrite (FeS$_2$). Soils and sediments rich in monosulfides (or "monosulfidic black ooze") tend to be very dark and soft. Monosulfides can react rapidly when they are disturbed (i.e., exposed to oxygen). Pyrite will tend to occur as more discrete crystals in the sediment and organic matter matrices and will react more slowly when disturbed.

According to Soil Taxonomy (Soil Survey Staff 2003), the term "sulfidic materials" applies to soils or sediments with a pH of >3.5, which if incubated as a layer 1 cm thick under moist conditions (field capacity) while maintaining contact with the air at room temperature shows a drop in pH of more than 0.5 to a pH value of 3.5 or less within 8 weeks. However, this definition of sulfidic materials has been established primarily within the context of coastal acid sulfate soil environments, where acidification is the primary environmental concern. For example, a "sulfuric horizon" (derived from sulfidic materials) is composed either of mineral or organic soil material (15 cm or more thick) that has both pH <3.5 and bright yellow jarosite mottles. A broader definition for sulfidic materials is required for inland environments because acidification is only one of the environmental concerns associated with them (*see below*). Thus, in this report, sulfidic materials will be loosely defined as any soil or sediment with a sufficient sulfide concentration to be of concern for acidification or for other environmental issues.

How do sulfidic material deposits form?

Sulfidic materials will develop when conditions are favourable to high rates of sulfate reduction (i.e. the use of SO$_4^{2-}$ instead of O$_2$ during microbial respiration). While sulfate reduction is a universal process in lake and wetland sediments, the rates of sulfate reduction on Lower River Murray floodplains may be higher now than prior to European settlement. The key requirements for high rates of sulfate reduction are: (*i*) a high concentration of sulfate in surface or groundwater, (*ii*) saturated soils and sediments for periods long enough to favour anaerobic conditions, and (*iii*) the availability of labile carbon to fuel microbial activity. Saline wetlands in the floodplain environment have all these conditions. In the Murray-Darling Basin, there is an ample supply of SO$_4^{2-}$ in saline environments because sulfate salts constitute 10 to 20% of the salinity (Herczeg et al. 2001). Numerous potential sources of carbon should also be available to fuel microbial activity in saline wetlands, including terrestrial leaf litter, macrophytes (sedges, etc), phytoplankton, and benthic algae (i.e. algae growing on or near the surface of the sediments).

What risks do sulfidic materials pose for the environment?

There are two possible categories of environmental risks associated with sulfidic materials: those occurring during formation and those associated with disturbance. For example, during their formation, monosulfides will coat the surface of wetland sediments and reduce the habitat available to benthic invertebrates (yabbies, clams, snails, etc). In addition, hydrogen sulfide (a toxic gas for many aquatic plants and other biota) is generated during the production of sulfidic materials. The process of sulfate reduction can also interfere with the cycle of carbon and of some of the key nutrients in freshwater, especially phosphorus (Waite 1997). High rates of sulfate reduction can make phosphorus more available to algae and, indirectly, could foster algal blooms.

Many potential environmental risks associated with sulfidic materials will arise when they are disturbed (i.e. resuspended in the water column or drained). These include,

Noxious odours: Hydrogen sulfide production (H$_2$S – the rotten egg smell) by drying sulfidic materials can decrease the aesthetic value of wetlands by generating noxious smells. Aside from the foul odour problem, H$_2$S is also of concern for human health at high concentrations. In addition, a number of malodorous organic-S gases (such dimethyl oligosulfides) can also be produced under the conditions favourable to H$_2$S production (Franzmann et al. 2001) and could contribute to noxious smell events.

Accumulation of radionuclides: In addition to iron, many other metals – including radioactive ones like uranium – can form mineral deposits in the presence of reduced sulfur. Thus, there is a possibility that, in some areas, the long-term accumulation of sulfidic materials can also lead to significant accumulation of radionuclides. Of special concern for water quality would be radium-226, a mobile progeny of the uranium-238 decay series (National Health and Medical Research Council 1996). An investigation is currently underway to characterise the accumulation of radionuclides in retention basins of the Murray-Darling Basin (B. Dickson and A. Giblin, CSIRO Exploration & Mining, *personal communication*).

Water column deoxygenation: When sediments rich in monosulfides are resuspended, they will rapidly oxidise, potentially removing most of the oxygen from the water column (Sullivan et al. 2002). This can lead to fish kills, especially in enclosed areas such as billabongs. Flushing of saline drains by high runoff events and resuspension of sediments during water level manipulations in wetlands could potentially induce deoxygenation events.

Acidification and elevated metal concentration: When sulfidic materials are drained and exposed to air, they oxidise and produce sulfuric acid (Sammut and Lines-Kelly 1996). If the amount of acidity produced exceeds the buffering capacity of water and sediments, acidification occurs. Prior to draining, materials that can cause acidification are called potential acid sulfate soil materials (PASS). These materials become actual acid sulfate soil materials (AASS) or sulfuric horizons once drained. In addition to lowering pH, activation of PASS materials can lead to significant increases in dissolved metal concentration in surface water, including toxic species such as aluminium and cadmium. The increase in solubility of these heavy metals under acidic conditions may be more harmful to biota than the low pH itself.

Riverland sulfidic material survey

The aim of this study was to assess whether or not deposits of sulfidic materials of environmental significance are present in Lower River Murray floodplains. This assessment was made by selecting eight wetlands in the Riverland region of South Australia with different salinities and water regimes. These ranged from saline to hypersaline evaporation basins to freshwater wetlands with natural wetting and drying cycles. The hypothesis tested was that sulfidic materials would be more likely to have accumulated in saline, permanently flooded environments as opposed to those with freshwater conditions and natural wetting and drying cycles. In addition to the Riverland wetlands, Bottle Bend Lagoon (Buronga, NSW) was also included in the survey because it had been the site of a severe acidification event during a water level draw down in 2002 (McCarthy et al. 2003). It was suspected that sulfidic materials had been involved in the acidification of Bottle Bend Lagoon.

If present, a second goal of the study was to characterise sulfidic materials from floodplain environments through a series of pedological, mineralogical and microbiological analyses. These would be used to understand the type of environmental risks associated with floodplain sulfidic materials, especially within the context of future salinity management initiatives.
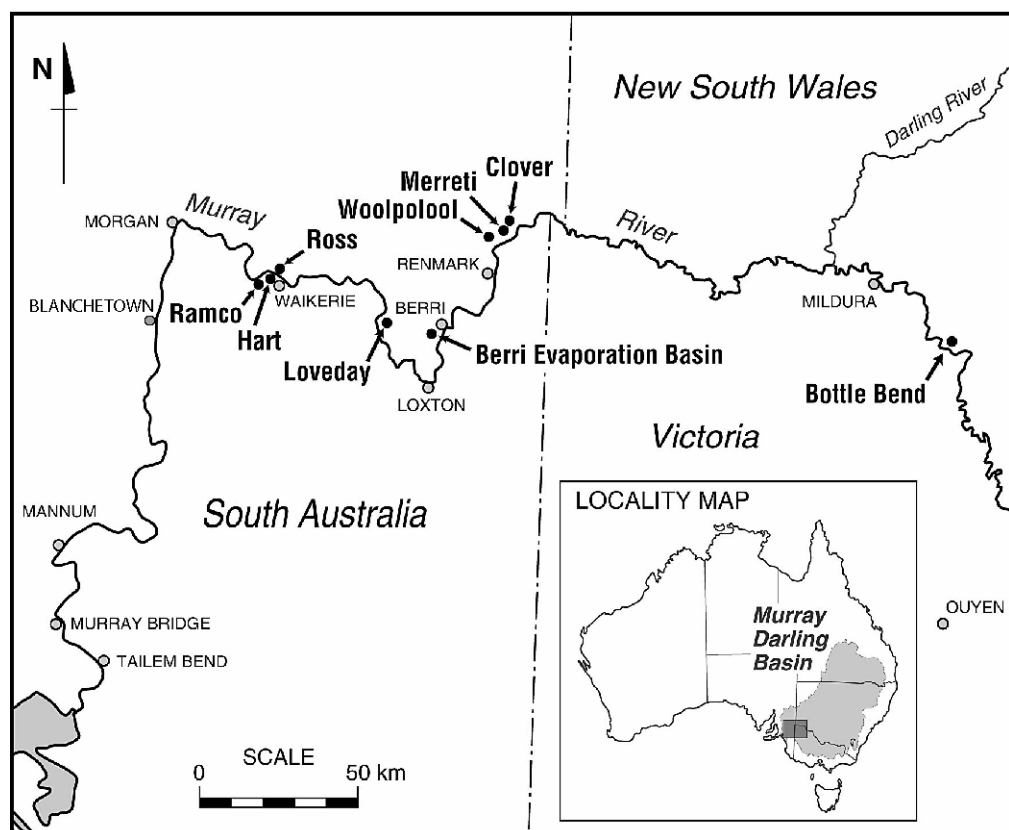
Figure 2: Location of the eight study sites in the South Australian Riverland and of Bottle Bend Lagoon in NSW.

Methods

Site selection and description

The Riverland region of South Australia spans from Blanchetown in the west to the NSW/Vic border in the east and includes approximately 300 river kilometres. Climate is semi-arid with a highly variable rainfall $(100 – 500$ mm $y^{-1})$ and a large potential evapotranspiration $(\sim 2000$ mm $y^{-1})$. In addition to dryland farming and tourism, the region is home to the largest and oldest irrigated agriculture industry in the state (mainly vineyards and orchards). In the western part, the riverine plain is 5–10 km wide and includes numerous floodplains, billabongs and anabranches, which form a part of the Bookmark Biosphere Reserve. In the eastern part (where most of the irrigated agriculture is based), the riverine plain narrows as it becomes encased by limestone cliffs. The predominant tree/shrub species in the floodplains are Black Box (*Eucalyptus largiflorens*), Redgum (*E. camaldulensis*) and Lignum (*Muehlenbeckia cunninghamii*), with bare soils and samphire spp. also common in more saline areas. The river throughout the Riverland is regulated through a series of locks and weirs to allow boat traffic and a permanent water supply for irrigation. Due to a combination of factors (see review in Jolly 1996), many of the floodplains are undergoing salinisation and some are already severely degraded. A number of management efforts are underway to mitigate floodplain salinisation, including greater irrigation efficiencies, the decommissioning of disposal basins formerly used for excess irrigation water, pumping schemes to decrease saline groundwater discharge and greater environmental flows.

Wetlands were selected to represent the range in salinity and water regime found in the study area, from hypersaline disposal basins to freshwater wetlands (Plate 1 to Plate 4). Where relevant for data analysis, the wetlands were grouped as either *disposal basins* and *natural wetlands*. Wetlands in the "disposal" group (Berri, Hart, Loveday and Ramco) are more impacted by anthropogenic activities than the ones in the "natural" group (Ross, Woolpolool, Merreti, Clover and Bottle Bend). However, all wetlands in the Lower

Murray probably have had some significant modifications to their hydroecology as a result of river regulation.

Disposal basins

In the Riverland region of SA, a number of former floodplain wetlands have been used as disposal basins for excess irrigation water for many decades. There are two types of disposal basins in the Murray-Darling Basin, those where water is primarily lost by evaporation and those designed to allow seepage to groundwater. Disposal basins within floodplains are of the evaporation type. They are generally isolated or have a limited connection to the river, except during larger floods. Because of evaporation and lack of flushing, salinity in disposal basins is elevated. In addition, some disposal basins are also impacted by irrigation-induced groundwater mounds which result in the discharge of saline groundwater to the floodplain.

Ramco and *Hart* lagoons are near the Waikerie irrigation district (Figure 2). The floodplain in this area is undergoing salinisation due to river regulation (impoundment by Lock 2), irrigation-derived groundwater mounds and the disposal of excess irrigation water to the lagoons. Vegetation health in the floodplain is poor and the lagoons have seawater-like salinities (Preiss, undated). Of significant interest for future studies, the lagoons are within the area of influence of the recently commissioned Waikerie IIB salt interception scheme. This scheme is specifically aimed at mitigating salinity in the floodplain (as opposed to limiting the discharge of saline groundwater to the river). The *Loveday Disposal Basin* near the city of Barmera is near one of the oldest irrigation districts in SA and is divided into a north and south basin by a causeway. At the time of the study, the south basin was dry and water levels in the north basin were very low due to a combination of increased irrigation efficiency and a drought in the Murray Basin. The low water levels were accompanied by very noxious smells (Plate 5). Only the north basin was sampled in this study. The *Berri Evaporation Basin* is located between the towns of Barmera and Berri and services a large irrigation district.

Natural wetlands

Ross Lagoon is a brackish to saline wetland in the Waikerie area but on the northern side of the river. Due to a better permanent connection to the river (and probably smaller impacts from irrigation) water quality in Ross is generally better than in nearby Ramco or Hart lagoons. *Woolpolool*, *Merreti* and *Clover* lakes are in the Bookmark Biosphere Reserve, approximately 20 km north-east of Renmark. This area of the floodplain is a part of the UNESCO-MAB Biosphere Reserve program that aims to combine both conservation and sustainable use of natural resources. Woolpolool Lagoon is brackish with a limited management regime of flooding and drying. Merreti Lagoon is fresh and has an active program of water level fluctuations to mimic natural conditions. Clover Lagoon is unregulated but has been dry since 1994. *Bottle Bend Lagoon* is located near Buronga, NSW. Prior to regulation, this wetland was probably ephemeral but it became permanent following the rise in river level that accompanied the building of the Mildura weir pool. Bottle Bend underwent a partial drying between March and December 2002 when a draw down of the weir pool and low flow conditions restricted its connection with the River Murray. An aquatic survey during the draw down (McCarthy et al. 2002) demonstrated that pH decreased to <3 and water electrical conductivity peaked at 33.1 mS/cm.
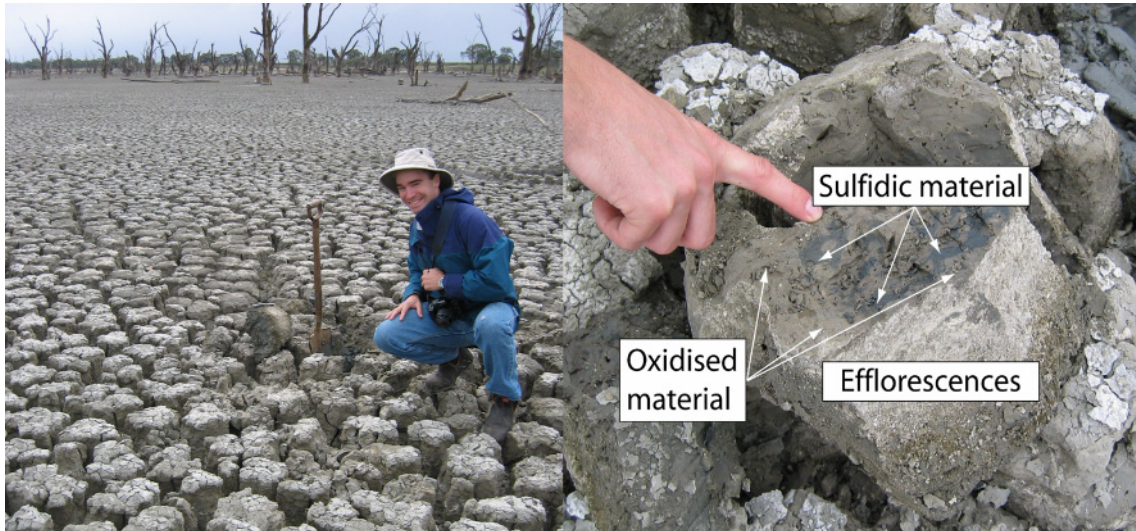
Plate 1: *Loveday Disposal Basin Pit 1 (near the causeway) showing large hexagonal peds typical of the drying phase in disposal basins, with ped detail. Despite being exposed to the atmosphere for several months, significant amounts of sulfides were still present within peds.*

Plate 2: *Loveday Disposal Basin in the vicinity of Pit 2 and 3 (near the irrigation museum), with details of the sulfidic black ooze.*

Plate 3: *The freshwater Merreti Lake and accompanying sediment profile, note the lack of accumulated sulfidic materials.*
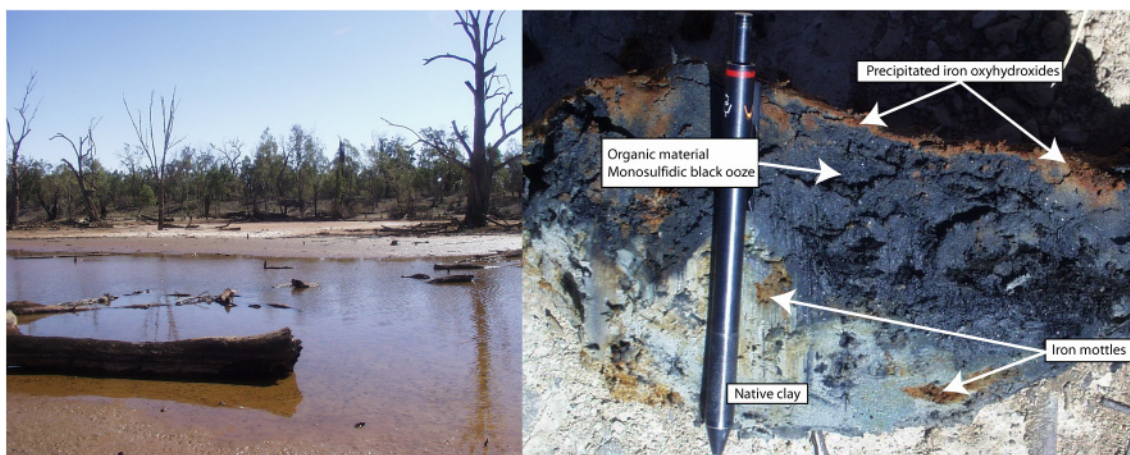
Plate 4: *Bottle Bend Lagoon showing extensive iron staining and detailed sediment profile displaying precipitated iron floc, organic matter, sulfidic sediments and native sediments with redoximorphic features.*

Plate 5: *News clipping, Murray Pioneer, 14 February 2003.*

Sample collection

One to three sites were sampled for each wetland. Where there was a range of conditions, for example different phases in the wetting-drying cycle or differing wetland morphology, qualitative judgement was used to select the sampling sites to reflect the differences. Notably, Ramco Pit 1 was ~0.2 m higher on the shoreline than the nearby Ramco Pit 2. Similarly, Loveday Pit 1 was in an area of the disposal basin left exposed for several months (Plate 1), while Loveday Pit 2 and 3 were near standing water (Plate 2). Submerged sediments were sampled using a corer whenever possible but were not always accessible by foot due to extremely soft substrates along some of the shorelines (especially in disposal basins). When submerged sediments were not accessible, samples were collected from pits dug as close to the shoreline as practical. Field tests (see below) and freezing of sub-samples for laboratory analyses were done within 30 min of collection (full details of sample collection equipment and methodology are given in Appendix 1).

Grab samples of surface water and/or pore water were collected at each site. Pore water was sampled by excavating to below the depth of free water and allowing the pit to fill. Water samples were split into unfiltered sub-samples for field measurement and alkalinity determination and filtered sub-samples (0.45 μm) for laboratory analysis. An aliquot of each filtered sample was placed in a tube containing the pre-prepared reagents for ferrous iron determination. A second sub-sample was collected for chloride analysis. The remaining filtered sub-sample was acidified to pH<2 with analytical reagent grade hydrochloric acid for laboratory analysis of major ions, nutrients and dissolved carbon.

Sample descriptions

Soil pits were dug to a depth of about 0.75 m and where possible a hand auger was used to sample soils down to 1.5 m. A representative profile face in the pit was selected and the master horizons demarcated and photographed. Soils were described according to the USDA Field book for describing and sampling soils, Version 2.0 (Schoeneberger et al. 2002) and Australian Soil and Land Survey Field Handbook (McDonald et al. 1990). Further details are given in Appendix 2.1. The following morphological features were described: horizon thickness (cm), horizon type (using nomenclature from: Schoeneberger et al. 2002; Soil Survey Staff 2003), horizon boundary, matrix colour (using soil Munsell colour notation), texture (McDonald et al. 1990), consistence (dry/force/strength), structure, pores/roots, concentrations, rock and other fragments, reaction or fizz to 1N HCl.

Sample analyses

Field Tests

We measured pH and Eh *in situ* in freshly collected cores or in sub-samples from visually distinct horizons within soil pits. The peroxide field test (a qualitative assessment of potential acid sulfate soil conditions) was performed on sub-samples according to ASSMAC Assessment Guidelines (NSW ASSMAC 1998; see Appendix 1).

Laboratory analyses

Water

Water samples were analysed for major ions (Ca, Mg, K, Na, total Fe, Fe(II), Cl, SO_4), alkalinity, nutrients (NH_4, $NO_3 + NO_2$, total dissolved N, filterable reactive P, total dissolved P, total dissolved S and filterable reactive Si) and total dissolved organic carbon. Method details are given in Appendix 1.

Soil and Sediment

Standard soil analyses were carried out on the sediment samples; these were pH, electrical conductivity and chloride in a 1:5 soil water extract, pH in 0.01M $CaCl_2$, total sulfur, chromium reducible sulfur, total carbon and carbonate content. Samples were also analysed for 'acid extractable' major and minor elements following microwave digestion. Selected samples were analysed for total major and minor elements including: heavy metals; metalloids; lanthanides; and actinides. These analyses were performed on the solution obtained following a mixed acid digestion (hydrofluoric + perchloric) using a combination of

ICP – OES and ICP – MS. For most analytes, this method gives the total amount present in the sample. Details are given in Appendix 1.

Mineralogy

Semi quantitative analysis of mineral composition was undertaken using power X-ray diffraction (XRD). Samples were finely ground and oven dried at 60°C then thoroughly mixed with an agate mortar and pestle. XRD patterns were recorded with a Philips PW1800 microprocessor-controlled diffractometer using Co K-alpha radiation. Analysis of the data was carried out using the program XPLOT (Raven 1990). Full method details are given in Appendix 1.6.

Microbiology

Until recently, the measurement of bacterial diversity and activity in sulfidic environments was a tedious and imprecise task. However, recent advances in molecular techniques are creating opportunities to better understand both the presence and activity of specific groups of microorganisms, without the need for complex culturing methods.

The survey of Riverland sulfidic materials was used to provide a preliminary assessment of the potential for molecular techniques to be used in this environment. Specifically, *i*) microbial diversity was assessed using the 16S ribosomal RNA gene sequence and *ii*) the potential for sulfide oxidation was measured through the presence of the sulfur oxidase B (*sox*B) functional gene in the sediments. The 16S ribosomal RNA gene is found in practically all organisms and is a standard tool to assess microbial diversity, as each species as its own genetic fingerprint for that gene. The *sox*B functional gene is present in all known sulfide oxidizers (it provides the code for an essential enzyme involved in the process of sulfide oxidation).

At each site, small subsamples (5–10 g) of sulfidic materials were collected from different sediment horizons and immediately preserved in 10 mL of 50 mM EDTA to prevent DNA degradation. Further details about the molecular techniques, sample extraction and sample analysis are provided in Appendices 1.7 and 1.8.

Results

Water chemistry

A wide range in water chemistry was found between wetlands (summary in Table 1; full dataset in Appendix 2). Salinity varied from fresh to hypersaline, with electrical conductivities ranging from 1.3 mS/cm in Merreti Lake to 120 mS/cm in pore water from the Berri Evaporation Basin. The pH values were neutral to alkaline (range 6.8 to 9.4) with the exception of the Loveday Disposal Basin and Bottle Bend Lagoon which were slightly acidic. Both calcium and bicarbonate were enriched relative to the seawater dilution line (Figure 3), with the exception of Bottle Bend Lagoon and Woolpolool Lake. Ferrous iron (Fe^{2+}) concentration was used as an indicator of the redox status of pore and surface water (with the presence of Fe^{2+} indicating more reducing environments). Fe^{2+} concentrations were generally less than the detection limit (0.05 mg/L) except in pore water from the Loveday, Ramco, Loveday and Berri disposal basins and in Bottle Bend Lagoon surface water (Appendix 2.2).

Table 1: *Summary results for Lower River Murray floodplain pore and surface waters.*

Location and site		EC (dS/m)	pH	Alkalinity (mg HCO_3^-/L)	SO_4^{2-} (mg/L)
Loveday Evap Basin	Site 1 Pit 1		7.6	600	440
	Site 2 Pit 2		6.5		8100
	Site 2 surface	16	4.5		
Ramco Lagoon	Surface	62	8.5	230	2100
	Inflow	2.4	8.3	810	210
	Pit 1	36	7.1	660	1700
	Pit 2	32	6.8	550	1200
Berri Evap. Basin	Surface	120	7.5	270	11000
Hart Lagoon	Pit 1	21	7.4	520	1400
Ross Lagoon	Surface	5.2	9.0	160	190
Woolpolool Lake	Surface	5	9.4	46	730
Merreti Lake	Surface	1.3	9.0	200	50
Bottle Bend Lagoon	Surface	13	5.5	2	280
Seawater		53	~8	150	1100
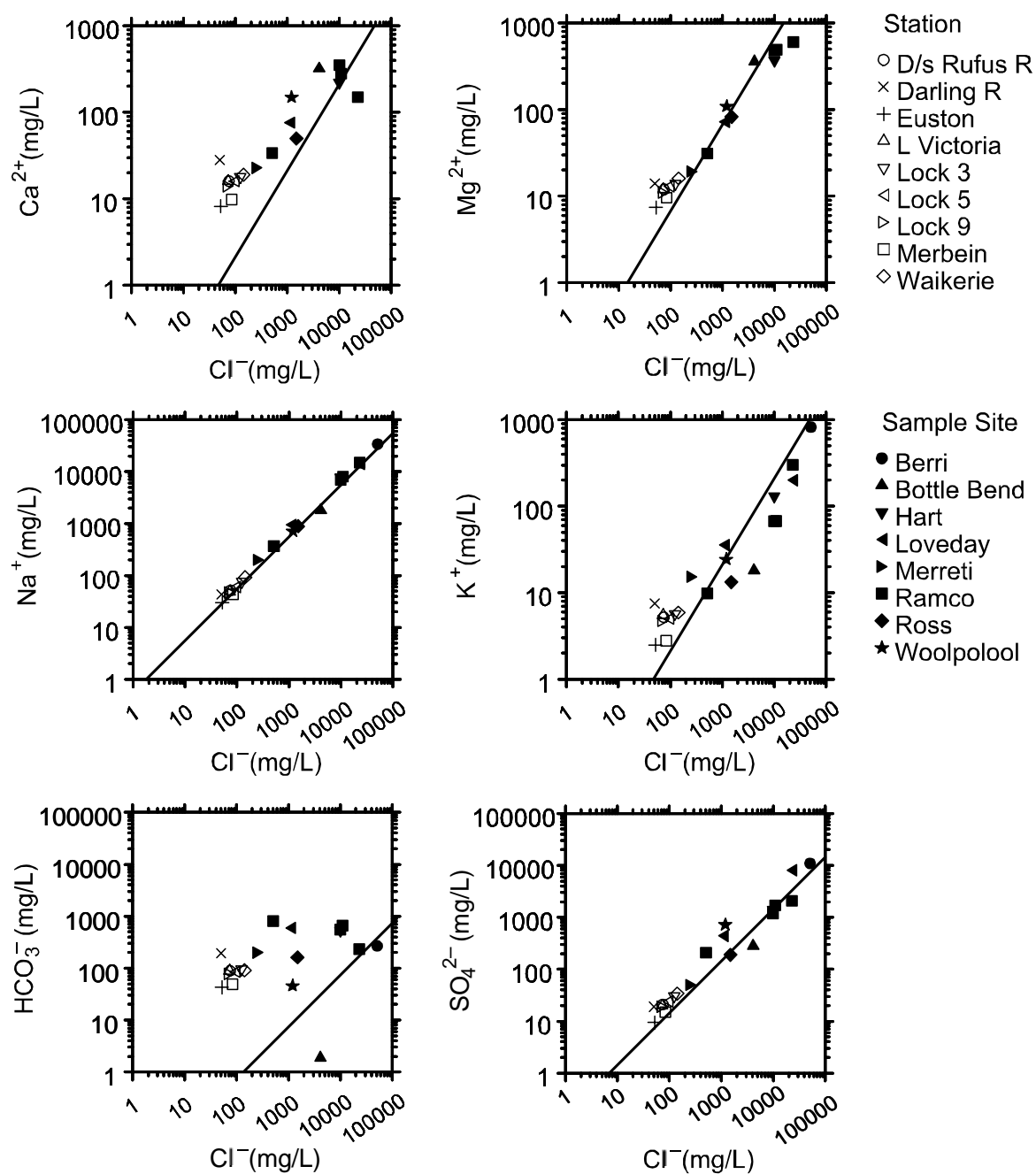
Figure 3: *Plot of major ion concentrations vs. chloride in Lower River Murray floodplain pore and surface waters. Mean values for compiled R. Murray sampling station data (Mackay et al. 1988) and the seawater concentration/dilution line are plotted on the graphs for comparison. Because solutes in waters from the Murray-Darling Basin are primarily derived from the deposition of marine aerosols (Herczeg et al. 2001), deviations from the seawater dilution line indicate changes in water composition through the interaction of the water with soil and rock minerals or microbiological transformations. Examples include pyrite weathering elevating sulfate concentration, calcite dissolution increasing carbonate/bicarbonate concentration and sulfate reduction at the sediment-water interface which removes sulfate from the water column.*

Sediment chemistry

Sediment texture and characterisation

Soil texture ranged from loamy sand and clayey sand to heavy clay in samples between wetlands (Appendix 2.1). In general, more sandy materials occur in the two wetlands, which had potential acid sulfate soil conditions and were at risk of acidification (i.e. Bottle Bend Lagoon and Loveday Disposal Basin).

Field measurements of the sediments

pH and Eh profiles

The redox environment is not always properly defined by field Eh measurements, especially when the overall redox potential is from of a combination of several redox couples. However, when the redox potential is dominated by one couple, Eh measurements are theoretically more meaningful. For example, Eh has been found useful in soils where the redox potential is primarily controlled by the Fe^{2+}/Fe^{3+} redox couple (Langmuir 1971; van Breeman 1973; van Breeman and Harmsen 1975; Bartlett 1986). In this case, constructing Eh-pH phase diagrams can assist in visualising the trends and patterns in the redox potential both within and between soil profiles. Furthermore, Eh (when expressed as pe = Eh(V)/0.059, or $-\log_{10}$ [e^- activity]) can be combined with pH and other redox sensitive species to better define the redox environment. For example, the equation to predict the conditions favourable for the reduction of ferric iron (Fe^{3+}) has been used to define the boundary between oxidising (or aerobic) and reducing soil environments (Bartlett 1986). For comparison, plots of data for four sediments on a traditional pH-pe diagram show clear trends between sediments but are less instructive to interpret patterns within sediment profiles (Figure 4). However, different patterns emerge when the same redox profiles are plotted in comparison to the equation for the reduction of amorphous ferrihydrite using (Stumm and Morgan 1996):

$$pe = pFe^{2+} + 16.02 - 3pH \qquad (1)$$

to construct the plots (Figure 5). For example, the profile from Bottle Bend Lagoon shows that the redox potential is close to the threshold for Fe^{3+} reduction near the surface but lower than this threshold at depth. This is consistent with the sediment textures observed in the field, which hinted at a layer of iron oxyhydroxides at the sediment-water interface and sulfides below (Plate 3). By contrast, in Merreti Lake the redox potential remained near the threshold for Fe^{3+} reduction throughout the profile and no visual evidence of sulfide accumulation (black sediments) was found (Plate 2). Another feature revealed by the diagrams is a tendency in disposal basins with sediments recently exposed by declining water levels (Ramco Pit 1 and 2, Loveday Pit 2 and 3) to be relatively more oxidised near the surface than at depth.

Peroxide field test

The reaction of a soil or sediment sample with hydrogen peroxide is used extensively in coastal ASS investigations as a qualitative test for the presence of sulfidic materials. The test has two attributes, reaction vigour and final pH. The vigour of the reaction estimates the amount of sulfidic material present and the final pH the neutralising capacity of the sample. Potential interferences are the presence of manganese nodules (false positive) and carbonates (increased reaction vigour). All samples tested had a vigorous response to the peroxide test. However, only samples from Bottle Bend Lagoon and those from some horizons at Loveday Disposal Basin had final pH values below 3.5 (Table 2 and Appendix 2.5).

Field pH

Field pHs in the disposal basin sediments were neutral to alkaline, ranging from 6.3 at the Berri Evaporation Basin to 8.6 at Loveday Disposal Basin (Appendix 2.5). Natural wetlands had slightly lower pHs ranging from 5.5 in dry sediment (Clover Lake) to 7.7 at Woolpolool Lake. An exception was the remaining shallow upper arm of Bottle Bend Lagoon where the sediment-water interface and precipitated iron oxyhydroxides had pH values of 4.5 and 5.2 respectively.
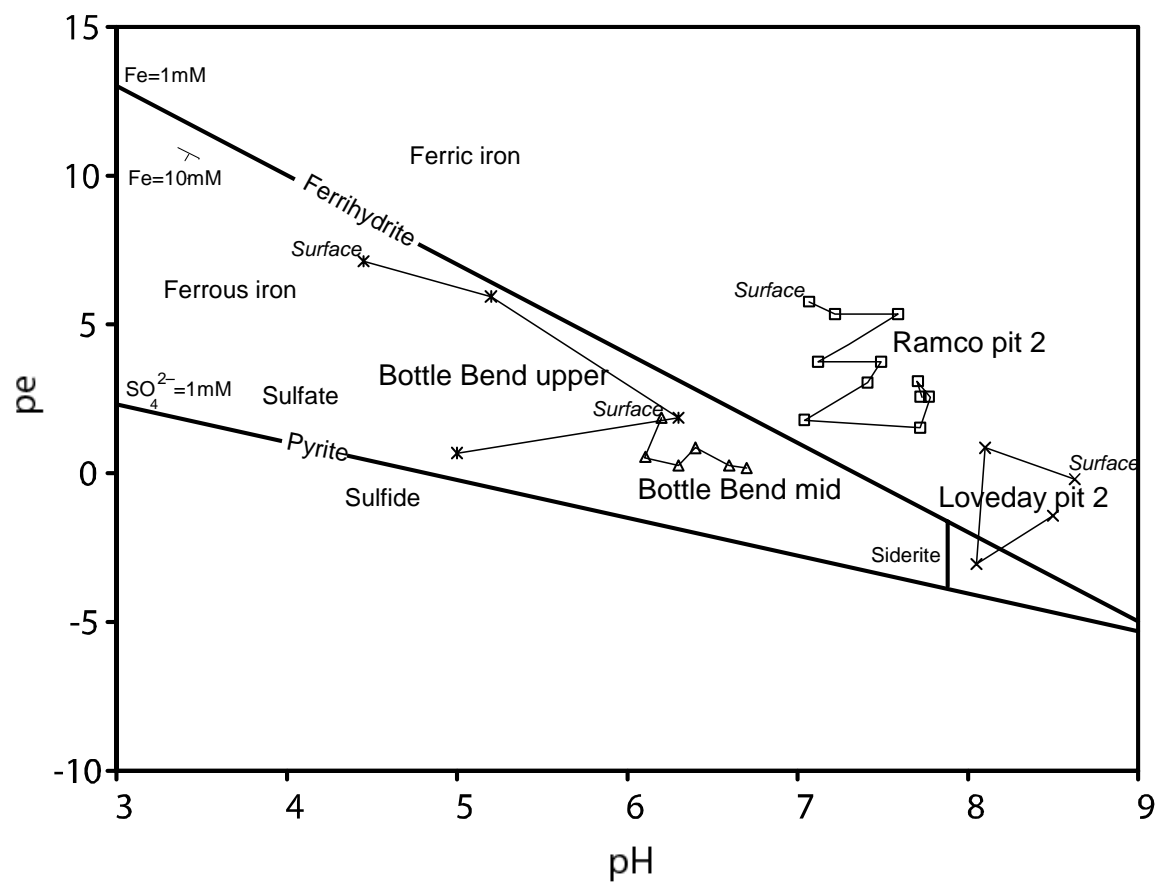
Figure 4: *pe-pH diagram showing iron and pyrite redox lines and siderite (FeCO₃) formation line. Field pe-pH data are plotted for selected profiles.*
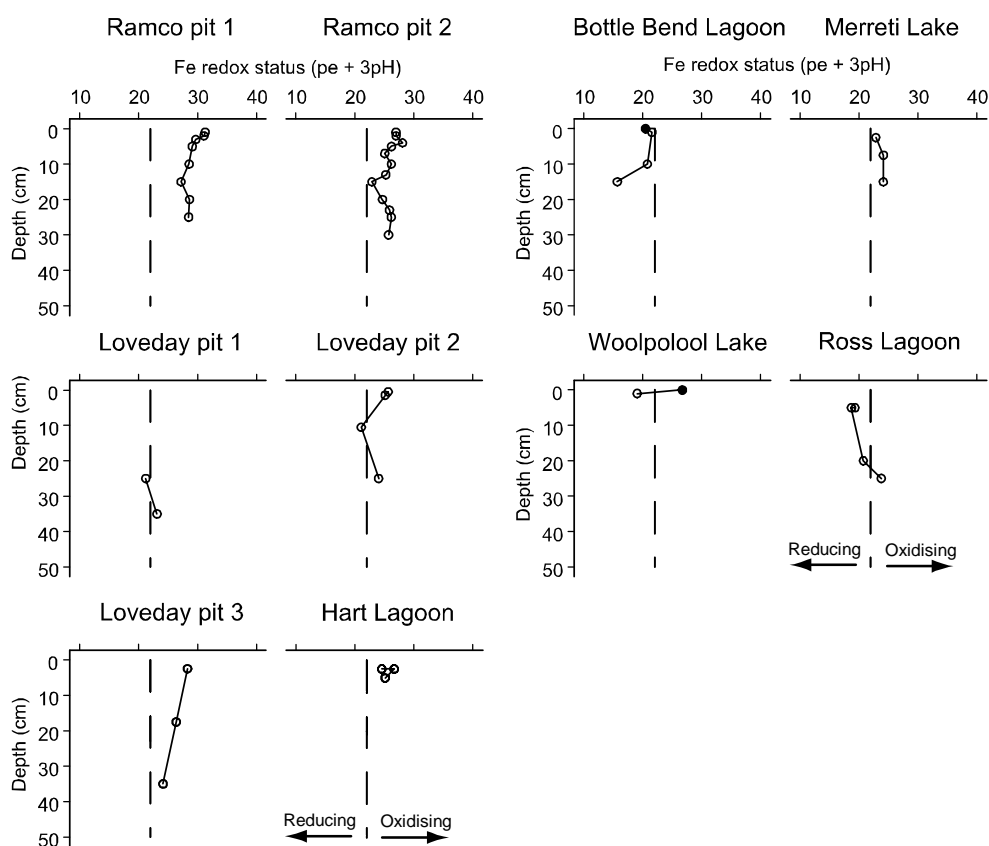
Figure 5: *Iron redox status for Lower River Murray floodplain sediment profiles. The vertical dashed line represents the Fe^{2+}/Fe^{3+} boundary when the ferrous iron concentration = 0.1 mg/L. Values to the left are 'reducing' and to the right 'oxidising'. Each 10-fold increase or decrease in ferrous iron concentration moves the line 1 unit to the left or right respectively. Filled circles for Bottle Bend Lagoon and Woolpolool Lake are for measurements made in the overlying water.*

Table 2: *Results for the peroxide oxidation field-test. A reaction to peroxide addition indicates the presence of sulfidic material. A final pH < 3.5 indicates a potential acid sulfate soil as the potential sulfidic acidity exceeds the neutralising capacity of the sediment and pore water.*

Location	Reaction	pH < 3.5
Loveday Evaporation Basin	Yes	**Some**
Ramco Lagoon	Yes	No
Berri Evaporation Basin	Yes	No
Hart Lagoon	Yes	No
Ross Lagoon	Yes	No
Woolpolool Lake	Yes	No
Merreti Lake	Yes	No
Clover Lake	Yes	No
Bottle Bend Lagoon	Yes	**Yes**
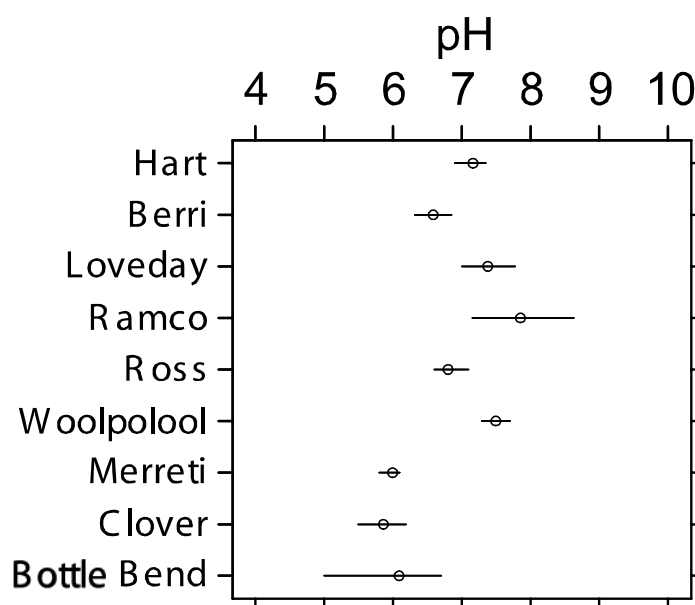
Figure 6: *Mean (circle) and range (horizontal line) for sediment field pH values.*

Soil laboratory analyses

Carbon

Carbon is present in soils and sediments in three broad forms: organic, inorganic (carbonates) and charcoal. In this study, we measured total carbon and carbonate carbon, with organic carbon calculated by difference. The estimates for organic carbon content will also include charcoal, which is of varying significance in Australian soils and sediments, however its inclusion will not affect this study. Total carbon and carbonate concentrations were higher in the disposal basins (mean of 2.2 and 1.0%C, respectively; Figure 7) relative to the natural wetlands (mean 0.90 and 0.27%C, respectively). However, the carbonate content in several wetlands was also below the detection limit of the analytical method used (0.06% as C or 0.5% as $CaCO_3$). To estimate the organic C content, a value of 0.03%C was used for carbonate concentration when below the detection limit. Organic matter was the main form of carbon in the natural wetlands (mean = 0.82%C; range 0.09% to 3.0%C) but disposal basins had greater organic C concentrations (mean = 1.6%C; range 0.05 to 6.8%C).
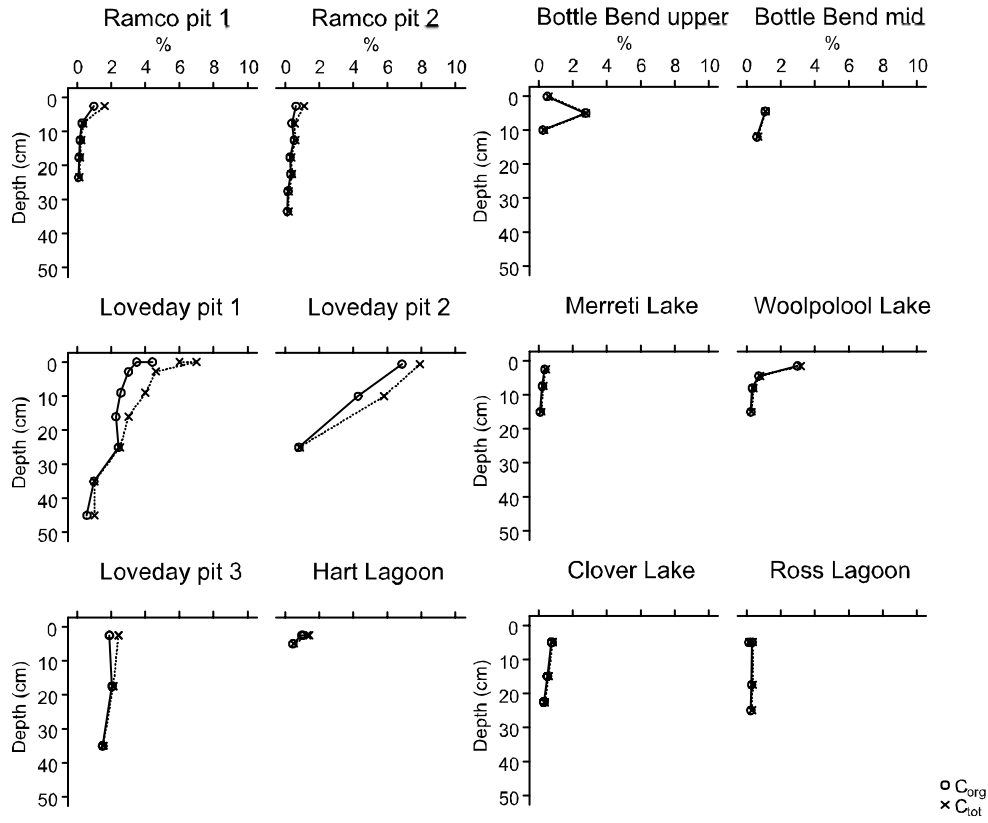
Figure 7: *Total and organic carbon profiles for Lower River Murray floodplain wetlands.*

Sulfur

Common analyses to quantify and speciate sulfur in sediments include total sulfur (S_{tot}), sulfate sulfur, acid volatile sulfur (AVS) or monosulfides, chromium reducible sulfur (S_{Cr}), pyrite and organic sulfur (S_{org}). In this study we measured total sulfur by combustion in a high temperature induction furnace and reduced sulfur (S_{red}) using the chromium reduction method. The relationship between these is:

$$S_{tot} = S_{red} + S_{oxid} + S_{org} \qquad (2),$$

where

$$S_{red} = S_{Cr} = AVS + pyrite \qquad (3).$$

Equation 2 was used to calculate oxidised plus organic sulfur ($S_{oxid+org}$) by difference.

Total and reduced sulfur concentrations were highest in the disposal basins (mean of 1.2 and 0.28 %S, respectively) when compared to the natural wetlands (mean of 0.14 and 0.04% S, respectively; Figure 8 and Appendix 2.7). To put these reduced S concentrations in perspective, they exceeded the recommended guidelines to trigger management action in the coastal ASS context in disposal basins and in some of the natural wetlands (Figure 8). There was a clear pattern in the distribution of S_{red} and $S_{oxid+org}$ within profiles in disposal basins (Figure 9). In the drier Ramco Pit 1, $S_{oxid+org}$ was highest near the surface but declined with depth, whereas this pattern was less pronounced in the wetter Ramco Pit 2. S_{red} concentrations were elevated (ca. 0.5%) within peds formed in drying sediments at Loveday Pit 1 but most of the total S was as $S_{oxid+org}$. A smaller proportion of the S was as $S_{oxid+org}$ in Loveday Pit 2 and 3.
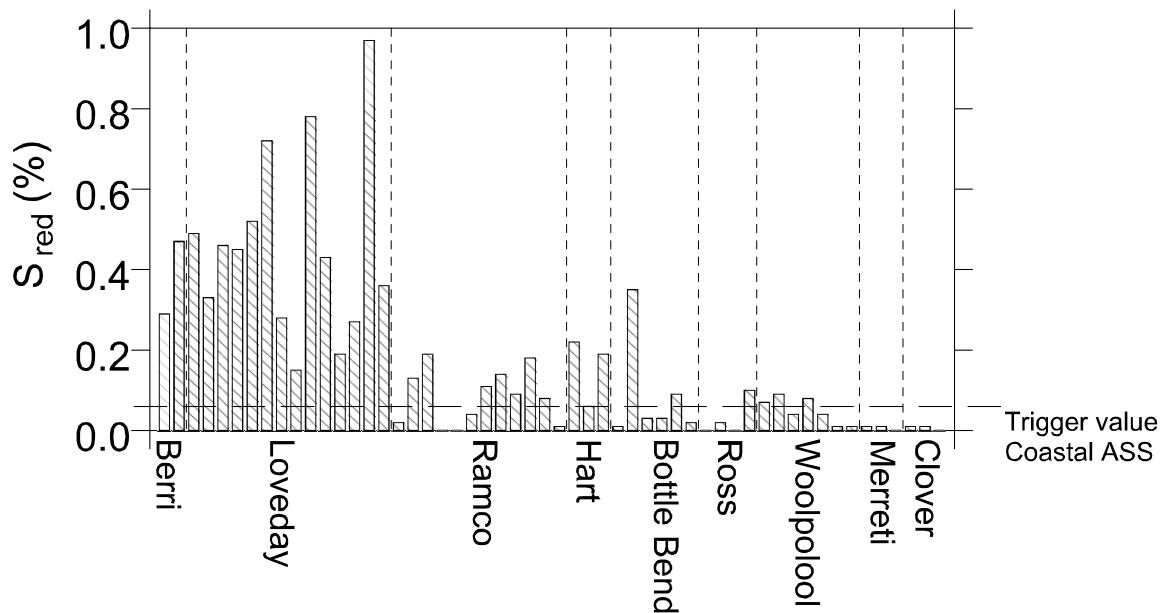
Figure 8: *Reduced sulfur in Lower River Murray floodplain samples. To put these values in perspective, the horizontal dashed line represents the trigger value (0.06%) for further investigation for medium textured sediments in coastal ASS environments (trigger values are 0.1% for fine textured and 0.03% for coarse textured sediments).*
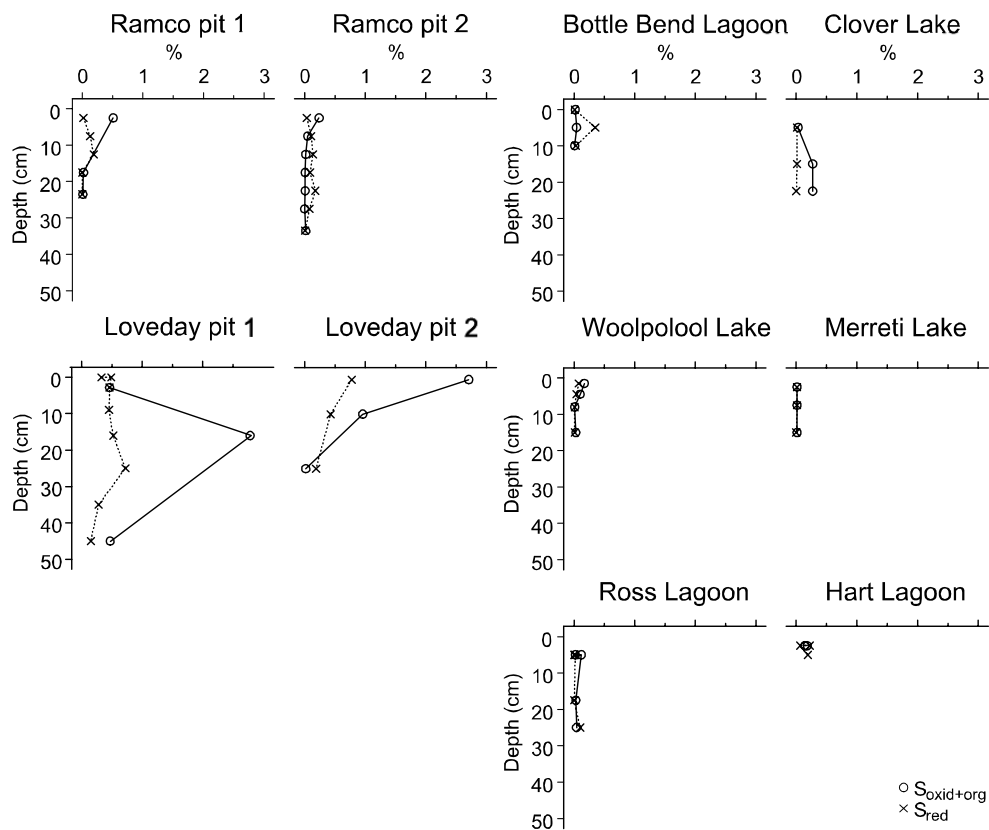
Figure 9: *Oxidised and reduced sulfur profiles for Lower River Murray floodplain wetlands.*

Gross and net acid generation potential

The gross acid generating potential, or potential sulfidic acidity, was calculated from the concentration of reduced sulfur. Each mole of reduced sulfur can generate 2 moles of acid (H^+) (see Equations 9 and 13 in *Discussion*). The acid generating potential was highest in the disposal basins, with a mean value of 170 millimoles H^+/kg and a range from 600 to 1 millimoles H^+/kg. In other wetlands, the mean acid generating potential was 28 millimoles H^+/kg, with a range of 2 to 220 millimoles H^+/kg. The trigger value of 0.06 %S for medium textured coastal sediments represents 37 millimoles H^+/kg.

The net acid generation potential (NAGP) is a measure used to assess the potential for acidification in acid mine drainage and coastal acid sulfate soil (NSW ASSMAC 1998). NAGP is the gross acid forming capacity minus the acid neutralising capacity (ANC) of a rock, soil or sediment.

$$NAGP = Acid\ generating\ potential - ANC \qquad\qquad (4)$$

We used the carbonate concentration as a measure of ANC and the reduced sulfur concentration to calculate the gross acid generating potential. Where the carbonate concentration was below the detection limit the NAGP was not calculated. Most sediments had excess ANC so that NAGP was negative (Figs 10 and 11). However, two horizons from the Loveday Evaporation Basin had positive NAGP values of 11 and 24 kg $CaCO_3$ (Figure 11). In addition, using our analytical detection limit for carbonates, at least one horizon from Bottle Bend Lagoon had a positive NAGP (Figure 10). The net acidification risk may be underestimated in the natural wetlands because $CaCO_3$ may be much lower than the detection limit in some cases. On the other hand, clays and dissolved carbonates in pore and surface water would also contribute to ecosystem ANC at the scale of the wetlands and were not included in our estimate of NAGP.
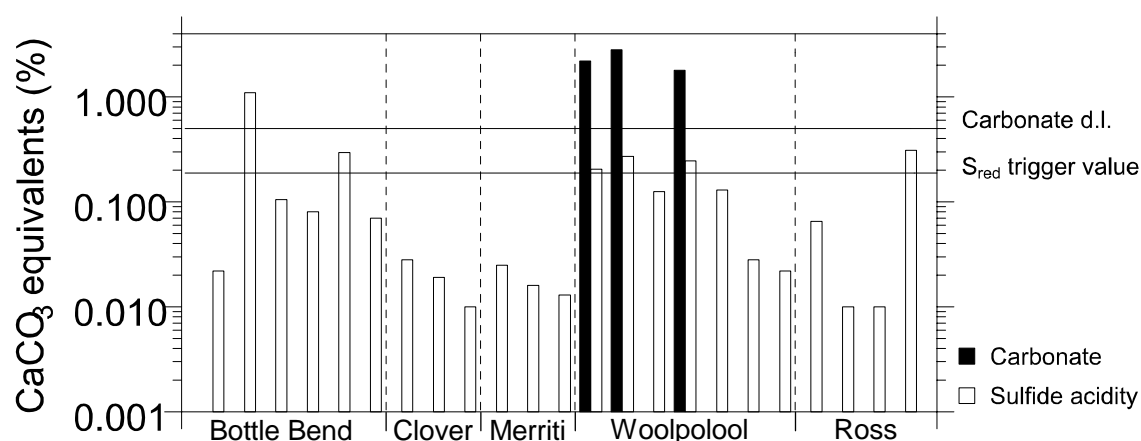
Figure 10: *Carbonate concentration and potential sulfidic acidity in samples from natural wetland areas. Results are expressed in calcium carbonate equivalents. The difference between carbonate and sulfide represents the net acid generation potential. The upper horizontal line represents the detection limit for the carbonate method used and the lower horizontal line the trigger value for reduced sulfur in medium textured sediments. Carbonate concentrations below the detection limit are not shown.*
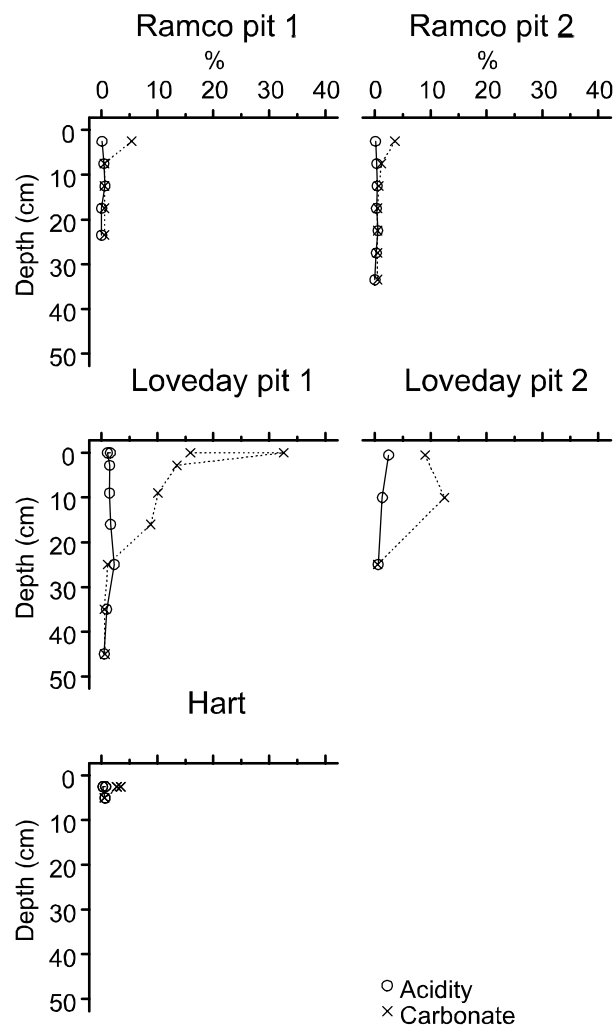
Figure 11: *Carbonate concentration and potential sulfidic acidity in samples from disposal areas. Results are expressed in calcium carbonate equivalents (%). The difference between carbonate and sulfide represents the net acid generating potential.*

General elemental composition of the sediments

Selected sediment samples were analysed for total major, minor and trace elements (Appendix 2.8 and 2.9) These concentrations were compared with sediment quality guidelines (ANZECC & ARMCANZ 2000). Nickel concentrations were above the interim sediment quality guideline (ISQG) trigger value of 21 mg/kg for some samples in wetlands other than Ross Lagoon and Merreti, but below the upper value of 52 mg/kg. Copper concentrations were above the trigger value of 65 mg/kg but below the upper value of 270 mg/kg in Ramco, Berri, Hart and Bottle Bend.

Mineralogy

XRD measurements were made to confirm the presence or absence of gypsum and pyrite in sediment samples. However, monosulfides are usually amorphous or poorly crystalline and not usually identifiable in XRD powder patterns. Pyrite was identified in 20 of the 29 samples (Table 3 and Appendix 2.10). In some samples from Ramco Lagoon, Hart Lagoon and Woolpolool Lake, gypsum was absent suggesting that organic sulfur rather than sulfate to be the major non sulfide sulfur present in the sample.

Table 3: *Sulfur minerals identified and inferred (when labelled with "?") in selected sediment samples.*

Site and sample description		Depth (cm) upper	lower	Reduced XRD	% S_{Cr}	Organic + oxidised XRD	% $S_{oxid + org}$
BBL upper	surface ppt	0	0.2	Pyrite	0.01		0.02
	sulfidic	0	10	Monosulfides?	0.35		0.04
	clay	10+			0.03		0.01
	dry	0	5		0.03	Gypsum	0.09
BBL mid		0	9	Pyrite	0.09		0.03
		9	15	Pyrite	0.02		0.01
Wpl L	Samphire	0	3	Pyrite	0.08	Organic?	0.17
		6	10	Pyrite	0.01		0.01
		10	20		0.01		0.03
Hart Lg	sulfidic	0	5	Monosulfides?	0.22	Organic?	0.14
Ramco	Pit 1	10	15	Pyrite	0.19		<0.01
	Pit 2	0	5	Pyrite	0.04	Organic?	0.24
		10	15	Pyrite	0.14		0.02
		20	25	Pyrite	0.18		0.01
BEB	sulfidic			Monosulfides?	0.29	Gypsum	1.09
	non-sulfidic			Monosulfides?	0.47	Gypsum	0.84
LDB	Site 1 Pit 1	0		Pyrite	0.33		1.40
		0.5	5	Pyrite	0.46	Gypsum	0.46
		5	12	Pyrite	0.45	Organic?	0.41
		12	20	Pyrite	0.52	Organic?	0.39
		20	30	Pyrite	0.72		<0.01
		30	40	Pyrite	0.28		<0.01
		40	50	Pyrite	0.15		0.07
	Site 2 Pit 2	0	1	Monosulfides?	0.78	Gypsum	2.71
		1	20	Monosulfides?	0.43	Gypsum	0.96
		20	30	Pyrite	0.19		0.02
	Site 2 Pit 3	0	5	Pyrite	0.27	Gypsum	0.86
		5	30	Pyrite	0.97		0.04
		30	40	Pyrite	0.36		<0.01

See Appendix 2.1 for site name abbreviations used in tables.

Table 4: *Minerals identified in samples according to acidic (pH <4) and alkaline (pH >4) conditions*

Mineral	Acid (<pH 4)	Alkaline (>pH 4)
	Abundance (Range %)	
Calcite (aragonite)	0	2 – 33
Halite	1 – 10	1 – 30
Pyrite	0.5 – 2	0.3 – 2
Gypsum	0 – 2	1 – 5
Quartz	30 – 66	8 – 50
Mica (illite)	10 – 30	15 – 20
Smectite	5 – 15	10 – 20
Other: (e.g. Kaolin, Orthoclase)	10 – 30	15 – 60

As expected, acidic environments have higher quartz and lower calcite (low neutralising) contents indicating the low buffering and neutralising capacity present in the samples. In general, samples from alkaline environments generally have high calcite, halite, gypsum and smectite contents (Table 4).

Salt Efflorescences

Evaporite minerals listed in Tables 3 and 4 and Appendix 2.10 only include those which could be identified with absolute certainty by powder XRD. Salt efflorescences consist of mainly halite, gypsum and calcite salts. Peak positions for the calcite phase suggest significant Mg substitution (Figure 12). The level of substitution varies between samples and in several samples (i.e. LDB 1.1, 2.1 and 2.3) approaches the theoretical limit. Samples with high amounts of aragonite show shifts in the "standard" peak positions due

to element substitution. The total element analysis suggests Sr as the dominant substituted element (Appendix 2.9).
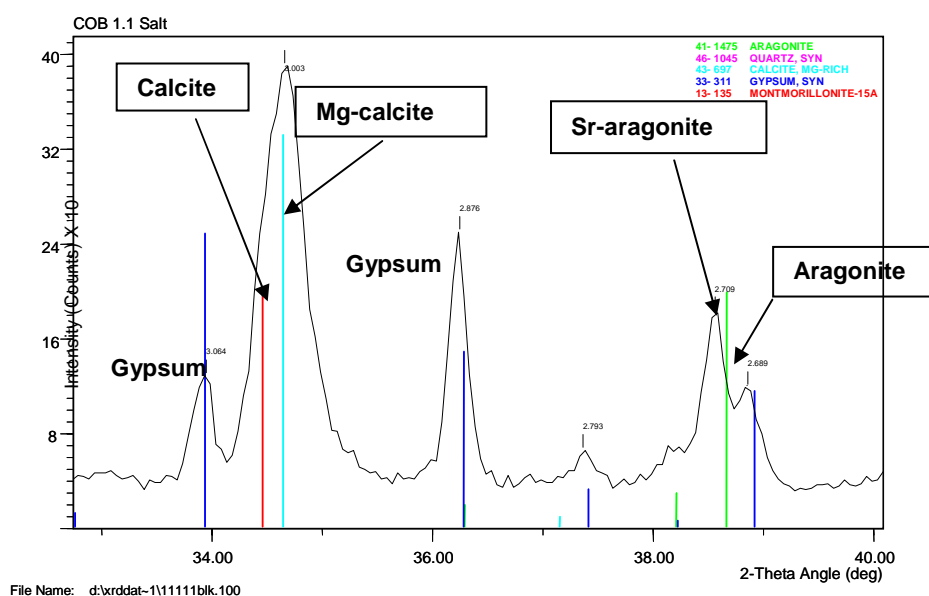
Figure 12: *XRD pattern of salt efflorescence in platy fragments from coatings on ped surfaces (Plate 1 and Figure 17). Aragonite peak shows shifts in the "standard" peak positions due to element substitution (XRF data suggests Sr as the dominant substituted element), Peak positions for calcite indicates significant Mg substitution and gypsum peaks have no shifts.*

Microbiology

Detection of soxB sulfur oxidase gene

The results of the detection of the *sox*B sulfur oxidase gene in DNA extracts from sulfidic sediments are summarised in Table 5. The *sox*B gene was detected in the majority of samples indicating that the biological potential for sulfide mineral oxidation was present in these samples (electrophoresis gels showing the detection of the *sox*B gene fragment are summarised in Appendix 3). A notable exception to this pattern was Bottle Bend Lagoon where only three out of the seven samples tested were positive for *sox*B gene presence. The levels of the *sox*B gene differed between sites and within sites (Table 5). For instance, samples from Ramco (no. 5, 6 and 44) and Bottle Bend (no. 34) displayed comparatively high levels of the *sox*B gene, indicating significant biological potential for sulfide mineral oxidation. These samples were either at the sediment-water interface or near the top of the sulfidic horizon, suggesting that the window for sulfide oxidation in the sediment profiles was narrow.

The detection of the biological potential for sulfide mineral oxidation in these environments is not unsurprising, as the chemolithoautotrophic S-oxidising bacteria are generally considered to be present in environments rich in S minerals. However, what these results do demonstrate is that molecular techniques can be successfully applied to the rapid detection of biological S mineral oxidiser populations in these environments.

Table 5: *Detection of* sox*B chemolithoautotrophic sulfur oxidase gene in floodplain sediments. See Appendix 1.8 for sample descriptions.*

Location	Sample No.	Presence of *sox*B gene
Ramco Lagoon Pit 1	1	++
Ramco Lagoon Pit 1	2	-
Ramco Lagoon Pit 1	3	-
Ramco Lagoon Pit 1	4	++
Ramco Pit 2	5	+++
Ramco Pit 2	6	+++
Ramco Pit 2	7	+
Ramco Pit 2	8	+
Ramco Lagoon Fringe	9	-
Ramco Lagoon Fringe	10	++
Ramco Lagoon Fringe	11	++
No sample	12	-
Hart Lagoon	13	+
Hart Lagoon	14	++
Hart Lagoon	15	++
Hart Lagoon	16	
Hart Lagoon	17	++
Hart Lagoon	18	+
No sample	19	
Ross Lagoon	20	++
Ross Lagoon	21	+
Lake Woolpolool	22	+
Lake Woolpolool	23	+
Lake Woolpolool	24	+
Lake Merreti	25	+
Lake Merreti	26	+
Bottle Bend Lagoon Site 1	27	+
Bottle Bend Lagoon Site 1	28	+
Bottle Bend Lagoon Site 1	29	-
Bottle Bend Lagoon Site 2	30	-
Bottle Bend Lagoon Site 2	31	-
Bottle Bend Lagoon Site 2	32	-
No sample	33	
Bottle Bend Lagoon Site 2	34	+++
Loveday Pit 1	35	+
Loveday Pit 1	36	+
Loveday Pit 3	37	+
Loveday Pit 3	38	+
Loveday Pit 2	39	+
Berri Evap Basin	40	+
Berri Evap Basin	41	+
Loveday Basin	42	+
Loveday Basin	43	+
Ramco Lagoon	44	+++
Ramco Lagoon	45	-

Bacterial population diversity analysis, application of 16S DGGE analysis to sulfidic sediments

Bacterial population diversity analysis, based on analysis of the bacterial 16S gene was successfully applied to all samples collected along the Murray Floodplain (examples of gel banding patterns are summarised in Appendix 3). Preliminary results showed both within and between site variability in community diversity (based on the gel banding patterns). However, a number of common distinct bands (species) appeared indicating a specific microflora is associated with sulfidic sediments.

The successful separation of species on DGGE gels provides the future opportunity to isolate and determine the genetic sequences of individual species, allowing the identification of species present in floodplain sediments based on their unique 16S gene sequences. In sulfidic sediments both oxidative and reductive process are known to proceed simultaneously, through a number of pathways and intermediate species with accumulation or destruction of sulfide the net of these processes. Extension of these studies to determine relative abundance of species and their activity will provide information to assist the estimation of both gross formation and net accumulation rates of sulfides in sediments.

Discussion

The survey clearly demonstrated that deposits of sulfidic materials are common in wetlands of Lower River Murray floodplains. The extent of these deposits is related to salinity and the extent and duration of waterlogging in the wetlands, with greater accumulation in saline, permanently flooded environments as opposed to those that are either fresh or with more regular wetting and drying cycles. Many of the sites with significant sulfidic material deposits also had environmental issues associated with them, including noxious smells in Riverland disposal basins and acidification at Bottle Bend Lagoon (McCarthy et al. 1993). The widespread occurrence of sulfidic materials also lends support to anecdotal evidence of deoxygenation events promoted by the resuspension of sediments during wetting/drying operations in some River Murray wetlands.

Our study has also highlighted that the issue of sulfidic materials in inland river environments has some similarities and differences with the one of coastal acid sulfate soils. In general, we found that the some of the tests used to assess the management risks in coastal ASS were also useful for the inland context. An exception was the standard manometric method used to measure carbonate concentration in sediments, which was not sensitive enough for inland environments. A significant difference between inland and coastal sulfidic material deposits is their age. In the River Murray floodplain environment, many of the sulfidic material deposits appear to have accumulated relatively recently (years to decades) whereas those in the coastal context would tend to be older (centuries to millennia). The age of formation will influence the properties of the sulfidic materials between these environments, including a more common occurrence of surficial, poorly consolidated and reactive monosulfide deposits in the inland context. Assessing the environmental risks associated with inland sulfidic materials could also be more complex because different risks are involved and could be site specific, whereas acidification is the main risk usually considered in coastal environments. While poorly understood at this stage, the deoxygenation risk may be the most widespread one within the context of the Murray-Darling Basin (Sullivan and Bush 2003). The current guidelines to assess the acidification risk in coastal ASS will also be useful for acidification in the inland context, but less useful to characterise the deoxygenation or aesthetic (noxious smell) risks. It is not possible to define these later risks at the present because 1) the mechanisms of deoxygenation and noxious smells generation are not well known and 2) there are no established management objectives for these risks.

In the following, the mechanisms of sulfidic materials formation and oxidation will be reviewed in more details to help understand how to quantify the acidification and deoxygenation risks for the inland context. Furthermore, the potential mechanisms of acidity generation during water level manipulations in River Murray wetlands will be assessed by reviewing the recent acidification event at Bottle Bend Lagoon. Preliminary recommendations for the management of sulfidic materials in River Murray floodplains and current knowledge gaps will be presented.

Rate limiting factors for the formation of sulfidic material in River Murray wetlands

An anaerobic environment and the availability of sulfate, carbon and iron are the potential rate-limiting factors in the formation of sulfidic material deposits. Holmer and Storkholm (2001) quote threshold sulfate concentrations ranging from 8 to 40 μM (0.8 to 4 mg SO_4/L) to induce sulfate reduction and Berner (1984) gives a value of 5 mM (490 mg SO_4/L) for the concentration where sulfate reduction rates are independent of sulfate concentration. Sulfate concentrations in all waters sampled were above the threshold value to induce sulfate reduction and in many cases, concentrations were high enough for sulfate reduction to be independent of concentration (Table 1). However, sulfate reduction rates could still be sulfate-limited by low sulfate diffusion rates across the sediment-water interface. These sulfate diffusion rates will be wetland specific as they are a function of the sulfate concentration gradient at the sediment-water interface and the physical properties of the sediments (Stumm and Morgan 1996). The formation of iron sulfide compounds also requires a source of reactive iron. However, unlike in an oceanic setting, iron availability is usually not limiting in terrigenous sediments characteristic of inland environments (but see Cook and Schindler 1983 and Carignan and Tessier 1988). Organic matter has a dual role in the formation of sulfidic materials as it contributes both to the generation of an anoxic environment (through aerobic decomposition) as well as providing the reductant for sulfate reduction. In addition, the type of the organic carbon present also

influences the extent and rate of formation of iron monosulfides and pyrite. The overall sulfate reduction process can be represented by the simple reaction (Berner 1984):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^{-}$$ (5).

A preliminary assessment of the rate limiting factors for sulfate reduction in Riverland wetlands can be made using sediment concentration data and empirical relationships developed by Raiswell and Berner (1985). These relationships compare sediment organic carbon concentration with percent reduced sulfur, the "degree of pyritization" (DOP) and the percentage of total iron. DOP is defined as (Berner 1970):

$$DOP_T = \frac{\text{pyrite iron}}{\text{total iron}}$$ (6),

where the subscript refers to whether total "T" or reactive "R" iron is used in the denominator of expression. Because organic carbon and Fe are often co-deposited, the DOP index helps to assess organic carbon limitation of iron sulfide formation independently from Fe concentration in sediments.

Disposal basins and natural wetlands had positive relationships between $\%C_{org}$ and $\%S_{red}$ (Figure 13) and between $\%C_{org}$ and DOP_T (Figure 14), suggesting that organic carbon availability is limiting iron sulfide formation. This is further supported by the lack of correlation between $\%C_{org}$ and $\%Fe_{total}$ in the same sediments (Figure 15). These relationships between $\%C_{org}$ and either $\%S_{red}$ or DOP_T are different between disposal basins and natural wetland, with steeper slopes and intercepts significantly greater than 0 in disposal basins. Possible interpretations for the differences in slopes include a greater lability of organic matter in disposal basins or a rate limitation of sulfate reduction in natural wetlands because of lower sulfate concentrations. Greater intercepts in disposal basins suggest an external source of iron sulfide to the sediments, possibly by the formation in a partially anoxic water column. Low oxygen concentrations in the water column is not an uncommon feature of River Murray wetlands, especially during summer (D. Baldwin, CSIRO Land and Water, *personal communication*).
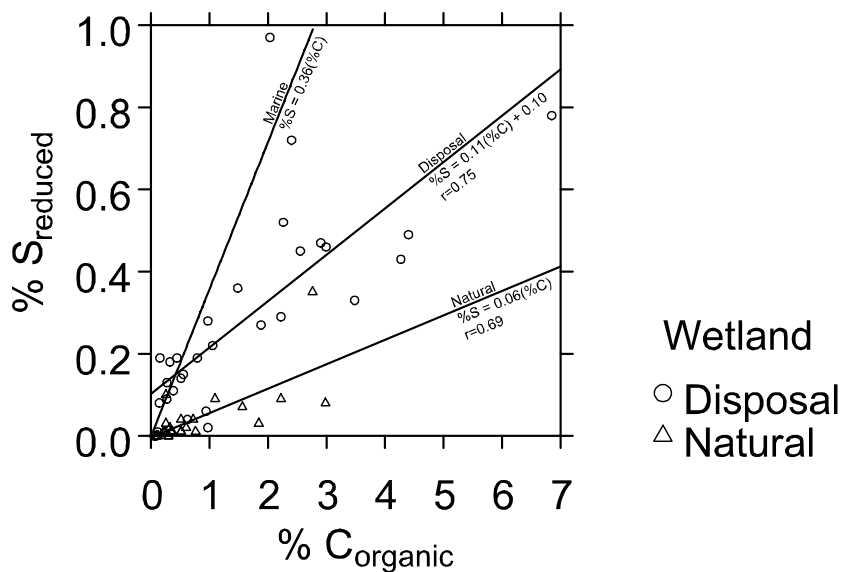
Figure 13: *Reduced sulfur versus organic carbon for Lower River Murray floodplain samples. (The line for normal marine sediments from Berner, 1984). All regressions coefficients statistically significant at P < 0.001, and Disposal basin intercept significantly different than 0 at P <0.05.*
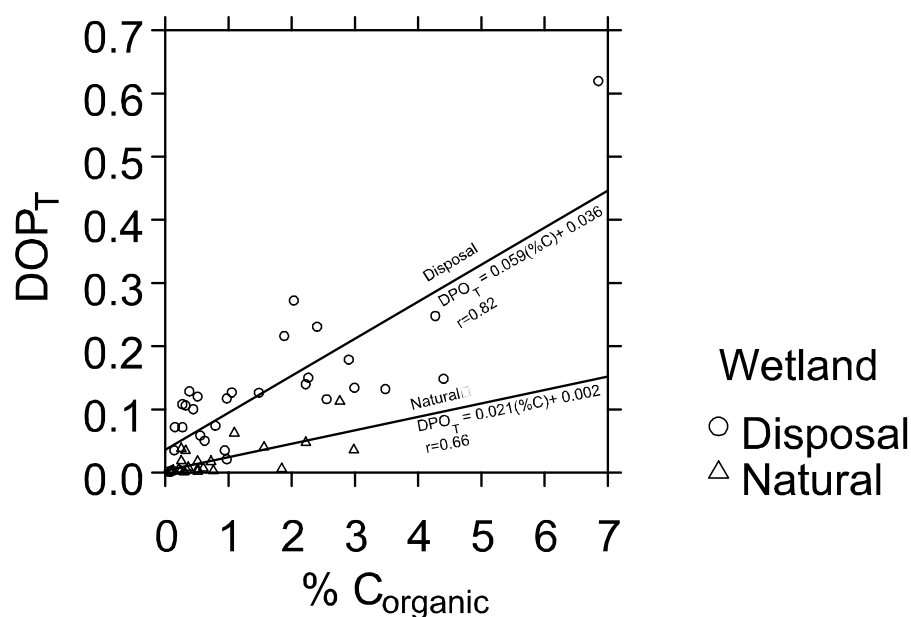
Figure 14: *The degree of pyritization (DOP_T) of total iron in sediment samples assuming all reduced sulfur is present as pyrite. All regression coefficients statistically significant at P <0.001 and the Disposal Basin intercept significantly different than 0 at P < 0.02.*
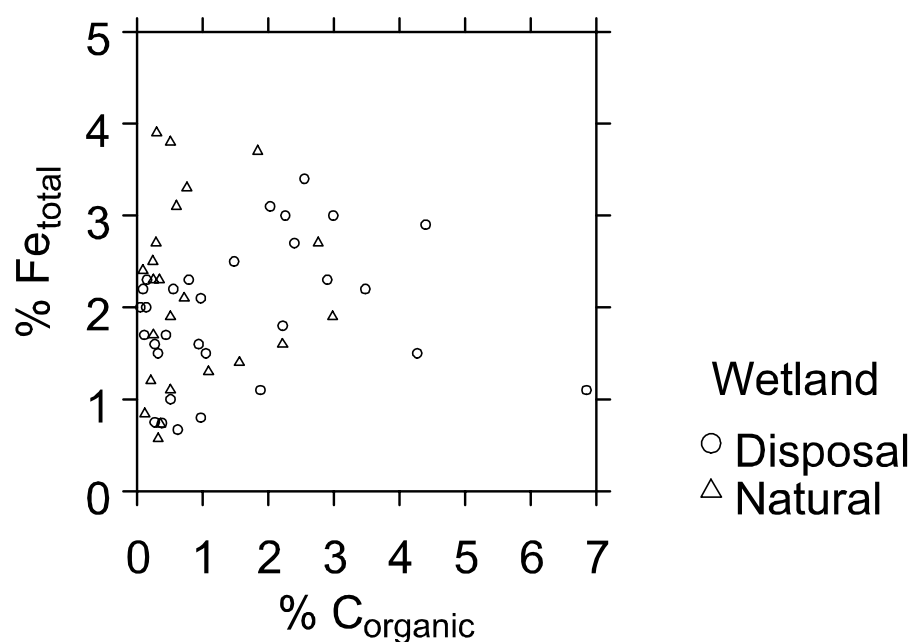
Figure 15: *Total iron vs. organic carbon. The iron content is poorly correlated with organic carbon (r=0.13).*

In summary, based on this preliminary investigation, the availability of reactive carbon limits the formation of sulfidic materials in River Murray wetlands. Iron and sulfate availability are either not limiting or are of secondary importance. There is also evidence of both syngenetic ("within water columns") and diagenetic ("within sediments") formation of sulfidic materials. The source of the elevated organic C concentrations found in saline wetland sediments is unclear at this stage. However, benthic algae could be an important source of primary production in this environment because of a suitable light environment and a high availability of nutrients. The favourable light environment would be promoted by the generally shallow depth of saline wetlands and the relatively lower turbidity levels promoted by higher salinities.

Mechanisms of deoxygenation and acid generation following the disturbance of sulfidic materials

Sulfidic materials are stable when left undisturbed in anoxic conditions. When they are exposed to oxygen (for example following a reduction in water level or by suspension in the water column), they react with oxygen to generate sulfuric acid (Figure 16). The chemical reactions during the oxidation of sulfidic materials will vary depending on whether monosulfides or pyrite is the main form of reduced S present. For iron monosulfides, the reaction sequence starts with the oxidation of sulfur by oxygen:

$$2FeS + 4O_{2\,(aq)} \rightarrow 2Fe^{2+} + 2SO_4^{2-} \tag{7}.$$

If oxygen is still present, this will be followed by ferrous iron oxidation and ferric iron hydrolysis

$$2Fe^{2+} + \tfrac{1}{2}\,O_{2(aq)} + 5H_2O \rightarrow 2Fe(OH)_3 + 4H^+ \tag{8}.$$

Note that this second set of reactions can be sluggish (especially at low pH or low O_2 concentrations) and may occur some distance away from the point of origin of the sulfidic materials. The overall reaction for monosulfide oxidation is:

$$2FeS + {}^9\!/_2\,O_{2(aq)} + 5H_2O \rightarrow 2Fe(OH)_3 + 2SO_4^{2-} + 4H^+ \tag{9}.$$
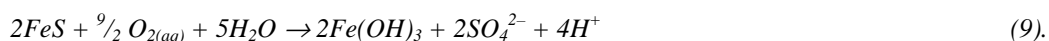
Thus, two moles of H^+ are generated for each mole of monosulfides that are oxidised. However, under conditions of limited oxygen availability, reaction (7) can deplete the oxygen in the water column without generating any acidity.

The sequence of reactions during pyrite oxidation are more complex. Both an inorganic sequence of reactions (10) to (12) and bacterially mediated reactions (*Metallogenium spp.*) have been proposed for the initial oxidation of pyrite. However, there is agreement that once the pH decreases to between 4 and 4.5 Thiobacillus ferroxidans greatly increases the rate of oxidation through a catalytic process (Nordstrom 1982). In more detail, the initial reaction includes two steps for the oxidation of sulfur:

$$FeS_2 + \tfrac{1}{2}\,O_{2(aq)} + 2H^+ \rightarrow Fe^{2+} + S^0_2 + H_2O \;(slow) \tag{10},$$

$$S^0_2 + 3O_{2(aq)} + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+ \;(fast) \tag{11},$$

followed by ferrous iron oxidation and ferric iron hydrolysis:

$$Fe^{2+} + {}^5\!/_2H_2O + {}^1\!/_4O_2 \rightarrow Fe(OH)_{3(s)} + 2H^+ \tag{12}.$$
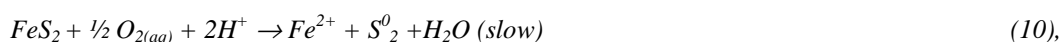
The overall reaction for pyrite oxidation is:

$$FeS_2 + {}^{15}\!/_4\,O_{2(aq)} + {}^7\!/_2H_2O \rightarrow Fe(OH)_3 + 4H^+ + 2SO_4^{2-} \tag{13}.$$
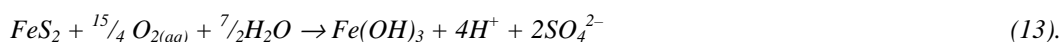
When the pH drops below 4, the rate of pyrite oxidation is increased by the catalytic oxidation of ferrous iron to ferric iron by the bacterium *Thiobacillus ferroxidans* (reaction 14), which acts as a pyrite oxidant (reaction 15) with regeneration of ferrous iron. These reactions are fast compared with reactions (10) and (12):

$$15Fe^{2+} + {}^{15}\!/_4O_{2(aq)} + 15H^+ \xrightarrow{\;T.\,ferroxidans\;} 15Fe^{3+} + {}^{15}\!/_2H_2O \tag{14},$$

$$FeS_{2(s)} + 14Fe^{3+}_{(aq)} + 8H_2O \rightarrow 15Fe^{2+} + 16H^+ + 2SO_4^{2-} \tag{15}$$
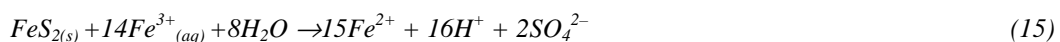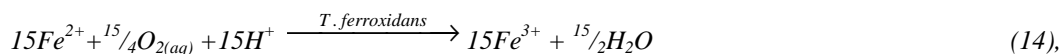
and are followed by further acid generation through the hydrolysis of ferric iron:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \tag{16}.$$

The overall reaction for the catalytic oxidation of pyrite is the same as for the one promoted by oxygen (equation 13).

There are some similarities and differences between the oxidation of monosulfides and pyrite. Both consume oxygen from the water column, either through the oxidation of sulfide sulfur (monosulfides), elemental sulfur (pyrite) or ferrous iron (both). Every mole of monosulfides oxidised produce two moles of acid (H^+), while each mole of pyrite produces four. One of the key differences between the two is the rapid initial reaction rate of monosulfides, which can result in complete deoxygenation of the water column (when sediments are resuspended) but a limited initial generation of acidity.
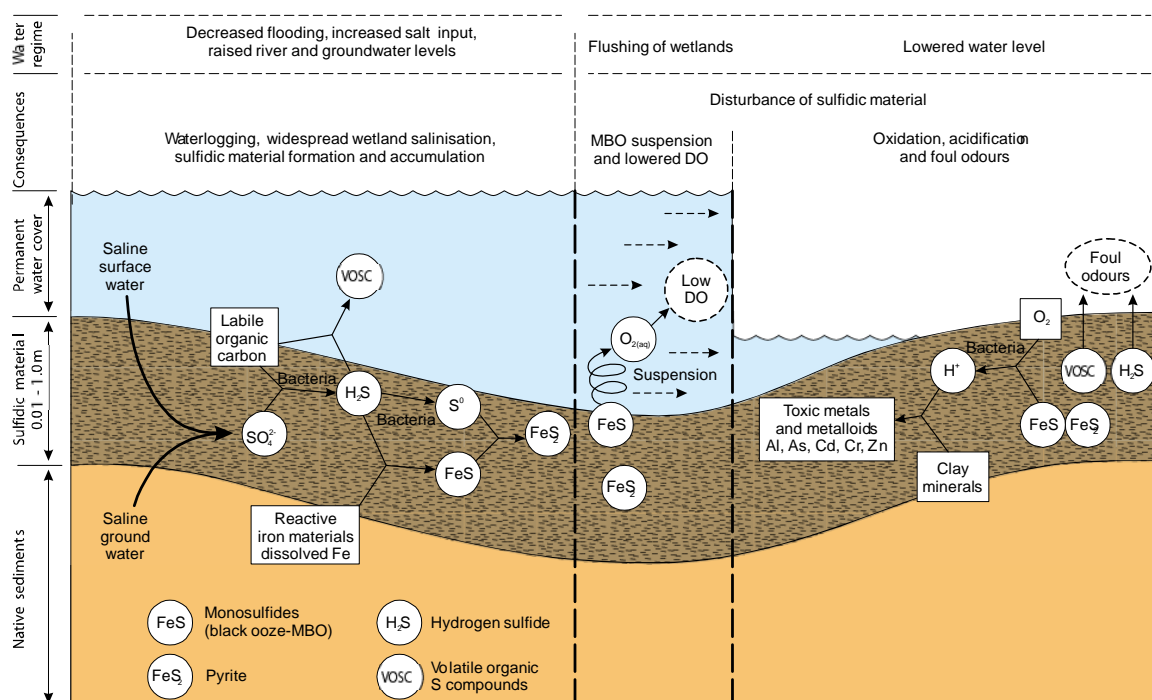
Figure 16: *Conceptual model of sulfidic material formation in River Murray wetlands and of the potential reactions following a disturbance.*

Acidification of Bottle Bend Lagoon

Bottle Bend Lagoon was formerly an ephemeral oxbow wetland that became permanently flooded following the construction of the Mildura Weir in 1927. In 2002, for the first time in decades, the wetland partially dried due to a combination of low river flows and the lowering of the Mildura weir pool for maintenance. During faunal surveys at the time of the draw down, McCarthy et al. (2003) observed that the remaining water in Bottle Bend (especially in the more isolated lower basin) became saline (EC >33 mS/cm) and acidic (pH <3). These conditions were also accompanied by low oxygen conditions, elevated concentrations of dissolved metals (including Fe, Al and Mn) and resulted in a massive fish kill. At the time of our survey (early 2003), water levels had partially recovered but the wetland was still weakly acidic (pH 5.5).

There are two potential mechanisms for the acidification of Bottle Bend Lagoon. First, it was demonstrated during this study that potential acid sulfate soil conditions were present. While the concentration of reduced S in the sediments were not exceptionally high, the potential for net acid generation was high because the sediments were poorly buffered. The potential for further acid generation appeared substantial in the exposed section of the wetland as the sulfidic layer had only been partially oxidised during the draw down (Plate 4). The elevated metal concentrations observed by McCarthy et al. (2003) are also consistent the acid and partially anoxic conditions of the water column (Stumm and Morgan 1996).

A second possible but less well understood mechanism for acidification at Bottle Bend Lagoon would be through the discharge of anoxic groundwater to the wetland caused by lower surface water levels. The impoundments on the Lower River Murray have induced increased lateral recharge rates and elevated water tables over large floodplain areas upstream of weirs (Jolly 1996). However, following a drop in surface water level, reverse hydraulic gradients can developed and floodplain groundwater can discharge back to surface water. As much of the groundwater in Lower River Murray floodplain is saline, such gradient reversals can also increase the salinity of wetlands (this is currently seen as a major disbenefit of using weir level manipulations for environmental flow purposes). Groundwater in the vicinity of Bottle Bend Lagoon is saline (McCarthy et al. 2003) and the significant increase in salinity during the draw down is consistent with an increased groundwater discharge to the wetland. However, in addition to being saline, groundwater in the floodplain environment can be anoxic and rich in dissolved metal species such as Fe^{2+} (Lamontagne

et al. *in press*) and probably Mn^{2+} (Appelo and Postma 1993). Once in the wetland, Fe^{2+} and Mn^{2+} would tend to consume O_2 and generate acidity. In the case of Fe this would occur, for example, through ferrous iron oxidation and ferric iron hydrolysis (equation 12) and for Mn through the oxidation of Mn^{2+} to insoluble manganese(IV) dioxide (Wetzel, 1983; Stumm and Morgan 1996):

$$Mn^{2+} + {}^1\!/_2 O_2 + H_2O \rightarrow MnO_2 + 2H^+ \tag{17}.$$

These oxidation reactions are sluggish at low pH but can also be catalysed by microorganisms (Stumm and Morgan 1996; King et al. 1999). Once the oxygen supply will be depleted in the water column, Fe^{2+} and Mn^{2+} will accumulate. In contrast to Fe and Mn, the elevated concentrations of total Al ($3.2 – 4.5$ mg L^{-1}) found by McCarthy et al. (2003) are less likely to originate from groundwater because Al is poorly soluble at the pH of the neighbouring groundwater (~6.1). Instead, the dissolved Al probably mostly originated from the dissolution of clays in the sediments due to the low pH (Appelo and Postma 1993).

The relative significance of sulfidic materials and anoxic groundwater discharge to generate acidification and deoxygenation in Riverland wetlands will require a more detailed analysis of the water and geochemical mass-balance during wetland wetting/drying operations. While salinity is generally the main water quality concern that is being considered for groundwater in the floodplain environment, the discharge of anoxic, metal-rich groundwaters could also be a significant issue in some environments.

Ped mineralogy in Loveday Disposal Basin

How fast will sulfidic materials oxidise during a wetland draw down phase will depend in part on the behaviour of the drying sediments, including through the formation of secondary minerals. Some insights into the behaviour of inland sulfidic sediments during the drying process were gained with a closer mineralogical examination of peds collected at Loveday Basin (Plate 1). Field observations at site 1 in LDB showed considerable amounts of thin platy coatings of white and grey salt efflorescences on the surface of peds in soil profiles when drained (Figure 17). These platy fragments with salt coatings were sampled and characterised together with other neighbouring zones and layers to try to understand the formation processes for the salt efflorescences.

A representative ped was chosen from those examined. This was carefully cleaved to expose both the interior with sulfidic material and the thin exterior coating with salt efflorescence (B in Figure 17). Based on these observations a conceptual model was constructed to describe the process of pyrite oxidation, leaching, element concentration and mineral formation/alteration (Figure 17).

In the underlying soil, pyrite was observed (A in Figure 17). In the interior pore network of the cleaved ped, unreacted pyrite, Mg-calcite and gypsum was detected (B in Figure 17). The presence of Mg-bearing calcites in the secondary salt efflorescence in platy fragments from coatings on ped surfaces (Plate 1 and Figure 17) was determined by the powder X-ray diffraction analysis (St Arnaud 1979) and is closely associated with high soluble Mg^{2+}/Ca^{2+} ratios in these soils. Aragonite is generally favoured over calcite to form in the presence of salts (i.e. Sr, Pb, Ba and $CaSO_4$), with Sr commonly inhibiting the alteration of aragonite to calcite.

A more detailed description of the processes of ped and salt efflorescences formation in oxidising sulfidic materials will be made in future studies. These processes are significant because:

- The dynamics of salt formation as a function of brine composition determines the type of salts that will be produced (including commercial salts);
- They determine the reactivity of carbonates to buffer acidification;
- They determine sulfide oxidation rates through the control on O_2 diffusion rates within drying sediment matrices.

A	B	C	D
Underlying sediment	**Interior of peds**	**Sides of peds**	**Top surface of peds**
Sulfidic material	Interior pore network	Exterior pore network	Surface pore network
Underlying unreacted or unoxidised layer	Inner unreacted or unoxidised zone Narrow front migrating or oxidation zone	Outer reacted zone with fluffy white salt efflorescence	Surface reacted zone with thin cracked plates of salt efflorescence
Water-saturated (108% moisture) and anaerobic: Eh = 80Mv pH = 8.6	Partly water-saturated (50-70 % moisture) and weakly anaerobic: Eh = 90Mv, pH = 7.8	Dry (8 % moisture) and aerobic. pH = 8.1	Dry (8 % moisture) and aerobic. pH = 8.1
Pyrite framboids - Py (M) Gypsum - Gy (M)	Pyrite framboids - Py (T) Mg- Calcite - Ct (M) Halite - Ht (M) Gypsum - Gy (T)	Gypsum – Gy (CD) Mg- Calcite - Ct (CD) Sr- Aragonite - At (M) Halite - Ht (T)	Mg- Calcite – Ct (CD) Sr- Aragonite – At (CD) Gypsum - Gy (T)
Bacterial reduction of sulfate and formation of Fe sulfides mainly pyrite.	Air space diffusion; Aqueous and solid phase oxidation of pyrite and geochemical reactions leading to crystallisation of Mg- calcite, halite, gypsum	Aqueous phase geochemical reactions (influx of Na, Ca, Mg and Sulfate), and concentration of porous gypsum, halite, Mg- Calcite and some Sr-Aragonite	Aqueous phase geochemical reactions: leaching of NaCl; influx of Ca, Sr and Mg, water lost by evaporation. Concentration effects resulting from High soluble Sr++/Ca++ ratios in soil solution form Sr-bearing aragonites, also formation of Mg-Calcite

D = dominant (>60%); CD = co-dominant (sum of components >60%); SD = sub-dominant (20 to 60%); M = minor (5 to 20%), T = trace (<5%)

Figure 17: *Conceptual model for formation of surface salt efflorescences on ped surfaces when sulfidic sediments in the Loveday Disposal Basin are drained. The model in based on a combination of the shrinking core model with reaction zones controlled by a combination of diffusion and chemical/ microbiological reactions.*

Application of molecular techniques

The study has demonstrated that a range of new molecular techniques could be applied to determine bacterial population diversity and functionality in sulfidic Murray floodplain sediments. In combination with chemical and mineralogical analyses, molecular tools add a significant biological dimension to the understanding of key processes and mechanisms occurring during the oxidation and (potentially) the formation of sulfide minerals in these environments. While still in the development stages, molecular techniques are quite promising because they will enable collection of information that cannot be obtained any other way or with as much ease. Additional research questions for the use of molecular techniques include:

- Determination of soxB gene expression (as opposed to presence only) to get one step closer to the measurement of actual rates of sulfide oxidation.
- Determination of the S reductase genes responsible for the biological reduction of S compounds in floodplain sediments.
- Identification of microbial and algal populations associated with active oxidation and reduction mechanisms using 16S sequencing.
- For management purposes, develop 16S fingerprinting techniques to predict where zones of oxidation and acid generation are most likely to occur.

How to quantify the risks associated with sulfidic materials?

The occurrence of sulfidic materials in some Lower River Murray wetlands is an environmental hazard. What risk these deposits represent will be context-specific, both for the type of risk that will be of concern (i.e., acidification, noxious smells, etc) and for the different factors that will contribute to the level of risk. It is outside the scope of this study to define risk management guidelines for inland deposits of sulfidic materials. However, some of the management considerations and risk factors that could be used to define these guidelines will be briefly reviewed.

Acidification risk

This risk is probably the easiest to define as a parallel can be made with the large body of research on coastal acid sulfate soil environments. For acidification, the risks factors include the:

- quantity of sulfide present;
- form of sulfide (pyrite vs. monosulfides);
- carbonate content of the sediments;
- clay content (texture) of the sediments;
- volume and alkalinity of the receiving water body.
- wetting-drying regime of the wetland.

The wetting-drying regime of wetlands can be an acidification risk depending on the mass-balance for alkalinity during a whole flooding cycle. In a closed system, the alkalinity generated during the formation of sulfidic materials will remain equal to their gross acid generation potential. However, in open systems, alkalinity can be lost while the acid generation potential can be preserved. For example, in coastal mangrove environments, part of the HCO_3^- generated by sulfate reduction can be flushed out to the ocean by tidal action, while sulfides remain stored in saturated sediments. In the inland wetland context, exporting high alkalinity water during draw down stages and filling with low alkalinity water during the filling stages could generate a net storage of acidity in wetlands over time.

Management targets can be proposed for the acidification risk because the impacts of acidification on biota are fairly well understood. In general, the most significant impacts of acidification occur once the carbonate alkalinity buffer is exhausted, which results in pH dropping from the circumneutral (>6) to acidic (<5) range. Once in the acidic pH range, the solubility of several dissolved metals (including Fe and Al) increases markedly. It is often these elevated metal concentrations that are harmful to biota rather than the low pH itself (Sammut and Lines-Kelly 1996). Thus, for River Murray wetlands, the management objective

to lower the acidification risk could be that the net acid generation potential (NAGP) of sulfidic materials and anoxic groundwater discharge remains less than the sediment carbonate alkalinity. This is a conservative approach because other potential sources of alkalinity (i.e., dissolved carbonates, ion-exchange with clays) are neglected.

Regional-scale risk factors in the Murray-Darling Basin?

It may not be a co-incidence that Bottle Bend Lagoon had a greater acidification risk than the wetlands and disposal basins surveyed in the South Australian Riverland. One noticeable difference between Bottle Bend and the other wetlands is that the former is located above the junction of the Murray with the Darling (Figure 2). While the Darling River contributes a small proportion of the flow to the Murray, the alkalinity of the River Murray doubles downstream from its junction with the Darling (from ~43 to 78–104 mg HCO_3^-/L; Mackay et al. 1988). Both a greater alkalinity in surface water and an increased likelihood of carbonate deposition in sediments should provide a greater protection from acidification in wetlands below the confluence with the Darling. While other factors could be involved (differences in local and regional geology, etc), the alkalinity of the source of surface water is probably a significant risk factor for wetland acidification in the MDB.

Methodology to assess the acidification risk

In general, the protocols used to measure the risk factors for acidification in the coastal ASS context appear applicable to inland environments. These have been reviewed extensively elsewhere (NSW ASSMAC 1998; Merry et al. 2003). A few additional recommendations would include:

- Ensure that sediments are not oxidised during transport to the laboratory (especially for the determination of the monosulfides content);
- Avoid grinding coarse carbonates and shells during sediment processing because these contribute little to the ANC under field conditions.
- When assessing the acidification risk of sediment leachates, use titratable acidity rather than pH as an indicator of acidity. The latter does not account for the presence of oxidisable and hydrolysable metal ions such as iron, manganese and aluminium (Cook et al. 2000; Hicks et al. 2002).

Deoxygenation risk

Deoxygenation may be the most widespread risk associated with sulfidic materials in the Lower River Murray because it may be significant even in areas that are naturally well-buffered against acidification. Currently there is no method of assessing the deoxygenation risk but some of the risk factors would include the:

- Potential for natural causes or management actions to resuspend sediments;
- Suspended sediment load;
- Sediment sulfide concentration and form;
- Water column residence time;
- Sulfide reaction rates;
- Salinity;
- Critical dissolved oxygen levels for the target species.

The residence time of suspended sediment is a function of sediment particle size and water velocity. Simpson et al. (1998) found iron monosulfides in resuspended sediment to react completely within 8 h. By comparison, Lu et al. (2003) estimated channel residence times of 10-15 h for eroded soil, which is likely to have a coarser particle size, and thus a shorter residence time than suspended sulfidic material. This indicates that, within the context of the MDB, sulfidic material should have sufficient time to react once suspended in the water column. The Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC and ARMCANZ 2000) recommend that dissolved oxygen levels remain above 85% of the saturation level. However, oxygen saturation levels decrease with increasing salinity and temperature. For a temperature of 25°C, this is equivalent to 7 mg/L in freshwater and 5.4 mg/L in water at a salinity of 25 mg/L. Thus, the deoxygenation risk will become greater at higher salinities because less oxygen will be present in the water column to start with. The guidelines also recommend measurement of diurnal variations as algae produce oxygen during the day, while algae and bacteria consume oxygen at night. Some of the bioassays used to characterise the environmental risks during dredging operations (Bonnet et al. 2000)

could be adapted to characterise the deoxygenation risk associated with the resuspension of inland sulfidic materials.

Human health and aesthetic risks

Foul odours

As well as creating unpleasant smells, sulfurous gases released from drying disposal basins have potential human health risks. Hydrogen sulfide has chronic health effects at long-term low exposure levels. VOSC's such as dimethyl sulfide are respiratory irritants and likely to adversely affect susceptible members of the public.

While an aesthetic and health concern, it is not clear how significant gaseous S loses are for the sulfur mass-balance of sulfide-rich wetlands. It is possible that gaseous S loses are small relative to the hydrological inputs and outputs of SO_4^{2-}.

Excavations

The health risks associated with H_2S would be especially significant when working with sulfidic materials in confined spaces. Engineering works that disturb sulfidic material will pose a risk to workers through the release and accumulation of hydrogen sulfide gas in excavations. Relevant occupational health and safety guidelines for work in confined spaces with the presence of hazardous gases need to be applied. For example, the Victorian Workcover guide to the application of the Occupational Health and Safety (Confined Spaces) Regulations 1996 lists as a hazard example, *seepage and build-up of natural contaminants from ground water and gases* (Workcover Victoria undated).

Heavy metals and sediment quality

Some sediments showed levels of copper and nickel above the trigger value specified by the Australian and New Zealand Guidelines for Fresh and Marine Water Quality interim sediment quality guidelines. When this occurs, the guidelines recommend further investigation of background levels and the "availability" of the metal through the use of other assays e.g., dilute acid extraction. Mineralisation, with mined deposits of copper does occur in the Lower Murray so these levels may reflect a locally elevated background, however further investigation is required for confirmation.

Managing sulfidic materials

Habitats most at risk

Based on the available information, it is possible to speculate on the parts of the Lower River Murray system most at risk from sulfidic materials. These would include:

- Disposal basins;
- Wetlands and anabranches made permanent by elevated weir pool levels;
- Wetlands and anabranches with a poor connection to the river at low flow or during weir pool draw downs;
- Terrestrial habitats over shallow (<2 m), saline water tables.

Habitats less likely to have significant sulfidic materials deposits would include:

- Freshwater wetlands with a natural wetting/drying regimes;
- Wetlands and anabranches that remain well connected to the main channel at low flows/low pool levels;
- Terrestrial habitats over deep, fresh water tables;
- The main river channels.

These habitats would be less at risk either because conditions would not be favourable to significant accumulations of sulfidic materials or because of the large dilution potential and alkalinity of the main river channels.

Options for management

Where sulfidic materials will be a significant environmental issue, a range of management options could be considered. These would include:

In the short term

- To keep wetlands sediments covered with fresh, alkaline water;
- To avoid resuspension of sediments during the management of water levels in wetlands;
- To add lime to prevent acidification.

In the longer term

- To remove the cause of salinity;
- To gradually oxidise sulfidic materials and export SO_4^{2-} out of the system with a carefully designed wetting/drying program;
- To excavate sulfidic materials and dispose safely;
- To carefully plan wetland wetting/drying programs to minimise the net loss of alkalinity over time;

The exposure time needed for sulfide-rich floodplain sediments to oxidise most of their reduced S is not clear at this stage. From the observations collected during this study, it is clear that sulfide deposits were only partially oxidised even following several months of exposure to the atmosphere. This is consistent with the relatively heavy texture of Lower River Murray floodplain sediments, which would tend to slow the rate of diffusion of oxygen in them. Likewise, there is currently not enough information to design an optimal wetting and drying cycle program in sulfide-rich wetlands that would eliminate sulfidic materials with minimum negative impacts.

Key knowledge gaps

The occurrence of significant deposits of sulfidic materials in the Lower River Murray environment has only been recently recognised. To devise management strategies for these materials, a number of outstanding tasks need to be addressed including:

- Complete a survey of the habitats suspected to have accumulated significant sulfidic material deposits in the Lower Murray;
- Further defining the regional-scale factors contributing to the acidification risk;
- Determine the rates at which sulfidic materials are formed or are oxidised under different salinity and water level management conditions;
- Assess the spatial variability in the distribution of sulfidic materials in representative wetlands;
- Identify the compounds responsible for the noxious smell problems and the optimal conditions under which these are produced and, conversely, minimised;
- Define the mass-balance for S and alkalinity during wetting-drying cycles in wetlands;
- Understand the role of sulfidic materials and of anoxic groundwater in causing wetland acidification and deoxygenation;
- Determine if monosulfides form a significant component of the reduced S pool in Riverland wetlands.

Outlook for management

The management of the floodplain environment to improve salinity will often involve draining formerly waterlogged soils and sediments. While the benefits of such actions will be valuable from an environmental point of view, these benefits could be partially offset by the environmental costs of exposing sulfidic materials to the atmosphere.

A number of immediate initiatives should be undertaken to help diminish the sulfidic materials risk in the floodplain environment. First, the awareness about sulfidic materials among stakeholders and managers is currently low. Thus, the first step is to educate stakeholders about sulfidic materials and the risk they pose to the environment. Efforts should be made to target groups whose activities affect the hydrology of the floodplain (wetland management groups, managers of salt interception schemes, etc).

The second step will be to provide managers with a set of criteria to quantify potential risks. Using the analogy of coastal acid sulfate soil environments, a guideline for the minimum chromium-reducible S concentration to initiate management action is one example. However, the criteria to be used are still to be defined within the context of the Lower River Murray floodplains. Thirdly, once an area has been identified at risk, a set of practical management options must be designed to minimise adverse environmental impacts associated with sulfidic materials. These will vary depending on whether noxious smells, deoxygenation or acidification is the principal risk.

References

ANZECC & ARMCANZ (2000) *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. National Water Quality Management Strategy Paper No 4, Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra. pp 3.5-1 to 3.5-10
http://www.deh.gov.au/water/quality/nwqms/introduction/

Appelo C.A.J., Postma D. (1993) *Geochemistry, Groundwater and Pollution*. Balkema, Rotterdam. 536p.

Bartlett R.J. (1986) Soil redox behaviour. *In*: Sparks D.L. (ed.), *Soil Physical Chemistry*, p179-208. CRC Press, Boca Raton, Fl.

Berner R.A. (1970) Sedimentary pyrite formation. *American Journal Science* **268**, 1–23.

Berner R.A. (1984) Sedimentary pyrite formation: an update. *Geochimica et Cosmochimica Acta* **48**, 605–615.

Bonnet C., Babut M., Ferard J.F., Martel L., Garric J. (2000) Assessing the potential toxicity of resuspended sediment. *Environmental Toxicology and Chemistry* **19**, 1290–1296

Caraco N.F., Cole J.J., Likens G.E. (1989) Evidence for sulfate-controlled phosphorus release from sediments of aquatic systems. *Nature* **341**, 316–318.

Carignan R., Tessier A. (1988) The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Québec. *Geochimica et Cosmochimica Acta* **52**, 1179–1188.

Cook F.J., Hicks W., Gardner E.A., Carlin G.D., Froggatts D.W. (2000) Export of acidity in drainage water from acid sulphate soils. *Marine Pollution Bulletin* **41**, 319–326

Cook R.B., Schindler, D.W. (1983) The biogeochemistry of sulfur in an experimentally acidified lake. *Ecological Bulletin* **35**, 115–127.

FAO/ISRIC/ISSS (1998) World Reference Base for Soil Resources. World Soil Resources Report, No.84. FAO, Rome.

Fitzpatrick R.W., Fritsch E., Self P.G. (1996) Interpretation of soil features produced by ancient and modern processes in degraded landscapes: V Development of saline sulfidic features in non-tidal seepage areas. *Geoderma* **69**, 1–29.

Franzmann P.D., Heitz A, Zappia L.R., Wajon J.E., Xanthis K. (2001) The formation of malodorous dimethyl oligosulfides in treated groundwater: The role of biofilms and potential precursors. *Water Research* **35,** 1730–1738.

Friedrich C.G., Quentmeier A., Bardischewsky F., Rother D., Kraft K., Kostka S., Prinz H. (2000) Novel genes for lithotrophic sulfur oxidation of *Paracoccus pantotrophus* GB17. *Journal of Bacteriology* **182**, 4677–4687.

George R. (2002) "Secondary acidification" an emerging problem in the wheatbelt. *Focus on Salt* **23**, 10.

Gippel C.J. (1995) Potential of turbidity monitoring for measuring the transport of suspended solids in streams. *Hydrological Processes* **9**, 83–87.

Herczeg A.L., Dogramaci S.S., Leaney F.W. (2001) Origin of dissolved salts in a large, semi-arid groundwater system: Murray Basin, Australia. *Marine and Freshwater Research* **52**, 41–52.

Hicks W.S., Bowman G.M., Fitzpatrick R.W. (2002) Assessing the acidity hazard of acid sulfate soil discharge waters. 5th International Acid Sulfate Soils Conference, Tweed Heads, NSW, 25th to 30th August 2002. Book of extended abstracts.

Holmer M., Storkholm P. (2001) Sulphate Reduction and Sulphur Cycling in Lake Sediments: a Review. *Freshwater Biology* **46**, 431–451.

Jolly I.D. (1996) The effects of river management on the hydrology and hydroecology of arid and semi-arid floodplains. *In*: Anderson MG, Walling DE, Bates DE (eds) *Floodplain Processes*. Wiley, New York, pp 577–609.

King H.M., Baldwin D.S., Rees G.N., McDonald S. (1999) Apparent bioaccumulation of Mn derived from paper-mill effluent by the crayfish *Cherax destructor* – the role of Mn oxidising bacteria. *The Science of the Total Environment* **226**, 261–267.

Lamontagne, S., Herczeg, A.L., Dighton, J.C., Jiwan, J.S., Pritchard, J.L. (*in press*) Patterns in nitrogen concentration in the floodplain of a subtropical stream (Wollombi Brook, New South Wales). *Biogeochemistry*

Langmuir D., Whitemore D.O. (1971) Variations in stability of precipitated ferric oxyhydroxides. p209-234. *In* Hem J.D. (ed.) *Nonequilibrium systems in natural water chemistry. Advances in Chemistry* **106**. American Chemical Society, Washington D.C.

Lu H., Moran C.J., Prosser I.P., Raupach M.R., Olley J., Petheram C. (2003) Hillslope erosion and

---

sediment delivery: A basin wide estimation at medium catchment scale, Technical Report 15/03, CSIRO Land and Water.

Mackay N., Hillman, T., Rolls J. (1988) *Water quality of the River Murray: Review of monitoring 1978–1986*, Murray–Darling Basin Commission, Canberra. 62p

Merry R.H, Fitzpatrick R.W., Barnett E.J., Davies P.G., Fotheringham D.G., Thomas B.P., Hicks W.S. (2003) *South Australian Inventory of Acid Sulfate Soil Risk (Atlas). Final project report to Coastal Acid Sulfate Soils Program (CASSP). March, 2003*. http://www.clw.csiro.au/staff/FitzpatrickR/publications.html

McDonald R.C., Isbell R.F., Speight J.G., Walker J., Hopkins, M.S. (1990) *Australian soil and land survey field handbook. 2nd Edition*, (Inkata Press, Melbourne).

McCarthy B., Conallin A., Walsh R. (2003) Aquatic survey of Bottle Bend Lagoon, near Buronga NSW: salinisation and acidification impacts. A CRC for Freshwater Ecology Consultancy Report for NSW Murray Wetland Working Group Inc. Report No. 2/2003. Murray-Darling Freshwater Research Centre, Albury, New South Wales, Australia.

Mukhopadhyaya P.N., Deb C., Lahiri C., Roy P. (2000) A *sox*A gene encoding a diheme cytochrome c and a sox locus, essential for sulfur oxidation in a new sulfur lithotrophic bacterium. *Journal of Bacteriology* **182,** 4278-4287.

National Health and Medical Research Council (1996) *Australian Drinking Water Guidelines*. Cat. No. 9627022. Commonwealth Government of Australia, Canberra, Australia. http://www.nhmrc.gov.au/publications/pdf/eh19.pdf

National Working Party on Acid Sulfate Soils (1999) *National strategy for the management of coastal acid sulfate soils*. NSW Agriculture, Wollongbar. http://www.mincos.gov.au/pdf/natass.pdf

Nordstrom D.K. (1982) Aqueous pyrite oxidation and consequent formation of secondary iron minerals. *In:* Kittrick J.A., Fanning D.S., Hossner, L.R. (eds), *Acid Sulfate Weathering*. SSSA Special Pub. No. 10, pp37-56. Soil Science Society of America, Madison.

NSW ASSMAC (1998) *Acid Sulfate Soil Manual*. New South Wales Acid Sulfate Soil Management Advisory Committee. NSW Agriculture, Wollongbar Agricultural Institute; Bruxner Highway, Wollongbar, NSW 2477.

Petri R., Podgorsek L., Imhoff J.F. (2001) Phylogeny and distribution of the soxB gene among thiosulfate-oxidising bacteria. *FEMS Microbiology Letters* **197,** 171–178.

Preiss K. (undated). Ramco Lagoon Health Study – Update. Riverland West Local Action Planning Association.

Raiswell R., Berner R.A. (1985) Pyrite formation in euxinic and semi-euxinic sediments. *American Journal of Science* **285**, 710–724.

Raven M.D. (1990) XPLOT version 3.2 user manual. Manipulation of X-ray powder diffraction data. CSIRO Division of Soils Technical Report No. 24/1990.

RMCWMB [River Murray Catchment Water Management Board] (2003) *Catchment Water management Plan for the River Murray in South Australia 2003 – 2008*. Government of South Australia. 244 p.

Rother D., Henrich H.J., Quentmeier A., Bardischewsky F., Friedrich C.G. (2001) Novel genes of the *sox* gene cluster mutagenesis of the flavoprotein SoxF, and evidence for a general sulfur-oxidising system in *Paracoccus pantotrophus* GB17. *Journal of Bacteriology* **183**, 4499–4508.

Sammut J., Lines-Kelly R. (1996) *An Introduction to Acid Sulfate Soils*. ISBN 0 642 24533 9. 23 p.

Schoeneberger P.J., Wysocki D.A. Benham E.C., Broderson W.D. (eds) (2002) *Field Book for Describing and Sampling Soils, Version 2.0*. Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE, USA.

Simpson S.L., Apte S.C., Batley G.E. (1998) Effect of short term resuspension events on trace metal speciation in polluted anoxic sediments. *Environmental Science & Technology* **32**, 620–625.

Soil Survey Staff (1999) Soil Taxonomy: A basic system of soil classification for making and interpreting soil surveys. 2nd edition. Agriculture Handbook No. 436. United States Department of Agriculture, Natural Resources Conservation Service, Washington.

Soil Survey Staff (2003) *Keys to Soil Taxonomy. 9th edition*. United States Department of Agriculture, Soil Conservation Service: Blacksburg. http://soils.usda.gov/technical/classification/tax_keys/

Self P.G. (1988) PC-PW1710 - user manual. PC based system for the control of a Philips PW1710 X-ray diffraction system. CSIRO Division of Soils Technical Memorandum No. 41/1988. 32pp.

Self P.G. (1989) PC-PW1800 - user manual. PC based system for the control of a Philips PW1800 X-ray diffraction system. CSIRO Division of Soils Technical Memorandum No. 1/1989. 29pp.

St Arnaud R.J. 1979. Nature and distribution of secondary soil carbonates within landscapes in relation to soluble Mg^{2+}/Ca^{2+} ratios . *Canadian Journal of Soil Science* **59,** 87–98.

Stumm W., Morgan J.J. (1996) *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters (3rd Edition)*. Wiley- Interscience NY, p459.

Sullivan L., Bush R. (2003) Geochemical aspects of sulphate salinity in the waterways of the Murray-Darling Basin. Ecological Society of Australia Conference: Abstracts. University of New England, Armidale.

Sullivan L.A., Bush R.T., Fyfe D. (2002) Acid sulfate soil drain ooze: Distribution, behaviour and implications for acidification and deoxygenation of waterways. *In*: Lin C, Melville MD, Sullivan LA (eds) *Acid Sulfate Soils in Australia and China*. Science Press, Bejing, China. pp 91–99.

van Breeman N. (1973) Calculation of ionic activities in natural waters. *Geochimica et Cosmochimica Acta* **37,** 101–107.

van Breeman N., Harmsen K. (1975) Translocation of iron in acid sulfate soils: I. Soil morphology, and the chemistry and mineralogy of iron in a chronosequence of acid sulfate soils. *Soil Science Society of America Proceedings* **39**, 1140–1148.

Waite T.D. (1997) Review of role of sulfur in nutrient release from sediments. Report prepared for the LWRRDC/MDBC National Eutrophication Management Program. Department of Water Engineering, University of New South Wales, Sydney.

Wetzel R.G. (1983). *Limnology, 2ⁿᵈ Edition*. Saunders, New York. 767p.

Workcover Victoria (undated) *Confined Spaces – Shafts, Tunnels and Trenches.* http://www.workcover.vic.gov.au/dir090/vwa/publica.nsf/0/465d2e1bebd7171eca256cd200826683/$FILE/_78dnmspj9dpim882je1gm6pbj40mi0kr8c5j78spc41a7arjeclm7681641a74pbecdk6aso.pdf

Appendix 1: Detailed methodology

1.1 Soil and sediment sampling

Core samples
A Dormer Engineering "Undisturbed Wet Sampler" was used to collect sediment cores in PVC tube. Cutting tip diameter is 34mm and PVC tube 35mm.

Pit samples
Pits were dug using a spade and grab samples taken from the interior of sediment blocks of around 10cm x 15cm x 20cm excavated with the spade.

Sample storage
Cores and grab samples were sub-sampled into 100g opaque polystyrene jars. Larger samples were placed in clip seal PVC bags. All samples were transferred to a portable freezer within 30 minutes of collection and were keep frozen until further processing occurred.

Sample preparation
Frozen samples were freeze-dried. They were then sieved to pass through a 0.5mm mesh.

1.2 Water sampling

Samples of surface and pore waters were obtained as grab samples. Electrical conductivity, pH and alkalinity were measured in the field on an unfiltered sample. A sub-sample for laboratory analysis was immediately filtered through a 0.45μm Supor membrane filter (Pall). A small volume of this was collected in a scintillation vial for chloride analysis, another aliquot was added to a pre-prepared vial containing 1,10 phenanthroline and other reagents for the later analysis of ferrous iron. The remainder was collected in a 125mL polyethylene bottle and preserved with analytical reagent grade hydrochloric acid for the analysis of major ions and nutrients. Prior to each sampling trip, all sampling bottles and filtering equipment were washed in P-free detergent and in a mild acid bath before thorough rinsing with distilled deionised water.

1.3 Field tests

pH and Eh measurement
Sulfides and hydrogen sulfide gas can poison the reference electrode of combined pH and combined ORP (Redox/Eh) electrodes. Double junction or similar (eg Ionode IJ series intermediate junction electrodes) should be used and the filling solution changed at regular intervals according to the manufacturers instructions. Eh values are always corrected to the value versus the standard hydrogen electrode (SHE) for reporting. Platinum electrodes can also be poisoned and the calibration should be regularly checked using solutions recommended by the manufacturer. Alternatively, Bartlett (1986) provides detailed instructions on the care and calibration of platinum electrodes. Note for field measurements where there are no strong redox couples, good calibration and response to Zobell's solution does not guarantee adequate field performance. The correction from the field meter reading depends on whether a silver-silver chloride or calomel reference was used and the concentration of the filling solution. Robust field meters should be used as the environment is aggressive.

Peroxide test
We recommend the use of analytical reagent grade hydrogen peroxide (30% vol/vol). Hydrogen peroxide is stabilised with acid (sulfuric or phosphoric) and technical grade can have both a low pH and considerable acidity. The pH of each batch of hydrogen peroxide should be tested and if necessary the pH of the hydrogen peroxide used in the field should be adjusted with a solution of sodium hydroxide. Note that once the pH has been adjusted the hydrogen peroxide decomposes and should be discarded at the end of the trip.

1.4 Analyte method reference for water samples.

Analyte	Method	Reference
Ca^{2+}, Mg^{2+}, Na^+, K^+	The samples were determined directly by ICP emission spectrometry. Any concentrations above the linear analytical range were diluted with 1% (v/v) HNO_3 before reanalysis.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 3120.
Cl^-, SO_4^{2-}	These anions were determined by ion chromatography (IC) using chemical suppression and electrical conductivity detection.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 4110.
Filterable Reactive Si	The filtered samples were analysed by segmented flow analysis (SFA) using ammonium molybdate and oxalic acid then reduced with ascorbic acid and determined colorimetrically at 815nm.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 4500-SiO2 Automated method for molybdate-reactive silica (modified). Perstorp Analytical Environmental EnviroFlow 3500 method procedure document 000595 (pers.com.)
Total dissolved Fe	(see Ca^{2+}, Mg^{2+}, Na^+, K^+)	
NH^{4+}	segmented flow analysis (SFA) using the sodium salicylate, sodium nitroferricyanide and DCIC in alkaline solution with citrate	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 4500-NH_3 G Automated phenate method (modified). Krom, M.D. (1980) Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. The Analyst, 105, 305-316.
$NO_3^- + NO_2^-$	Determined by segmented flow analysis (SFA). Nitrate reduced to nitrite by Cu/Cd. Total nitrite then determined colorimetrically after reaction with sulfanilamide and NEDD in acid solution.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 4500-NO_3 F Automated cadmium reduction method.
Filterable reactive P	Determined by segmented flow analysis (SFA) using ammonium molybdate and potassium antimony tartrate in the presence of ascorbic acid at pH 1.0 to form a molybdenum blue colour.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 4500-P F Automated ascorbic acid reduction method.
Total dissolved P	The samples were determined directly by ICP emission spectrometry. Lower detection limits were achieved using an ultrasonic nebulizer sample introduction system.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 3120.
Dissolved organic carbon	Thermal combustion of filtered solution to form CO_2 that is determined by IR detection. Inorganic carbon removed before analysis or determined separately.	Standard Methods for the Examination of Water and Wastewater (1999). 20th edition American Public Health Assoc., American Water Works Assoc., Water Environment Federation. Method 5310 B High-temperature combustion method.
Total dissolved N (TDN) and Dissolved organic N (DON)	TDN was measured by thermal combustion to NO_2 and measurement by thermoluminescence. DON was the difference between TDN and (NH_4^+ + NO_3^-).	SKALAR Analytical B.V. 2000. FormacsHT TOC/TN Analyzer user manual.
Fe^{2+}	Modified phenanthroline method	T. Hislop and M. Grace, Water Studies Centre, Monash University, *pers. comm.*

1.5 Analyte method reference for soil samples.

Analyte	Method	Reference
Electrical Conductivity (EC) $pH_{1:5}$, $pH_{0.01M\ CaCl_2}$ and Cl^-	These parameters were determined in a 1:5 soil water extract. pH and EC are measured directly in the extract and chloride in a filtered sub-sample.	Australian Laboratory Handbook Of Soil And Water Chemical Methods 3A1,4A1, 4B2 and 5A2
Total carbon and total sulfur	These analytes were determined by high frequency induction furnace with infra red detection (LECO CNS2000)	Australian Laboratory Handbook Of Soil And Water Chemical Method 6B3,
Carbonate	Carbonate is determined manometrically	Australian Laboratory Handbook Of Soil And Water Chemical Method 19B1
Chromium reducible sulfur	Reduced sulfur was determined by reacting the sample with Cr powder in HCl, followed by collection of the evolved $H_2S_{(g)}$ and its titration	Southern Cross University Environmental Analysis Laboratory: NSW ASSMAC (1997) *Acid Sulfate Soils Laboratory Methods Guidelines November 1997*. New South Wales Acid Sulfate Soil Management Advisory Committee. NSW Agriculture, Wollongbar Agricultural Institute, Bruxner Highway, Wollongbar, NSW 2477.
Acid extractable elements	A multi-element acid leach followed by ICP OES analysis of the digest	US EPA Method 3051: Microwave assisted acid digestion of sediments, sludges, soils, and oils. *In* Test Methods for Evaluating Solid Waste, 3rd edition, 3rd update; U.S. Environmental Protection Agency: Washington, DC, 1995.
Total elements	A mixed acid digestion (incl. $HF/HClO_4$) of the sample followed by ICP-OES and ICP-MS. Al, Ba, Cr, Ti, W, Zr, Sn are acid soluble values only. K may report low due to the solubility of potassium perchlorate.	AMDEL Methods IC3E,M and R

1.6 Mineralogy

Semi quantitative analysis of mineral composition was undertaken using power X-ray diffraction (XRD). Samples were finely ground with an agate mortar and pestle (salt efflorescences) or in a McCrone micronizing mill under ethanol (1g sub-sample for 10 minutes) and oven dried at 60°C then thoroughly mixed in an agate mortar and pestle. Powdered samples were lightly pressed in aluminium sample holders for X-ray diffraction analysis. XRD patterns were recorded with a Philips PW1800 microprocessor-controlled diffractometer using Co K-alpha radiation, variable divergence slit, and graphite monochromator. Diffraction patterns were recorded in steps of 0.05° 2 theta with a 3.0 second counting time per step, and logged to permanent data files using instrument control programs developed by Self (1988, 1989). Analysis of the data was carried out using the program XPLOT (Raven, 1990). Codes used to indicate abundance are: D - dominant (>60%), CD - co-dominant (sum of components >60%), SD - sub-dominant (20 to 60%), M - minor (5 to 20%), T - trace (<5%), nd - not detected.

1.7 Microbiological analyses

Background

The suite of genes encoding for sulfur oxidation in chemolithotrophes has recently been identified (Rother *et al*. 2001). The sulfur oxidase (*sox*) gene cluster comprises nine genes; *sox*XYZABCDEF (Friedrich *et al*. 2000). The *sox*B gene encodes for a diheme cytochrome c enzyme and has been shown to be essential for chemolithotrophic sulfur oxidation (Mukhopadhyaya et al. 2000). This functional gene has also been identified in representatives of all known groups of chemolithotrophic sulfur oxidising bacteria, making it an ideal candidate for our functional molecular approach. Degenerate oligonucleotide primers have been designed that amplify a conserved 1000 base pair region of the *sox*B gene sequence (Petri et al. 2001), allowing us to apply our existing functional molecular techniques to the study of sulfur oxidising chemolithotrophic bacteria activity and presence in sulfidic sediments.

DNA extraction and amplification

Two steps are required in the measurements of the *sox*B and 16S gene in environmental samples: *i*) DNA (for *sox*B) and RNA (for 16S) must be extracted from samples and *ii*) they must be amplified (i.e., replicated thousands of times) to enable quantification by gel electrophoresis.

DNA and RNA were first extracted by centrifuging the EDTA-preserved sediment samples at 15000rpm for 12 minutes and decanting the water. DNA was then extracted from the soil using a MoBio UltraClean Soil DNA Isolation Kit as per the manufacturers instructions. All extracts were subsequently tested for the presence of the *sox*B and 16S genes.

Samples were then amplified by Polymerase Chain Reaction techniques (PCR). Very briefly, the principle of the technique is to add specific "primers" to the extracted DNA or RNA samples that will target the specific gene segments of interest (Table 1). The targeted gene sequences are then replicated by repeated cycles of DNA denaturation (i.e., splitting the DNA helix by heating) in the presence of the polymerase enzyme (to produce identical copies of the DNA or RNA).

Table 1.1. Oligonucleotide primers used for PCR amplification of the soxB and 16S genes.

Functional Gene	Oligonucleotide Primer Sequence		PCR Product
SoxB			1000bp
SoxB-432F	GAY GGN GGN GAY CAN TGG		
SoxB-1446B	CAT GTC NCC NCC RTG YTG		
16S			
27F-GC	CGC CCG CCG CGC GCG GCG GGC	GGG	510bp
	GCG GGG GCC CGG GGG GAG		
	AGT TTG ATC CTG GCT CAG		
534R	ATT ACC GCG GCT GCT GG		

All PCR amplifications were performed on an Eppendorf Mastercycler Gradient Thermocycler. Products were analysed by electrophoresis on 2% agarose gels (Roche Agarose MP) followed by a 15 min staining with ethidium bromide (0.5mg litre). The reagents used were:

- Promega Taq DNA Polymerase
- Promega $MgCl_2$ 25mM
- Promega PCR buffer 10X
- Promega DNTP's
- Roche bovine serum albumin
- Promega 100bp DNA ladder
- Invitrogen low DNA mass ladder (for quantification)

soxB
The PCR reaction mix (20µL) contained 200uM each deoxyribonucleoside triphosphate, 1.25mM $MgCl_2$, 1X PCR buffer, 1U Taq polymerase, 400ng/µL bovine serum albumin, 1.0uM of each of the forward and reverse primer (soxB-432F and soxB-1446B) and 5ul of DNA template.

After a denaturation step of 5 min at 94°C, amplification reactions were performed with 10 cycles of a denaturation step of 1 min at 94°C, a primer annealing step of 1 min at 55°C, and an extension step of 2 min at 72°C. This was followed by 25 cycles of denaturation step of 1 min at 94°C, a primer annealing step of 1 min at 48°C, and an extension step of 2 min at 72°C A final 5 min extension step at 72°C was performed.

16S
The PCR reaction mix (20µL) contained 200µM each deoxyribonucleoside triphosphate, 1.5mM $MgCl_2$, 1X PCR buffer, 1U Taq polymerase, 400ng/µL bovine serum albumin, 0.2µM of each of the forward and reverse primer (27F-GC and 534R) and 5µL of DNA template.

After a denaturation step of 4 min at 94°C, a "touchdown" PCR was performed. This consisted of a denaturation step of 1 min at 94°C, a primer annealing step of 1:30 min, and an extension step of 1:30 min at 72°C. During the first 10 cycles, the annealing temperature was decreased by 1.0°C every cycle, starting at 64°C until it reached a touchdown of 54°C. The additional 20 cycles were performed at an annealing temperature of 54°C. After 30 cycles a final 8 min extension at 72°C was performed.

1.8 Identification numbers for microbiological analysis samples.

Location	Sample No.	Description
Ramco Lagoon Pit 1	1	Old Shoreline 0–5cm
Ramco Lagoon Pit 1	2	Old Shoreline 5–10cm
Ramco Lagoon Pit 1	3	Old Shoreline 10–15cm
Ramco Lagoon Pit 1	4	Old Shoreline Surface algal mat
Ramco Pit 2	5	Black sulfidic material 0–2cm
Ramco Pit 2	6	0–5 cm
Ramco Pit 2	7	5–10cm
Ramco Pit 2	8	10–15cm
Ramco Lagoon Fringe	9	Highly sulfidic 0–2cm
Ramco Lagoon Fringe	10	Sulfidic consolidated 2–4cm
Ramco Lagoon Fringe	11	0–2cm sandy material
No sample	12	
Hart Lagoon	13	0–2cm sandy material
Hart Lagoon	14	2–4cm
Hart Lagoon	15	5–10cm
Hart Lagoon	16	Oxidised material
Hart Lagoon	17	Sulfidic 'block'
Hart Lagoon	18	oxidised crack between 'blocks'
No sample	19	
Ross Lagoon	20	Mottled sandy layer at 5cm
Ross Lagoon	21	Blue sandy clay >15cm
Lake Woolpolool	22	Surface 5 cm inundated with water
Lake Woolpolool	23	10–13 cm inundated samphire?
Lake Woolpolool	24	Sulfidic layer from track 5m from Samphire
Lake Merreti	25	2m lake margin green algae
Lake Merreti	26	20–23 mottled zone
Bottle Bend Lagoon Site 1	27	0–5 cm sulfidic layer
Bottle Bend Lagoon Site 1	28	15–20cm clay above decayed wood
Bottle Bend Lagoon Site 1	29	>30cm sandy clay no mottles present
Bottle Bend Lagoon Site 2	30	0–1 cm Fe rich sulfidic zone
Bottle Bend Lagoon Site 2	31	10–12 cm mottled clay–mainly mottle
Bottle Bend Lagoon Site 2	32	>20 mottled clay/sand
No sample	33	
Bottle Bend Lagoon Site 2	34	surface sheen at waters edge above FeO surface coating
Loveday Pit 1	35	5–12 cm
Loveday Pit 1	36	20–30 cm
Loveday Pit 3	37	0–5 cm
Loveday Pit 3	38	5–30 cm
Loveday Pit 2	39	1–20 cm
Berri Evap Basin	40	11 Nov 03 – Offshore sulfidic ooze (1 – 20 cm)
Berri Evap Basin	41	11 Nov 03 – Offshore sulfidic ooze (1 – 20 cm)
Loveday Basin	42	11 Nov 03 – Sulfidic ooze near Pit 3 (1 – 20 cm)
Loveday Basin	43	11 Nov 03 – Sulfidic ooze near Pit 3 (1 – 20 cm)
Ramco Lagoon	44	11 Nov 03 – Offshore sulfidic ooze (1 – 20 cm)
Ramco Lagoon	45	11 Nov 03 – Shoreline sulfidic ooze (1 – 20 cm)

Appendix 2: Detailed results

2.1 Soil description and morphology.

Sample ID	Location	Site description	Depth (cm)	Colour (Munsell)	Texture	Consistence (dry)	Structure	Pores	Roots	HCl fizz	Concentrations (CAX=carbonate GYX=gypsum SAX=salts)
BBU1	BBL upper	surface precipitate	0 – 0.2	10YR5/6	CS	S	m	1,vf	none	NE	none
BBU2		sulfidic	0 – 10	5Y5/2; 10YR4/4	ZCL	S	m	2,f	2,f	NE	none
BBU3		clay	10+	5Y6/2; 2.5Y5/4	LC	MH	m	1,vf	1,f	NE	none
BBU4		dry	0 – 5	2.5Y5/2;7.5YR5/6	MC	VH	m	1,f	1,vf	NE	none
BBL1	BBL middle		0 – 9	10YR4/2	LS	L	sg	2,f	2,f	NE	none
BBL2			9 – 15	5Y6/1; 2.5Y 6/6	MC	HA	m	2,f	none	NE	none
CL1	Clover L		0 – 10	5Y5/1	LC	HA	abk	2,f	1,vf	NE	none
CL2			10 – 20	5Y4/1	LC	HA	abk	2,f	2,vf	NE	none
CL3			20 – 25	5Y4/2	MC	HA	abk	2,f	2,vf	NE	none
ML1	Merreti L		0 – 5	2.5Y5/2	CS	S	m	1,vf	none	NE	none
ML2			5 – 10	5Y6/2	SCL	S	m	1,f	none	NE	none
ML3			10 – 20	5Y6/2; 10YR4/4	HC	EH	m	1,f	2,f	NE	none
WL1	Wpl	Track	mbo	5Y5/1	ZL	S	m	1,vf	1,f	SL	none
WL2		Track	0 – 3	2.5Y4/1	ZCL	S	m	1,vf	1,f	SL	none
WL3		Track	3 – 7	5Y5/2	MC	SH	m	1,vf	2,vf	VS	none
WL4		Samphire	0 – 3	5Y4/2	ZCL	S	m	1,vf	1,ff	VS	CA, f,1,C,S
WL5		Samphire	3 – 6	5Y5/1	MC	SH	m	1,vf	1,vf	NE	none
WL6		Samphire	6 – 10	2.5Y5/2	MC	SH	m	1,vf	1,f	NE	none
WL7		Samphire	10 – 20	5Y5/2	HC	VH	m	1,vf	1,vf	NE	none
RL1	Ross Lg	sulfidic	0 – 10	2.5Y5/2	LS	L	sg	1,f	1,f	VS	none
RL2		non-sulfidic	0 – 10	2.5Y6/2	CS	S	sg	1,f	1,f	NE	none
RL3		mottled	15 – 25	2.5Y5/2	SL	S	m	1,f	1,f	NE	none
RL4		clay	25+	5Y5/2	MC	SH	m	1,f	none	VS	none
HL1	Hart Lg	sulfidic	0 – 5	5Y4/1	ZLC	SH	m	1,vf	none	SL	none
HL2		cracks	0 – 5	5Y4/1	ZLC	SH	m	1,vf	none	SL	none
HL3			5+	5Y5/1	ZLC	SH	m	1,vf	none	VS	SF, c,2,C
RVSS2.1.1	Ramco	Pit 1	0 – 5	5Y6/2	ZCL	S	m	1,vf	none	ST	none
RVSS2.1.2			5 – 10	5Y5/2	ZCL	SH	m	1,vf	none	SL	none
RVSS2.1.3			10 – 15	5Y5/2	MC	SH	m	1,vf	none	VS	none
RVSS2.1.4			15 – 20	5Y5/2	MC	SH	m	1,vf	none	NE	none
RVSS2.1.5			20 – 27	5Y6/2	MC	SH	m	1,vf	none	NE	none

2.1 (continued).

Sample ID	Location	Site description	Depth	Colour (Munsell)	Texture	Consistence (dry)	Structure	Pores	Roots	HCl fizz	Concentrations (CAX=carbonate GYX=gypsum SAX=salts)
RVSS2.2.1	Ramco	Pit 2	0 – 5	5Y5/2	LS	L	sg	1,vf	none	ST	none
RVSS2.2.2			5 – 10	5Y6/2	LS	L	sg	1,vf	none	ST	none
RVSS2.2.3			10 – 15	5Y5/2	LS	L	sg	1,vf	1,vf	VS	none
RVSS2.2.4			15 – 20	5Y6/2	LS	L	sg	1,vf	none	NE	none
RVSS2.2.5			20 – 25	5Y5/2	LC	SH	m	1,vf	none	NE	none
RVSS2.2.6			25 – 30	5Y5/2	LC	SH	m	1,vf	none	NE	none
RVSS2.2.7			30 – 37	5Y5/2	LC	SH	m	1,vf	none	NE	none
BEB1	BEB	sulfidic		5Y3/1	LC	SH	m	1,f	1,vf	VE	none
BEB2		non-sulfidic		5Y5/3	HC	VH	m	1,f	none	VE	none
CBD2.1	LDB	Site 2 Pit 2:	0 – 1	5Y6/3	LC	SH	pl	1,f	2,f	VE	none
CBD2.3			1 – 20	5Y6/3	LC	SH	pl	1,f	2,f	SL	none
CBD2.4			20 – 30	5Y4/1	LC	MH	m	1,f	2,f	NE	none
CBD3.1		Site 2 Pit 3:	0 – 5	5Y4/2	LC	MH	m	1,f	2,f	VE	none
CBD3.2			5 – 30	5Y4/1	MC	MH	m	1,f	2,f	VS	none
CBD3.3			30 – 40	5Y4/1	MC	MH	m	1,f	2,f	NE	none
CBD1.1		Site 1 Pit 1:	0	5Y8/2			pl				
CBD1.2			0 – 0.5	5Y8/1			pl				
CBD1.3			0.5 – 5	5Y5/2	LC	EH	m	1,vf	none	VE	none
CBD1.4			5 – 12	5Y4/1	LC	EH	m	1,vf	none	VE	none
CBD1.5			12 – 20	5Y4/1	MC	EH	m	1,vf	none	VE	none
CBD1.6			20 – 30	5Y4/1	MC	EH	m	1,vf	none	VS	none
CBD1.7			30 – 40	5Y5/1	MC	EH	m	1,vf	none	VS	none
CBD1.8			40 – 50	5Y4/1	MC	EH	m	1,vf	none	VS	none

Abbreviations used in the tables: Lagoon – Lg, Lake – L, precipitate – ppt, Loveday Disposal Basin – LDB, Berri Evaporation Basin – BEB, Woolpolool – Wpl, Bottle Bend Lagoon – BBL, Veg. Survey No.2 – VS2

Soil pits were dug to a depth of about 0.75m and where possible a hand auger was used to sample soils down to 1.5m. A representative profile face in the pit was selected and the master horizons demarcated and photographed.
Soils were described according to the USDA Field book for describing and sampling soils, Version 2.0 (Schoeneberger et al., 2002) and Australian Soil and Land Survey Field Handbook (McDonald et al., 1990; See also Glossary for soil texture criteria). Soils were classified according to Soil Taxonomy (Soil Survey Staff, 1999) and The World Reference Base for soil resources (WRB) (FAO, 1998).

The following morphological features were described:

Horizon thickness (cm).

Horizon type using horizonation nomenclature from: Soil Taxonomy (Soil Survey Staff, 1999) and Schoeneberger et al., (2002). Where: p = ploughed layer, z = pedogenic salts more soluble than gypsum, y = pedogenic gypsum, m = strong cementation; t = clay accumulation, k = pedogenic carbonates, n = high ESP; g=strong gley; a = highly decomposed organic matter, c = concretions, e = moderately decomposed organic matter).

Horizon boundary (Bnd) (mm): VA= very abrupt(<5), A=abrupt(5-20), C=Clear (20-50), G=Gradual (50-150), D=Diffuse (>150). / S=Smooth, W=Wavy, I=Irregular, B=Broken.

Matrix colour, mottle colour using the standard soil Munsell colour notation, mottle type abundance, size contrast).

Texture, using the Australian Soil and Land Survey: Field Handbook: McDonald et al. (1990) (see Glossary). Where: S=Sand, LS=Loamy Sand, CS=Clayey Sand, SL=Sandy Loam, L=Loam, ZL=Silty loam, ZCL= Silty Clay Loam, SCL=Sandy Clay Loam, ZLC= Silty Light Clay, MC=Medium Clay, HC=Heavy clay.

Consistence (dry/force/strength): L=Loose; S=Soft; SH= Slightly Hard; MH= Moderately Hard; HA = Hard; VH=Very Hard; EH= Extremely Hard; R= Rigid; VR= Very Rigid.

Structure, gr=Granular; abk=Angular blocky; sbk=Subangular blocky; pl=Platy; WEG=wedge; sg= single grain; m=Massive); pr=Prismatic; cpr=Columnar; PO=Polyhedral; Grade: 0=structureless/apedal; 1=weak; 2=moderate; 3=strong. Size (mm): vf(<2); f(2-5); m(5-10); co(50-100); vc(100-500); ec(>500).

Pores/roots: none=No roots or pores; 1=Few (<1/area); 2=Common (1-5/area); 3=Many(>5/area). Size Class: (mm): MACROPORES of DIAMETER (mm): vf= Very fine(<2), f=Fine(1-2), m=Medium(2-5); co=Coarse (>5); vc= (>5); Dt= Dendtitic; IG= Irregular; TU=Tubular; VE= Vesicular. Cracks = see reference #5.

Concentrations: FD=finely disseminated, M=Masses, N=Nodules, C=concretions; X=Crystals, CA = calcite/carbonates, GY=Gypsum, SA=Salts, B= biological; SF=Shell fragments; RS=root sheaths; SI =Silica, CB= Clay bodies. ABUNDANCE (%): f= few(<2), c=Few(2-20), m=Many (>20).SIZE (mm): 1=Fine(<2); 2=Medium(2-6); 3= Coarse(6-20); 4=Very Coarse(20-76); 5=Extremely Coarse(>76). SHAPE: C= cylindrical; D=Dendritic; I = Irregular; P=Platy; R= Reticulate; S=Spherical; T=Threads; LOCATION: MAT=matrix; PED faces= APF; on surfaces along pores = SPO; on surfaces along root channels = RPO. CONTRAST: S=Sharp, C=Clear, D=Diffuse

Rock and other fragments (texture modifiers): gravelly (15-35%), very gravelly (35-<60), extremely gravelly (60-90); WD = Woody; MK = Mucky, PT = Peaty; CEM = Cemented; GYP = Gypsiferous.

Reaction or fizz to 1N HCl (H2)/calcareous: NE= Noneffervescent/ no bubbles; VS= very slightly effervescent; SL=slightly effervescent; ST=strongly effervescent; VE= violently effervescent.

2.2 Field measurements of water samples.

Site and sample description			Date	EC (mS/cm)	pH	T (°C)	Fe^{2+} (mg/L)
LDB	Site 1	Pit 1	15/04/2003	5.2	7.6		
	Site 1	Pit 4	15/04/2003	66	6.5		
	Site 1	Pit 1	11/08/2003	60	5.4		16
	Site 2	Surface	3/04/2003	16	4.5		
Ramco Lg	VS2	Pit 1	1/04/2003	36	7.1		0.15
		Pit 2	1/04/2003	32	6.8		0.59
		Pit 3	1/04/2003	58	6.8		<0.05
		Inflow	1/04/2003	2.4	8.3		<0.05
		Surface	1/04/2003	62	8.5		<0.05
		Surface	11/08/2003	79	8.8		
		Pit	11/08/2003	38	7.6		
BEB	Near Centre	Pit 1	2/04/2003	120	7.5		<0.05
		Pit 1	11/08/2003	78	7.5	11.7	2.6
		Surface	11/08/2003	43	8.8	14.2	
Hart Lg	Near "Spit"	Pit 1	1/04/2003	21	7.4		<0.05
Ross Lg	Near Road	Pit 1	1/04/2003	5.2	9.0		<0.05
Wpl L	SE shore	Surface	2/04/2003	5.1	9.4		<0.05
Merreti L	Near Post 155	Surface	2/04/2003	1.4	9.0		<0.05
BBL	Site 1	Surface	3/04/2003	13	5.5		0.20 & 0.24

2.3 Laboratory measurements of water samples.

(Results are in mg/L)

Site and sample description			Ca	Mg	Na	K	Total Fe	Cl	SO$_4$	Alk. as HCO$_3^-$	Acidity as HCO$_3^-$	NH$_4$-N	NO$_3$-N	PO$_4$-P	Total S	Total P	DOC	Total Dissolved N
LDB	Site 1	Pit 1	76	72	950	36	0.0	1100	440	596		8.5	0.65		150	0.8	15	12
	Site 2	Pit 2	1200	1600	14000	200	3.4	23000	8100			29	0.03		2500	3.4	160	46
Ramco Lg		Inflow	34	31	370	9.8	<0.1	510	210	813		0.29	0.36	0.02	70	<0.1	7.1	1.0
	VS2	Pit 1	280	490	8000	67	<0.1	11000	1700	662		2.0	0.07	0.05	610	1.0	12	2.6
	VS2	Pit 2	350	480	6900	67	1.1	10000	1200	552		4.2	0.03	0.09	410	0.9	7.2	4.8
		Surface	150	600	15000	300	<0.1	23000	2100	233		0.2	0.04	0.12	750	1.4	45	4.7
BEB	Near centre		1200	2800	34000	820	<0.1	51000	11000	267		31	0.03	0.51	3600	4.7	220	45
Hart Lg	Near "Spit"	Pit 1	220	370	7700	130	<0.1	10000	1400	524		0.78	0.11	0.14	460	0.3	8.4	1.4
Ross Lg	Near Road		50	83	880	13	<0.1	1500	190	161		0.25	0.03	0.05	66	0.2	37	2.9
Wpl L	SE shore		150	110	720	24	<0.1	1200	730	46		0.25	0.04	0.01	220	0.1	20	2.0
Merreti L	Near Post 155		23	19	200	15	<0.1	260	50	199		0.81	0.62	0.35	18	0.4	47	3.6
BBL	Site 1		320	360	1800	18	0.7	4100	280	2	9.1	7.0	0.06	0.05	85	<0.1	6.3	7.5

2.4   River Murray water quality data.

(from Mackay et al. 1988. All results are in mg/L unless otherwise indicated. TSS values were calculated using the equation of Gippel 1995)

Sampling Station	Na			K			Ca			Mg			Cl		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		min	max		min	max		min	max		min	max		min	max
Euston	30	8.9	96	2.5	0.1	6.5	8.2	4.3	19	7.4	3.2	21	52	13	180
Merbein	43	18	104	2.8	0.9	6.6	9.8	5.8	3.6	9.6	5.5	23	84	14	195
Darling River	43	17	134	7.5	1.5	14	28	8.2	50	14	4.5	35	50	9	134
Lock 9	47	16	86	4.7	1.9	9.3	14	5	25	11	4	21	72	16	159
L Victoria	49	24	72	5.5	3.1	8.3	16	10	22	12	8	17	73	36	120
D/s Rufus R. Jn.	52	23	93	5.2	3.3	9.8	16	9	29	12	5	21	75	21	327
Lock 5	60	23	120	5	2.5	9.1	16	8	31	13	6	22	94	28	207
Lock 3	76	28	140	5.7	3.4	9.8	18	9	29	14	8	21	124	36	298
Waikerie	92	37	176	5.9	3.5	9.2	19	9	31	16	8	25	143	40	361

Sampling Station	HCO_3			SO_4			Turbidity (NTU)			TSS		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		min	max		min	max		min	max		min	max
Euston	43	23	80	9.5	1.2	7.8	34	6	120	45	12	147
Merbein	49	22	85	15	2.1	33	29	3.5	122	40	10	149
Darling River	194	38	308	19	3.9	61	109	10	500	134	17	594
Lock 9	78	27	190	19	5	35	74	11	470	92	18	558
L Victoria	89	46	124	20	7	47	91	22	560	112	31	664
D/s Rufus R. Jn.	89	48	167	21	7	36	82	14	470	102	22	558
Lock 5	85	27	176	24	9	45	83	16	400	103	24	476
Lock 3	95	29	176	30	8	48	85	14	410	105	22	488
Waikerie	91	36	176	34	15	53	83	15	380	103	23	452

2.5 Sediment sample field measurements.

Site and sample description			Depth (cm) ud	Depth (cm) ld	pH	$pH_{H_2O_2}$	Eh (mV vs. SHE*)
LDB	Site 1	Pit 1	1	5	7.2	6.9	
			5	12	7.3	6.6	
			12	20	7.3	6.7	
			20	30	7.3	6.2	-40
			30	40	7.7	6.2	-3
			40	50	8.6	7.9	
	Site 2	Pit 2	0	1	8.6	7.3	-12
			0	3	8.1	6.6	50
			1	20	8.1	6.6	-180
			20	30	8.5	2.6	-85
		Pit 3	0	5	7.9	6.1	270
			5	30	8.0	3.2	180
			30	40	7.8	2.0	40
Ramco Lg	Pit 1		1		7.0		600
			2		7.2	7.5	550
			3		7.3		470
			5		7.3	6.6	430
			10		7.2	6.4	410
			15		7.3	7.1	310
			20		7.5	7.1	360
			25		7.6		340
	Pit 2		1		7.1		340
			2		7.2	7.3	320
			4		7.6		320
			5		7.3	7.5	260
			7		7.1		220
			10		7.5	6.3	220
			13		7.4		180
			15		7.0	5.8	110
			20		7.7	4.7	90
			23		7.8		150
			25		7.7	7.9	180
			30		7.7		150
BEB			0	5	6.9		
			0	5	6.3		

Electrochemical potentials are referenced to the Standard Hydrogen Electrode (SHE) as the measured potential depends on the reference electrode used.

2.5 (continued).

Site and sample description			Depth (cm)		pH	$pH_{H_2O_2}$	Eh (mV vs. SHE[*])
			ud	ld			
Hart Lg		sulfidic	0	5	6.9	7.1	230
		crack	0	5	7.4	7.8	270
		clay	5	5	7.2	5.9	200
Ross Lg		water					240
		s/w interface	0				220
		black sulfidic	0	10	6.9	6.2	-81
		sandy	0	10	6.6	5.9	-65
		mottled zone	15	25	6.6	5.8	55
		clay	25		7.1	7.2	150
Wpl L	samphire	water			8.9		0
		MBO	1		7.7	7.2	-240
		clay	3	6		6.2	
		clay	6	10		6.2	-130
		clay	10	20		7.3	-230
	track	water			9.1		0
		MBO	1		7.3	6.4	-310
		clay	3	7		6.1	-150
Merreti L			0	5	6.1	4.6	270
			5	10	6.1	5.4	340
			10	20	5.8	6.5	400
Clover L		dry	0	10	5.9		
			10	20	5.5		
			20	25	6.2		
BBL	mid		1		6.7		10
			3		6.6		15
			5		6.4	2.9	50
			7		6.3		15
			10		6.1		30
			15		6.2	2.2	110
	upper	water	0		4.5		420
		surface ppt	1		5.2		350
			10		6.3		110
			15		5.0		40

[*] *Electrochemical potentials are referenced to the Standard Hydrogen Electrode (SHE) as the measured potential depends on the reference electrode used.*

2.6 Moisture, electrical conductivity, pH and soluble chloride in sediment samples.

Sample ID	Moisture	E.C.	pH	Soluble Cl
	%	(1:5 soil:water) dS/m		%
CBD1.1	7.6	4.9	8.1	0.34
CBD1.2	8.2	3.2	8.1	0.13
CBD1.3	32	17	8.0	2.8
CBD1.4	50.9	4.5	7.8	0.40
CBD1.5	73.1	4.5	7.8	0.42
CBD1.6	108	1.3	8.6	0.13
CBD1.7	44	0.54	9.2	0.028
CBD1.8	39.6	0.83	9.0	0.32
CBD2.1	130	68	8.6	16
CBD2.3	182	71	8.7	17
CBD2.4	41	3.4	8.9	0.57
CBD3.1	69	38	8.4	6.6
CBD3.2	113	15	8.3	2.6
CBD3.3	50	2.1	8.8	0.26
RVSS2.1.1	46	33	8.4	5.5
RVSS2.1.2	29	5.8	8.9	0.93
RVSS2.1.3	32	3.0	9.2	0.41
RVSS2.1.4	28	2.3	9.5	0.29
RVSS2.1.5	24	2.0	9.5	0.25
RVSS2.2.1	41	25	8.6	4.2
RVSS2.2.2	37	6.4	8.9	1.1
RVSS2.2.3	46	4.0	8.8	0.59
RVSS2.2.4	29	2.2	9.3	0.30
RVSS2.2.5	26	1.9	9.4	0.24
RVSS2.2.6	27	1.9	9.4	0.24
RVSS2.2.7	24	1.8	9.4	0.23
BEB1	52	47	8.4	8.6
BEB2	37	44	8.4	8.3
HL1	50	24	8.8	4.1
HL2	49	22	8.5	3.7
HL3	38	6.0	8.6	1.0
RL1	19	12	9.4	2.1
RL2	23	3.6	9.1	0.53
RL3	38	3.3	8.9	0.42
RL4	34	2.5	9.4	0.29
WL1	54	4.0	8.5	0.34
WL2	77	5.0	8.3	0.54
WL3	22	5.5	8.6	0.53
WL4	88	1.9	8.3	0.12
WL5	36	1.5	8.4	0.054
WL6	29	0.60	8.6	0.039
WL7	27	0.87	8.7	0.041
ML1	25	0.59	7.6	0.07
ML2	20	0.40	7.2	0.043
ML3	29	0.31	7.2	0.030
CL1	8	7.8	6.6	1.4
CL2	18	9.0	6.8	1.2
CL3	22	8.5	7.0	1.1
BBL1	54	1.2	5.8	0.18
BBL2	47	0.82	5.0	0.12
BBU1	38	3.4	5.7	0.53
BBU2	79	6.4	6.2	1.1
BBU3	27	1.8	5.0	0.27
BBU4	35	11	5.8	2.3

2.7 Sulfur, carbon, calculated acidity and acid base accounting for sediment samples.

(Negative net acid generating potential (NAGP) indicates residual neutralisation capacity. NAGP was not calculated when the inorganic carbon was below the detection limit.)

Sample ID	Sulfur			Carbon			Acidity		Acid – Base Accounting
	Total	Cr reducible	Oxidised + organic S	Total	Inorganic C	Organic C ($C_{tot}-C_{inorg}$)	Acidity	Lime equivalent	NAGP (Acidity - Carbonate)
	S_T %	S_{Cr} %	S_{ox+org} %	C_{tot} %	C_{inorg} %	C_{org}%	moles H^+/t	kg $CaCO_3$/t	kg $CaCO_3$/t
CBD1.1	7.4	0.33	7.1	7.4	3.9	3.5	210	11	- 320
CBD1.2	6.3	0.49	5.8	6.3	1.9	4.4	310	16	- 140
CBD1.3	0.92	0.46	0.46	4.6	1.6	3.0	290	15	- 120
CBD1.4	3.8	0.45	3.3	3.8	1.2	2.6	280	14	- 87
CBD1.5	3.3	0.52	2.8	3.3	1.1	2.3	320	16	- 72
CBD1.6	0.72	0.72	0.00	2.5	0.13	2.4	450	23	11
CBD1.7	0.28	0.28	0.00	1.0	<0.06	1.0	180	9.0	
CBD1.8	0.62	0.15	0.47	0.62	0.07	0.55	93	4.7	- 1.3
CBD2.1	3.5	0.78	2.7	7.9	1.1	6.8	490	25	- 66
CBD2.3	1.4	0.43	0.96	5.8	1.5	4.3	270	14	-110
CBD2.4	0.21	0.19	0.02	0.85	<0.06	0.82	120	6.0	
CBD3.1	1.1	0.27	0.86	2.4	0.54	1.9	170	8.5	- 37
CBD3.2	1.0	0.97	0.04	2.1	0.07	2.0	600	30	24
CBD3.3	0.33	0.36	- 0.03	1.5	<0.06	1.5	230	12	
RVSS2.1.1	0.53	0.02	0.51	1.6	0.65	0.97	11	0.55	- 53
RVSS2.1.2	0.12	0.13	- 0.01	0.33	<0.06	0.30	82	4.1	
RVSS2.1.3	0.18	0.19	- 0.01	0.21	<0.06	0.18	120	6.0	
RVSS2.1.4	0.02	0.00	0.02	0.15	<0.06	0.12	1	0.06	
RVSS2.1.5	0.02	0.00	0.01	0.11	<0.06	0.08	1	0.06	
RVSS2.2.1	0.28	0.04	0.24	1.1	0.43	0.62	23	1.2	- 35
RVSS2.2.2	0.16	0.11	0.05	0.52	0.15	0.38	68	3.4	- 8.7
RVSS2.2.3	0.16	0.14	0.02	0.59	0.08	0.51	86	4.3	- 2.3
RVSS2.2.4	0.10	0.09	0.01	0.33	<0.06	0.30	58	2.9	
RVSS2.2.5	0.19	0.18	0.01	0.38	<0.06	0.35	110	5.5	
RVSS2.2.6	0.08	0.08	0.00	0.20	<0.06	0.17	49	2.5	
RVSS2.2.7	0.02	0.01	0.02	0.17	<0.06	0.14	3	0.16	
BEB1	1.4	0.29	1.1	4.6	2.3	2.2	180	9.0	- 190
BEB2	1.3	0.47	0.84	5.1	2.2	2.9	290	15	- 170
HL1	0.36	0.22	0.14	1.4	0.33	1.1	140	7.0	- 20
HL2	0.24	0.06	0.18	1.3	0.40	0.94	40	2.0	- 32

Sample ID	Sulfur			Carbon			Acidity		Acid – Base Accounting
	Total	Cr reducible	Oxidised + organic S	Total	Inorganic C	Organic C $(C_{tot} - C_{inorg})$	Acidity	Lime equivalent	NAGP (Acidity - Carbonate)
	S_T %	S_{Cr} %	S_{ox+org} %	C_{tot} %	C_{inorg} %	C_{org} %	moles H$^+$/t	kg CaCO$_3$/t	kg CaCO$_3$/t
HL3	0.14	0.02	0.12	0.38	0.06	0.32	13	0.65	- 4.4
RL1	0.02	0.00	0.02	0.18	<0.06	0.15	2	0.10	
RL2	0.03	0.00	0.03	0.35	<0.06	0.32	2	0.10	
RL3	0.14	0.10	0.04	0.31	<0.06	0.28	62	3.1	
RL4	0.18	0.19	- 0.01	0.50	0.06	0.44	120	6.0	
WL1	0.32	0.07	0.26	1.8	0.27	1.6	41	2.1	- 20
WL2	0.36	0.09	0.27	2.6	0.33	2.2	54	2.7	- 25
WL3	0.33	0.04	0.29	0.57	<0.06	0.54	25	1.3	
WL4	0.25	0.08	0.17	3.2	0.22	3.0	49	2.5	- 16
WL5	0.14	0.04	0.10	0.78	<0.06	0.75	26	1.3	
WL6	0.02	0.01	0.01	0.40	<0.06	0.37	6	0.28	
WL7	0.04	0.01	0.03	0.30	<0.06	0.27	4	0.22	
ML1	0.02	0.01	0.01	0.42	<0.06	0.39	5	0.25	
ML2	0.01	0.01	0.01	0.27	<0.06	0.24	3	0.16	
ML3	0.01	0.00	0.01	0.15	<0.06	0.12	3	0.13	
CL1	0.04	0.01	0.03	0.82	<0.06	0.79	6	0.28	
CL2	0.28	0.01	0.27	0.57	<0.06	0.54	4	0.19	
CL3	0.27	0.00	0.27	0.36	<0.06	0.33	2	0.10	
BBL1	0.12	0.09	0.03	1.1	<0.06	0.80	59	3.0	
BBL2	0.03	0.02	0.01	0.66	<0.06	0.63	14	0.70	
BBU1	0.03	0.01	0.02	0.57	<0.06	0.54	4	0.22	
BBU2	0.39	0.35	0.04	2.8	<0.06	2.8	220	11	
BBU3	0.04	0.03	0.01	0.31	<0.06	0.28	21	1.1	
BBU4	0.11	0.03	0.09	1.9	<0.06	1.9	16	0.80	

2.8 Analytical results for acid extractable elements.

(All results are in mg/kg.)

Sample ID	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn
CBD1.1	33000		120	140000			28	19	22000	6400	11000	1700		4900	16	740		19000		47
CBD1.2	48000		130	64000			42	24	29000	10000	12000	1100		2500	30	810		12000		60
CBD1.3	49000	<10	86	56000	<10	12	43	23	30000	11000	10000	1200	<10	21000	24	460	25	13000	<10	60
CBD1.4	55000		54	42000			48	33	34000	11000	7200	1100		4800	30	460		9700		67
CBD1.5	47000		49	34000			42	25	30000	9600	7600	880		5100	22	380		10000		57
CBD1.6	46000	<10	47	6600	<10	11	42	18	27000	10000	5600	420	<10	2500	23	200	25	9000	<10	54
CBD1.7	40000	<10	33	1700	<10	<10	36	12	21000	8500	4000	160	<10	1300	17	110	22	3200	<10	45
CBD1.8	40000		32	2300			37	14	22000	8100	4000	150		1600	20	130		2600		44
CBD2.1	19000	<10	350	39000	<10	<10	16	<10	11000	7200	26000	580	<10	120000	<10	1400	13	38000	<10	26
CBD2.3	26000	<10	200	45000	<10	<10	22	12	15000	8300	18000	860	<10	110000	<10	700	15	17000	<10	34
CBD2.4	43000	<10	32	2400	<10	12	39	15	23000	7300	4400	140	<10	6700	26	170	22	2700	<10	49
CBD3.1	16000	<10	91	23000	<10	<10	17	<10	11000	4400	8800	360	<10	44000	<10	240	12	14000	<10	25
CBD3.2	47000	<10	47	5100	<10	14	41	17	31000	8800	6100	350	<10	19000	20	240	25	13000	<10	57
CBD3.3	46000	<10	30	2400	<10	10	43	16	25000	8400	4600	190	<10	3800	22	190	25	4400	<10	56
RVSS2.1.1	14000	<10	84	25000	<10	<10	16	11	8000	4500	7900	580	<10	38000	<10	250	10	6800	<10	18
RVSS2.1.2	26000	<10	30	1700	<10	<10	27	16	16000	6400	3300	220	<10	7900	17	160	15	1900	<10	29
RVSS2.1.3	39000	<10	36	1100	<10	<10	35	18	23000	9200	4900	260	<10	5500	14	120	20	2400	<10	44
RVSS2.1.4	40000	<10	34	1000	<10	<10	37	18	22000	9400	5000	160	<10	4600	16	78	20	230	<10	43
RVSS2.1.5	33000	<10	31	910	<10	<10	31	16	20000	6700	4600	150	<10	4000	10	67	20	170	<10	40
RVSS2.2.1	12000	<10	61	15000	<10	<10	12	<10	6700	4000	5400	390	<10	29000	<10	140	<10	3400	<10	13
RVSS2.2.2	13000	<10	21	5700	<10	<10	14	<10	7400	3800	2100	230	<10	8200	<10	69	<10	1900	<10	16
RVSS2.2.3	18000	<10	23	3600	<10	<10	17	<10	10000	4700	2500	240	<10	5000	<10	91	12	2100	<10	22
RVSS2.2.4	15000	<10	<20	1300	<10	<10	15	<10	7500	4300	1700	130	<10	2700	<10	35	11	1200	<10	16
RVSS2.2.5	27000	<10	25	1200	<10	<10	25	11	15000	6500	3000	170	<10	3000	<10	76	17	2100	<10	28
RVSS2.2.6	34000	<10	32	1000	<10	<10	32	16	20000	7700	3900	160	<10	3800	13	110	21	920	<10	37
RVSS2.2.7	31000	<10	27	1000	<10	<10	30	16	17000	7400	3500	130	<10	3200	13	77	20	140	<10	34
BEB1	30000	<10	110	80000	<10	<10	27	71	18000	9200	15000	520	<10	58000	15	330	20	16000	<10	55
BEB2	39000	<10	120	77000	<10	<10	34	55	23000	11000	13000	790	<10	61000	18	450	24	17000	<10	60
HL1	26000	<10	51	11000	<10	<10	24	17	15000	7000	5800	290	<10	28000	25	220	16	4500	<10	36
HL2	25000	<10	59	15000	<10	<10	22	17	16000	6100	7200	960	<10	25000	11	520	16	3200	<10	35
HL3	28000	<10	35	2400	<10	<10	28	15	17000	6200	3400	200	<10	8300	15	140	16	2600	<10	34

2.8 (continued).

Sample ID	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn
RL1	9000	<10	28	2200	<10	<10	<10	<10	5700	2100	2000	260	<10	15000	<10	81	<10	1900	<10	12
RL2	13000	<10	<20	260	<10	<10	12	<10	8400	2200	1300	110	<10	4700	<10	59	<10	250	<10	14
RL3	41000	<10	43	940	<10	<10	34	19	27000	7600	4000	290	<10	6600	16	110	19	410	<10	40
RL4	33000	<10	31	2100	<10	<10	29	16	23000	7200	4000	150	<10	4600	15	81	16	1300	<10	32
WL1	24000	<10	50	13000	<10	<10	22	10	14000	7100	7000	220	<10	2900	<10	270	16	3600	<10	27
WL2	28000	<10	51	15000	<10	<10	25	17	16000	8300	6900	230	<10	4500	17	310	18	4000	<10	39
WL3	36000	<10	38	4000	<10	<10	33	21	19000	10000	4600	140	<10	5700	20	130	20	3500	<10	40
WL4	35000	<10	80	12000	<10	<10	34	22	19000	10000	7700	250	<10	1600	20	420	21	2800	<10	47
WL5	41000	<10	34	3800	<10	<10	37	22	21000	11000	4300	130	<10	1100	19	170	20	1500	<10	45
WL6	39000	<10	29	2200	<10	<10	35	19	23000	10000	3900	110	<10	1100	16	120	21	230	<10	40
WL7	40000	<10	33	1800	<10	<10	34	23	25000	10000	4100	170	<10	1500	18	86	23	360	<10	43
ML1	13000	<10	<20	550	<10	<10	12	12	7300	3200	1400	66	<10	780	<10	130	10	190	<10	18
ML2	22000	<10	23	740	<10	<10	19	16	12000	5300	2200	97	<10	560	<10	110	14	84	<10	27
ML3	38000	<10	35	1200	<10	<10	32	22	24000	9300	4100	220	<10	700	20	130	17	36	<10	42
CL1	57000	<10	53	2700	<10	11	48	13	33000	17000	6900	250	<10	7600	27	340	28	790	<10	65
CL2	63000	<10	67	3200	<10	12	52	13	38000	20000	7900	300	<10	11000	32	290	28	3600	<10	71
CL3	66000	<10	79	2400	<10	13	55	11	39000	21000	8000	290	<10	11000	30	240	28	3200	<10	74
BBL1	15000	<10	<20	1300	<10	<10	14	11	11000	3700	1700	160	<10	2200	<10	150	14	450	<10	20
BBL2	29000	<10	<20	1900	<10	12	27	14	27000	7100	3100	380	<10	5600	17	310	21	4200	<10	35
BBU1	28000	<10	<20	1100	<10	24	27	16	17000	6700	2700	200	<10	1400	20	88	20	650	<10	35
BBU2	56000	<10	29	3600	<10	15	51	20	37000	13000	7000	410	<10	9400	25	490	37	1700	<10	73
BBU3	16000	<10	<20	1100	<10	<10	14	16	13000	4200	1500	190	<10	920	<10	170	15	1600	<10	21
BBU4	59000	<10	25	1500	<10	13	54	26	31000	13000	3900	210	<10	1200	24	260	31	450	<10	63

2.9 Analytical results for the total element analysis of selected sediment samples by mixed acid digestion.

(All results are in mg/kg)

Sample ID	Al	Ba	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Nb	Ni	P	Pb	S	Ti	V	Zn
CBD1.1	42700	350	138000	36	41	22700	10500	11700	1650	7850	8	26	650	6	19000	2750	56	76
CBD1.2	58000	300	58800	44	40	29700	14200	10400	1100	23700	12	33	450	8	12200	3750	74	84
CBD1.3	71500	360	53300	54	60	37400	16900	8800	1100	9250	14	38	470	<5	9500	4600	94	90
CBD1.4	57200	310	50800	49	38	32000	14900	8750	900	9300	12	33	340	24	9900	4100	78	74
CBD1.5	54300	310	20900	46	34	29700	14900	6200	450	6500	12	28	240	10	8600	4200	76	70
CBD1.6	50600	310	12300	48	52	28600	14500	4750	260	5550	12	24	175	14	3150	4000	72	52
CBD1.7	54800	360	12600	46	46	30700	15600	5200	260	6100	12	25	170	6	2750	4300	78	60
CBD2.1	24800	175	46000	21	26	13500	8850	25000	550	100000	6	18	1200	<5	37100	1650	38	56
CBD2.3	32900	210	49900	27	48	18000	10700	17500	800	97600	8	20	600	8	15800	2150	45	50
CBD2.4	54300	320	22700	50	31	27100	13700	5550	230	9550	12	29	200	14	2300	4000	76	74
CBD3.1	29700	280	34400	37	45	20400	11300	9600	410	47800	8	20	230	6	13600	2800	40	43
CBD3.2	60000	310	19900	54	37	34200	15400	7200	400	23500	12	29	290	12	12700	4400	82	76
CBD3.3	58700	350	20200	49	43	28900	15700	5800	270	8650	14	28	280	18	4250	4650	84	70
RVSS2.1.3	53400	350	10900	43	46	28200	16600	5600	310	10700	12	26	155	8	2450	4050	66	50
RVSS2.2.1	24600	300	22600	25	78	18900	11300	5650	470	30400	8	16	175	10	3300	2350	30	36
RVSS2.2.3	36200	360	14400	33	56	23800	14800	3650	370	11000	8	19	170	8	2100	2900	42	40
RVSS2.2.5	43400	340	13800	44	34	25000	15500	3950	280	8850	12	23	170	18	2050	3750	52	44
BEB1	35900	230	80700	31	76	20000	11400	14900	500	69400	6	23	350	<5	16200	2350	56	86
BEB2	46300	250	75700	35	58	23700	12600	12800	750	57400	8	28	400	<5	17200	2800	68	90
HL1	38700	300	20300	37	240	23700	13900	6600	380	32600	10	21	290	8	4400	3500	52	50
WL6	49300	350	16600	46	41	26900	16400	4900	185	5400	10	24	165	14	200	3550	68	54
WL7	51400	360	12100	44	39	28800	16700	4700	230	5900	12	28	115	12	550	3700	68	48
BBL1	33000	320	16600	42	140	25500	15200	3100	320	6350	8	24	240	10	1500	2400	36	45
BBL2	65600	410	20800	62	40	31100	19800	5450	260	5650	14	28	320	16	350	4500	88	72
BBU1	34000	320	15400	35	72	19700	14900	3150	250	8600	10	16	180	12	500	2900	35	43
BBU2	43100	340	12700	45	43	33200	15200	4300	430	10700	10	24	310	<5	4300	2950	47	56
BBU3	46400	370	13400	35	38	22900	16700	3950	270	7150	10	30	115	14	550	3350	50	49
BBU4	63600	390	14700	60	49	37700	17800	7850	430	12800	14	35	460	26	1500	4100	76	82

2.9 (continued).

Sample ID	Ag	As	Bi	Cd	Ce	Co	Cs	Ga	In	La	Mo	Nd	Rb	Sb	Se	Sm	Sn	Sr	Te	Th	Tl	U	W	Y
CBD1.1	0.1	5	0.2	<0.1	39.5	10.5	3.7	13	<0.05	24	0.6	22	60	0.5	0.5	4.6	2.6	5950	<0.2	9.5	0.3	2.8	5.5	15.5
CBD1.2	0.4	5	0.3	<0.1	50	13	4.5	16	0.05	29.5	1.1	27	74	0.5	<0.5	5.5	2.9	1350	<0.2	11.5	0.4	3	1.4	18.5
CBD1.3	0.2	7	0.4	0.1	62	16	5.5	20.5	0.05	36.5	0.9	34.5	94	0.5	<0.5	7	3.7	1300	<0.2	14.5	0.6	2.8	2.3	23
CBD1.4	0.1	6	0.3	<0.1	50	13	4.3	16	<0.05	30.5	0.9	28.5	74	0.5	<0.5	6	3	1000	<0.2	12	0.4	2.2	2.5	19.5
CBD1.5	0.4	6	0.2	<0.1	56	11.5	3.8	15	<0.05	33	1	30	70	0.5	<0.5	6.5	2.7	340	<0.2	12.5	0.4	2.5	1.8	19.5
CBD1.6	0.4	6.5	0.3	<0.1	62	11	4.2	15.5	0.05	35	2.2	31.5	80	1	0.5	6.5	3.4	290	<0.2	14	0.4	2.8	3.7	20.5
CBD1.7	<0.1	5	0.3	<0.1	56	9	3.8	15	<0.05	32.5	1.7	30	72	0.5	<0.5	6.5	3	240	<0.2	13.5	0.4	2.5	2.8	18.5
CBD2.1	0.3	5	<0.1	<0.1	21	5.5	1.8	6.5	<0.05	12.5	0.5	11.5	32	<0.5	1	2.4	1.5	1800	<0.2	4.6	0.1	1.65	0.7	8
CBD2.3	0.3	4.5	0.2	<0.1	30.5	7.5	2.7	10	<0.05	18	0.8	16.5	48	<0.5	0.5	3.5	2	1300	<0.2	7	0.3	2.1	0.9	12
CBD2.4	0.2	3	0.2	<0.1	52	10	3.7	14.5	<0.05	30	1.8	27	70	0.5	<0.5	5.5	2.6	210	<0.2	12	0.3	2.4	2.1	18.5
CBD3.1	0.2	5	0.1	<0.1	32	7	1.9	7	<0.05	19	3.2	17	43.5	<0.5	1	3.5	1.8	850	<0.2	8	0.2	2	1.8	11
CBD3.2	0.2	7	0.3	<0.1	54	11.5	4	16	<0.05	30.5	1.3	29	74	1	0.5	6	2.8	270	<0.2	12	0.4	2.5	1.6	19
CBD3.3	0.2	4	0.3	<0.1	66	12.5	4.8	18	0.05	38.5	1.1	35.5	88	1	<0.5	7.5	3.4	220	<0.2	15	0.5	3.2	3.5	23.5
RVSS2.1.3	0.2	7	0.3	<0.1	70	12.5	4.4	16.5	<0.05	39.5	3.8	34.5	88	0.5	<0.5	7	3.5	160	<0.2	17	0.5	2.9	3.2	20
RVSS2.2.1	0.1	6.5	0.1	<0.1	35.5	6.5	1.8	7.5	<0.05	21	2.3	18	52	1.5	<0.5	3.6	2.8	500	<0.2	9	0.3	2.1	8	12.5
RVSS2.2.3	0.3	5	0.1	<0.1	44	6.5	2.4	9.5	<0.05	26.5	2	22.5	60	1	<0.5	4.6	2.7	180	<0.2	11	0.3	1.65	3.2	13.5
RVSS2.2.5	0.6	5.5	0.2	<0.1	72	7	3	11	<0.05	42.5	3.9	37.5	66	0.5	<0.5	7.5	2.6	145	<0.2	19	0.4	2.7	4.2	21.5
BEB1	0.5	4.5	0.2	<0.1	32	9.5	2.8	11	<0.05	18.5	3	18	50	0.5	0.5	3.7	3	3800	<0.2	8	0.3	6.5	1.2	12
BEB2	0.4	5.5	0.3	<0.1	42	12	3.9	14	<0.05	24.5	3	22.5	64	0.5	0.5	4.7	3.9	3450	<0.2	9.5	0.4	7.5	1.3	16
HL1	0.2	12.5	0.3	<0.1	62	10	2.9	12	<0.05	36.5	5	32.5	64	1	<0.5	6.5	3	300	<0.2	15.5	0.5	3.6	2.5	19
WL6	0.4	6.5	0.3	<0.1	60	10	4	15.5	<0.05	35.5	3	33	86	1	<0.5	7	3.1	185	<0.2	14.5	0.5	2.3	23.5	20.5
WL7	0.2	8	0.3	<0.1	52	8.5	3.7	14	<0.05	31.5	2.3	28.5	76	1	<0.5	6	3	135	<0.2	13	0.4	1.9	2.9	18
BBL1	0.3	8.5	0.2	<0.1	56	10	2.4	8.5	<0.05	32.5	6	28	66	1	<0.5	5.5	2.6	120	<0.2	13.5	0.5	2.2	4.2	17.5
BBL2	0.3	6.5	0.5	<0.1	72	14	7	23	0.05	44.5	0.9	38	125	1	<0.5	8	5	185	<0.2	19	0.7	3.2	4	22
BBU1	0.6	6.5	0.2	<0.1	52	4.7	2.6	9.5	<0.05	32	4	26.5	72	1	<0.5	5.5	2.7	160	<0.2	14	0.4	2.2	3.8	15.5
BBU2	0.5	7	0.3	<0.1	54	11	3.4	11.5	<0.05	33.5	3.1	27	78	1	<0.5	5.5	2.9	115	<0.2	13.5	0.4	2.1	3	14
BBU3	0.5	6	0.3	<0.1	70	23.5	4.4	14	<0.05	41	1.3	35.5	96	1	<0.5	7	3.7	135	<0.2	16.5	0.6	2.1	3.2	21
BBU4	0.3	10.5	0.5	<0.1	78	17.5	7	22	0.05	45	1.8	39	130	1.5	<0.5	8	4.8	165	<0.2	18.5	0.8	3.1	2.9	23.5

2.9 (continued).

Sample ID	Hf	Dy	Er	Eu	Gd	Ho	Lu	Pr	Tb	Tm	Yb
CBD1.1	2	3.2	1.85	0.99	3.7	0.56	0.24	5.5	0.6	0.25	1.7
CBD1.2	3	3.8	2.1	1.15	4.5	0.69	0.28	6.5	0.7	0.3	2
CBD1.3	3	4.7	2.7	1.45	5.5	0.87	0.36	8.5	0.9	0.35	2.6
CBD1.4	3	4.1	2.3	1.25	5	0.75	0.32	7	0.76	0.3	2.2
CBD1.5	3	4.4	2.3	1.2	5	0.74	0.31	7.5	0.78	0.35	2.3
CBD1.6	3	4.3	2.4	1.25	5	0.78	0.32	8	0.83	0.3	2.4
CBD1.7	3	4.1	2.2	1.2	4.8	0.74	0.33	7.5	0.76	0.3	2.3
CBD2.1	<1	1.65	1	0.48	2	0.33	0.13	2.8	0.31	0.15	0.95
CBD2.3	2	2.4	1.4	0.7	2.8	0.45	0.19	4.2	0.45	0.2	1.35
CBD2.4	3	3.9	2.3	1.1	4.5	0.72	0.31	7	0.7	0.3	2.2
CBD3.1	2	2.3	1.3	0.64	2.8	0.41	0.19	4.2	0.43	0.2	1.35
CBD3.2	3	4	2.2	1.2	4.8	0.73	0.32	7	0.76	0.3	2.2
CBD3.3	3	5	2.9	1.45	6	0.95	0.39	9	0.93	0.4	2.8
RVSS2.1.3	4	4.5	2.5	1.15	5.5	0.77	0.34	9	0.84	0.35	2.5
RVSS2.2.1	3	2.4	1.45	0.66	3	0.47	0.23	4.6	0.45	0.2	1.55
RVSS2.2.3	3	2.9	1.65	0.8	3.4	0.52	0.24	6	0.54	0.25	1.7
RVSS2.2.5	4	4.3	2.4	0.94	5.5	0.77	0.32	9.5	0.82	0.3	2.3
BEB1	2	2.6	1.5	0.75	3.1	0.47	0.2	4.4	0.46	0.2	1.5
BEB2	2	3.4	1.9	0.98	3.8	0.6	0.26	5.5	0.6	0.25	1.85
HL1	4	4	2.3	1.05	5	0.73	0.33	8.5	0.81	0.3	2.3
WL6	3	4.6	2.6	1.3	5.5	0.83	0.36	8	0.87	0.35	2.6
WL7	3	4	2.2	1.15	4.7	0.74	0.32	7	0.77	0.3	2.2
BBL1	3	3.4	1.85	0.8	4.4	0.6	0.25	7	0.64	0.25	1.7
BBL2	4	4.9	2.7	1.35	6	0.87	0.38	10	0.94	0.35	2.7
BBU1	3	3.2	1.8	0.76	4.1	0.57	0.27	7	0.63	0.25	1.85
BBU2	3	3.3	1.65	0.86	4.1	0.57	0.25	7	0.62	0.25	1.75
BBU3	4	4.5	2.4	1.15	5.5	0.78	0.34	9	0.83	0.35	2.4
BBU4	4	5	2.7	1.45	6	0.89	0.38	10	0.94	0.35	2.6

2.10 XRD analysis of selected sediment samples.

(Results are semi-quantitative for the mineral and give a compositional range)

Sample ID	Q	Mi	Al	Or	Ka	Sm	Ha	Py	Ca	Ar	Gy	Do	Un	Dominant S form	
														Reduced	Organic + oxidised
BBU1	D	M	M	M	T	T	T	?T	-	-	-	-	-	Pyrite	
BBU2	D	M	M	M	T	T	T	-	-	-	-	-	-	MBO	
BBU3	D	SD	M	M	T	T	T	-	-	-	-	-	-	MBO	
BBU4	CD	CD	T	T	M	CD	T	-	-	-	T	-	-	MBO	Gypsum
BBL1	D	M	M	M	T	-	T	?T	-	-	-	-	-	Pyrite	
BBL2	CD	CD	T	T	M	CD	T	?T	-	-	-	-	-	Pyrite	
WL4	D	M	M	M	T	M	T	?T	T	-	-	-	-	Pyrite	Organic
WL6	D	SD	M	M	T	M	T	?T	T	-	-	-	-	Pyrite	
WL7	D	SD	T	T	T	M	-	-	T	-	-	-	-	MBO	
HL1	D	M	M	T	T	T	M	-	M	?T	-	-	-	MBO	Organic
RVSS2.1.3	D	SD	M	M	T	M	T	?T	-	-	-	-	-	Pyrite	
RVSS2.2.1	D	M	M	M	T	T	M	?T	T	-	-	-	-	Pyrite	Organic
RVSS2.2.3	D	M	M	M	T	T	T	?T	T	-	-	-	-	Pyrite	
RVSS2.2.5	D	M	M	M	T	T	T	?T	-	-	-	-	-	Pyrite	
BEB1	CD	M	T	T	T	M	CD	-	M	CD	T	-	-	MBO	Gypsum
BEB2	CD	M	T	T	T	M	CD	-	M	CD	T	-	-	MBO	Gypsum
CBD1.1	CD	M	T	T	T	T	T	T	CD	CD	M	-	-		
CBD1.2	CD	M	T	T	T	M	M	T	CD	-	T	-	-	Pyrite	Gypsum
CBD1.3	D	M	T	T	T	SD	T	T	SD	-	T	-	-		
CBD1.4	D	M	T	T	T	M	T	T	SD	-	T	?T	-		
CBD1.5	D	M	T	T	T	M	-	T	T	-	-	-	-	Pyrite	
CBD1.6	D	M	T	T	T	M	-	T	-	-	-	-	-	Pyrite	
CBD1.7	D	M	T	T	T	T	-	T	-	-	-	-	-		
CBD2.1	M	M	T	T	T	T	D	-	M	-	T	-	M	MBO	Gypsum
CBD2.3	M	M	T	T	T	T	D	-	M	-	T	-	T	MBO	Gypsum
CBD2.4	D	M	T	T	T	M	T	T	T	-	-	-	-	Pyrite	
CBD3.1	D	M	T	T	T	T	M	?T	M	-	T	-	-	Pyrite	Gypsum
CBD3.2	D	M	T	T	T	M	M	T	T	-	-	-	-	Pyrite	
CBD3.3	D	M	T	T	T	M	T	T	T	-	-	-	-	Pyrite	
CDB efflorescence 1	M	-	T	-	-	T	-	-	CD	CD	T	-	-		
CDB efflorescence 2	SD	M	T	T	T	M	-	T	D	-	T	-	-		
CDB efflorescence 3	M	T	-	-	T	T	T	-	CD	M	CD	-	-		

Q=Quartz, Mi=Mica, Al=Albite, Or=Orthoclase, Ka=Kaolin, Sm=Smectite, Ha=Halite, Py=Pyrite, Ca=Calcite, Ar=Aragonite, Gy=Gypsum, Do=Dolomite, Un=Unidentified
D=Dominant (>60%), CD=co-dominant (sum of phases >60%), SD=sub-dominant (20-60%), M=minor(5-20%), T=trace (<5%).

Appendix 3:   Gel electrophoresis

Summary of gel electrophoresis analyses for presence of DNA (Figure 3.1), soxB functional gene (Figures 3.2 and 3.3) and 16S ribosomal RNA (Figures 3.4 and 3.5).
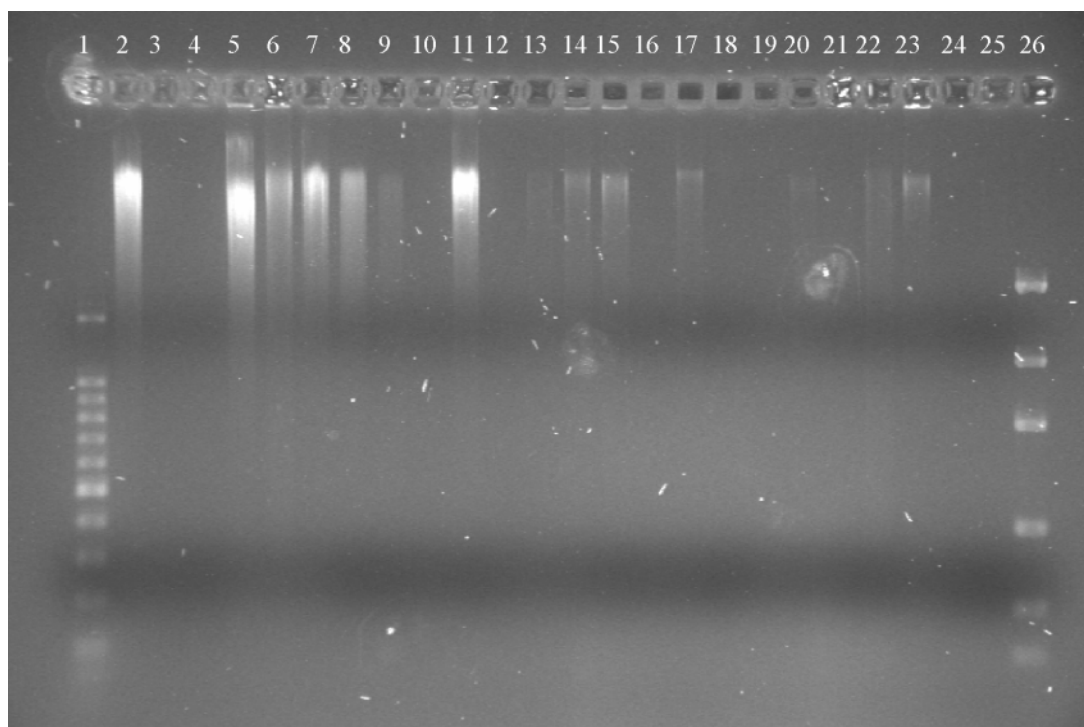

Figure 3.1: *Murray floodplain sediment DNA extracts. **Lane 1** 100bp DNA ladder; **Lane 2- 25** Soil DNA extracts numbers 1-11, 13-18, 20-26; **Lane 26** DNA quantification marker.*
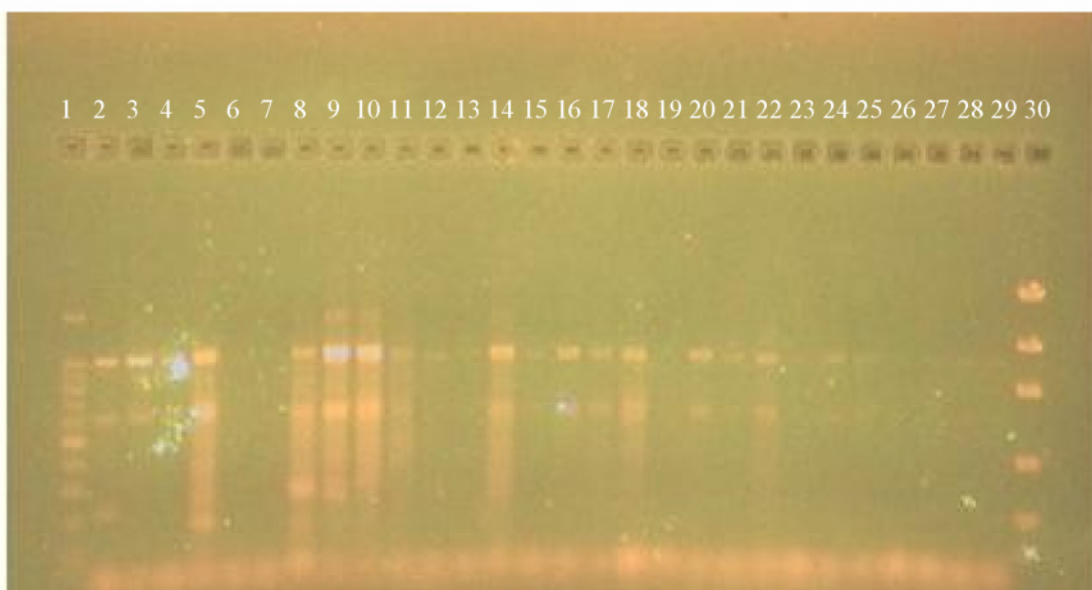
Figure 3.2: soxB functional gene DNA detected in extracts from Murray Floodplain sediments . 1000 bp PCR product visualised on 2% agarose gel. **Lane 1** 100bp DNA ladder; **Lane 2-4** A.caldus soxB positive control 1000bp product; **Lanes 5-29** soil extracts #1-11, 13-18, 20-27; **Lane 30** DNA quantification marker.
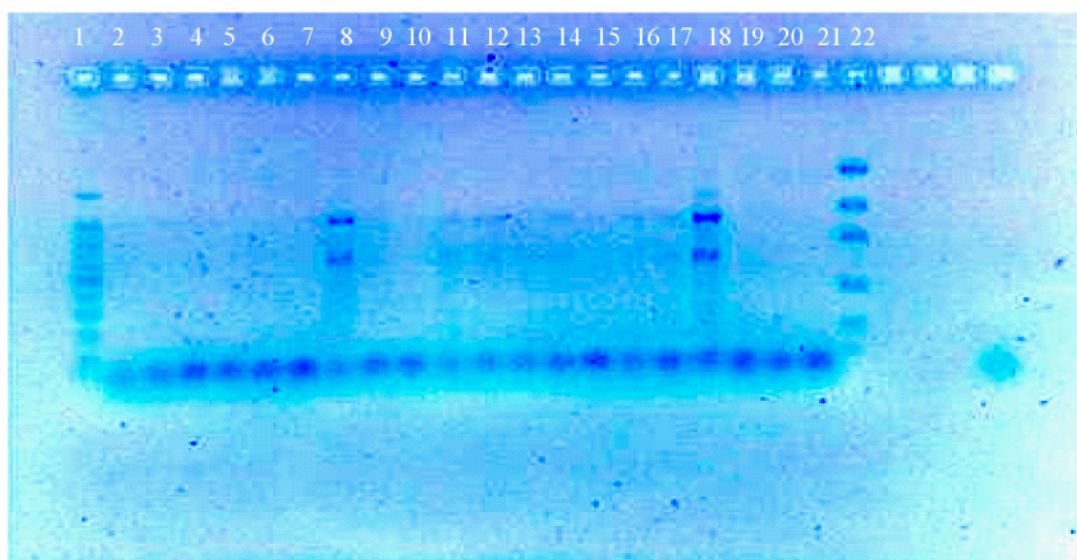

Figure 3.3: soxB functional gene DNA detected in extracts from Murray Floodplain Sediments. 1000 bp PCR product visualised on 2% agarose gel. **Lane 1** 100bp DNA ladder; **Lane 2-18** soil extracts #28-32, 34-45; **Lane 19-21** reagent blanks; **Lane 22** DNA quantification marker.
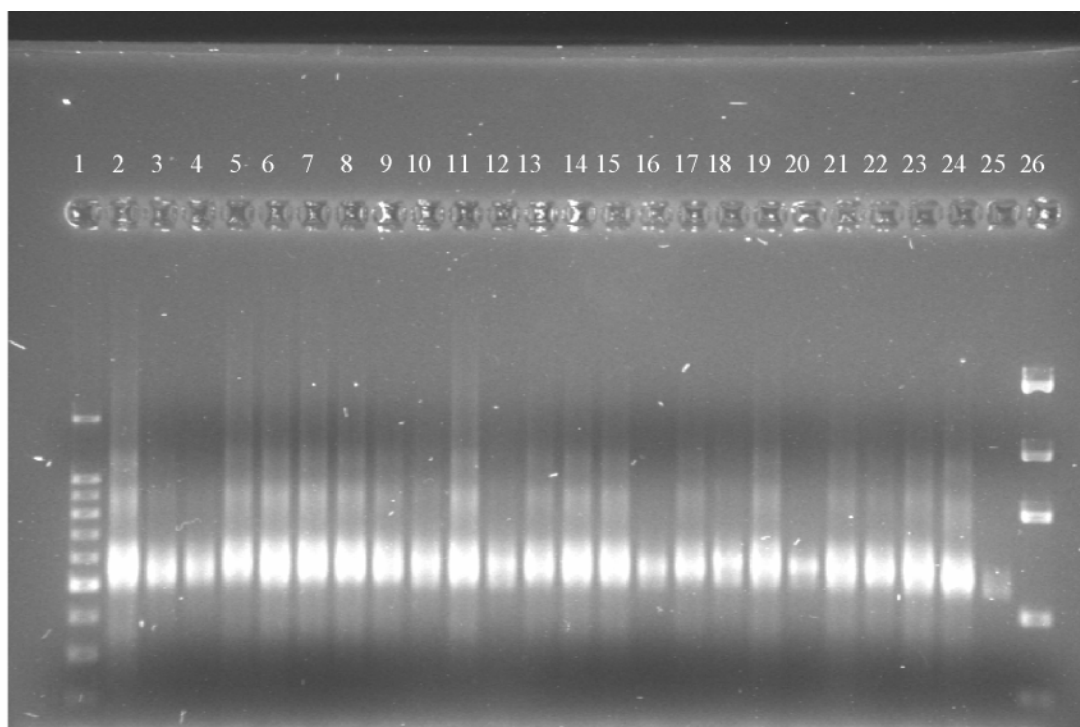
Figure 3.4: *16S functional gene DNA detected in extracts from acid sulphate soils . 510 bp PCR product visualised on 2% agarose gel.* **Lane 1** *100bp DNA ladder;* **Lane 2-25** *soil extracts #1-11, 13-18, 20-26;* **Lane 26** *DNA quantification marker.*
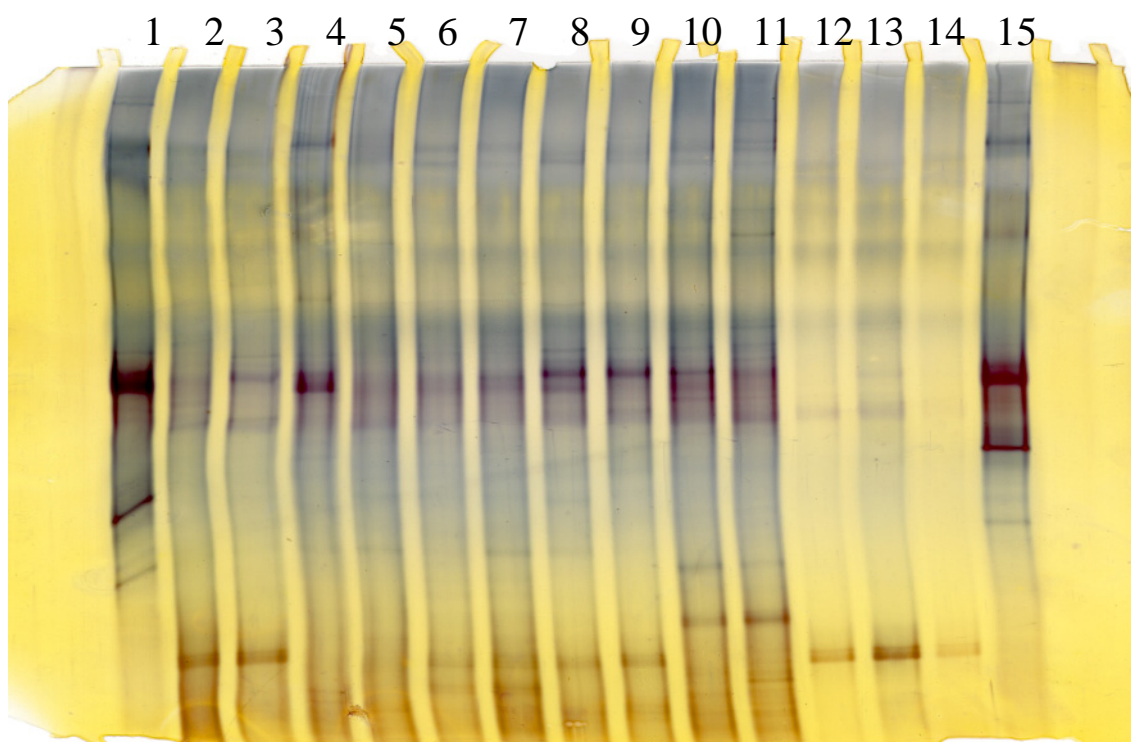

Figure 3.5: *Microbial population diversity, examples of 16S DGGE analysis of bacterial populations in Murray Floodplain Sediment DNA extracts. Acrylamide formamide/urea gel. Lane 1. E. coli, 2. Ramco Lagoon, 3. Ramco Lagoon, 4. Ramco Lagoon, 5.Berri E.B., 6.Berri E.B., 7.Loveday, 8.Loveday, 9.Loveday, 10.Loveday, 11.Loveday, 12.Ross Lagoon, 13. Ross Lagoon 14. Ross Lagoon, 15. E. coli.*