

Monitoring of the Lower Lakes based on the Ecotoxicological Assessment of Selected sites

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Ann Marie Jolley and Liz Barnet, Department of Environment, Water and Natural Resources, as part of the Coorong, Lower Lakes and Murray Mouth Recovery Program

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Executive Summary

Drought from 2007 to mid-2010 caused large expanses of previously long-inundated sediments and subaqueous soils to be exposed around the margins of lakes Alexandrina and Albert in South Australia. This exposed acid sulfate soil (ASS) material became progressively oxidised over increasing depths in the soil profiles. The resultant formation of sulfuric materials ($\text{pH} < 4$) produced significant water quality and ecological problems.

The main focus of earlier field investigations of ASS environments in lakes Alexandrina and Albert was measuring and analysing the physico-chemical parameters at the two lakes. Based on the seven years of monitoring (2007-2014), the subaqueous soils in the Lower Lakes are in a transient state and the build-up of sulfide is likely to continue under saturated conditions. The surface of subaqueous soils in many areas has returned to a circumneutral pH, which has formed an effective reactive barrier to upward acid and metal fluxes. Recent monitoring has found that the subsoils in many areas of the Lower Lakes have remained acidic, buffered by hydroxysulfate minerals such as natrojarosite (Shand *et al.* 2012). The hazards posed by acidity and contaminants in deeper layers however are poorly known.

Sediments constitute an association between organic and inorganic particles and living organisms. They provide a habitat for many aquatic organisms (bacteria to macroinvertebrates) and are a major repository for many of the more persistent contaminants that are introduced into the water environment. Contaminants in sediments may be directly toxic to aquatic life or can be a source of chemicals for bioaccumulation in the food chain. Although certain contaminants are highly sorbed to sediment, these may still be available to the biota. The sediment-bound contaminants may become resuspended from wetland beds into the water column by physical (water currents and wind) or biological processes. Toxicity tests measure the cumulative effects of all bioavailable contaminants and their interactions as mixtures.

The overall aim of this project is to perform an ecological assessment of subaqueous soils in the Lower Lakes to provide a better understanding of the health and potential impacts of recovered ASS.

This interim report presents ecotoxicity and chemical data collected during the 2014 sampling year at the Point Sturt North and Boggy Creek sites. Those sites were selected for surface water and sediment sampling in 2014. They have been permanently inundated since late 2010 and potentially have recovered, given the four years elapsed since the return of water. Surface water samples collected from the two sites were evaluated for their toxicity using a microbial assay for risk assessment (MARA), a duckweed (*Lemna* sp.) bioassay, a waterflea (*Ceriodaphnia dubia*), a freshwater shrimp (*Paratya australiensis*) and embryo-larval stages of Murray cod (*Maccullochella peelii*) under laboratory conditions. Survival, growth and fecundity were used as endpoints for these bioassays.

Subaqueous soil profiles were sampled from Point Sturt North (up to 67 cm with four distinct layers) and Boggy creek (up to 62 cm with five distinct layers). Whole sediment bioassays were conducted on each of these sediment sub-layers at different depths to assess their contribution towards toxicity. Laboratory cultured, second-instar midge larvae (*Chironomus tepperi*) were used for sediment toxicity assessment. Survival, growth, emergence and sex ratios were the endpoints used for sediment bioassays. Pore water collected from the subaqueous soil sub-layers was also subjected to ecotoxicological assessment using MARA and *Ceriodaphnia dubia*.

The chemical characterisation of surface water, pore water and whole sediment samples at sites included measurements of (i) pH, electrical conductivity (EC), and dissolved oxygen (DO), (ii) alkalinity/acidity, (iii) total organic carbon, (iv) the major anions (Cl, NO₃, Ammonia, PO₄, SO₄), (v) the major cations (Al, Fe, Mn, Na, K, Ca, Mg), and (vi) trace elements (As, Cd, Co, Cr, Cu, Ni, Zn).

Conclusions from ecotoxicological assessment of ASS components

Surface water

- Surface water from Boggy Creek and Point Sturt North generally was not associated with reproduction impairment in *Ceriodaphnia dubia* waterfleas.
- Low toxicity was observed when shrimp and fish larvae were exposed to surface waters from Boggy Creek and Point Sturt North.
- In general, elemental concentrations in surface waters were below those considered to be of risk to aquatic organisms.

Pore water

- Pore water collected from deeper sections of the sediment profile from Boggy Creek (3-13, 13-27 and 27-47 cm) and Point Sturt North (12-25, and 25-42 cm) were severely toxic to water fleas during both acute and chronic exposures. Microbial toxicity of the sediments varied from low to moderate.
- Metal concentrations in pore water from deeper cores were above their guideline trigger values at both sites. Combinations of Al, Co, Mn, Ni, Cu, Zn and As at low pH and high EC could be contributing to this toxicity.

Whole sediments

- At Boggy Creek, midge larvae survival was not affected during 5-day exposure. Percentage emergence of midge larvae was impacted when exposed to sediments from the upper four layers. However, exposure to the layer at 47-67 cm did not impact midge emergence. Sex ratios were skewed in midge larvae exposed to the sediment layers from 0-3 and 27-47 cm depth at Boggy Creek.
- At Point Sturt North, midge larvae survival was not affected. Both the growth and emergence of midge larvae were impacted when exposed to sediments from 12-25 and 25-42 cm depths. Sex ratios were not skewed in midge larvae exposed to any sediments.

The ecotoxicological assessment of surface water, pore water and sediments at different depths at the two sites, four years after inundation, confirms that the contaminants generated at the ASS impacted sites, at deeper sediment depths, are potentially severely toxic to aquatic organisms. If this monitoring was undertaken during or straight after the water had returned, the surface sediments may also have posed this risk level to aquatic organisms.

This is an interim report and part of a three year investigation. A final synthesis report will be written which will include all investigations of the Lower Lakes Ecotoxicology project.

Implications

- In sub aqueous soils, under acidic conditions, a combination of stressors such as pH, conductivity and metals may adversely affect the growth and reproduction in the aquatic organisms inhabiting sites where ASS are present.
- Sediments at deeper profiles could cause an upward flux of contaminants that may pose a moderate to high level risk to the biota inhabiting Lower Lakes sites where ASS are present.

Recommendations

- Further ecotoxicological monitoring is recommended to assess the spatial and temporal variation in the toxicity at selected Lower Lakes sites to address seasonal changes in partitioning of contaminants and their bioavailability in subaqueous soils.
- The development of rapid monitoring tools and modelling approaches should be considered. They would utilise chemical, physical and microbial parameters to enable assessment of sediment health and impacts of stress-induced changes.
- Mesocosm studies involving drying and wetting of sediments should be included in future monitoring studies so as to better integrate chemical and ecotoxicological investigations.
- In the present study, a mixture of metals including Al, Cu, Zn, As, Mn and Co were above ANZECC/ARMCANZ guideline values in the pore water collected from the sediments at some sites. As the potential for multiple chemical exposure increases, the question raised is whether the toxicity of mixtures of chemicals is simply additive or whether there is potentiation of toxicity. The general consensus has been that chemicals interact by concentration addition, however past studies have demonstrated that concentration addition of the components of a mixture does not always reflect the overall interaction of a mixture. Risk assessment procedures should account for mixtures of contaminants present in a given system.
- Pore water Al had the highest hazard quotient. Thus, it becomes obvious that Al is a significant hazard associated with ASS. Unfortunately, there is a notable shortage of literature on the biological response of the aquatic biota to Al released from ASS. The locations and soil characteristics of ASS are well defined throughout the literature. Similarly, Al is recognised as a highly toxic element when bioavailable. However, Al forms a range of chemical species, little is known about speciation, bioavailability and toxicity when it comes to a system dominated by ASS. The current Al guideline is applicable at pH <6.6. The ANZECC/ARMCANZ water quality guidelines require review for aluminium, particularly in relation to deriving guideline value(s) for aluminium toxicity in lower pH water. The sediment guidelines for aluminium should also be reviewed.

1 Background

From 2007 until mid 2010, reduced inflows from the River Murray to lakes Alexandrina and Albert, South Australia occurred as a consequence of persistent drought in south east Australia including the Murray-Darling Basin. The combination of decreasing water levels and gently sloping near-shore lake beds caused large areas of previously continuously-inundated sediments and subaqueous soils to be directly exposed to the atmosphere. With continued lowering of water levels, acid sulfate soil (ASS) materials became progressively oxidised over increasing depths in the soil profiles. The resultant formation of sulfuric materials ($\text{pH} < 4$) produced significant soil, water quality and ecological problems.

Increased rainfall within the Murray-Darling Basin catchment from March 2010 caused a rapid rise in water levels and inundation of the sulfuric materials that had formed in the dried margins of the Lower Lakes. The main focus of earlier field investigations of Acid Sulfate Soil (ASS) environments in lakes Alexandrina and Albert was measuring and analysing the physico-chemical parameters at the two lakes (Baker *et al.*, 2010; 2011; 2013a; 2013b and Baker and Shand. 2014; Fitzpatrick *et al.*, 2008a; 2008b; 2008c; 2009 and 2010a). These investigations provided information on the scale of the problem and timescales of recovery of ASS around the margins of the lakes. Based on the seven years of monitoring (2007-2014), Baker and Shand (2014) concluded that the soils in the Lower Lakes are in a transient state and the build-up of sulfide is likely to continue under saturated conditions. Since November 2009, the physical and chemical properties of seventeen study areas have been monitored, providing good spatial coverage of the recovery of ASS around the Lower Lakes (Figure 1). The surface of subaqueous soils in many areas has returned to a circumneutral pH, which has formed an effective reactive barrier to upward acid and metal fluxes. Recent monitoring has found that the subsoils in many areas of the Lower Lakes have remained acidic, buffered by hydroxysulfate minerals such as natrojarosite (Shand *et al.* 2012). The hazards posed by acidity and contaminants in deeper layers however are poorly known.

The aim of this study is to build on previous monitoring programs to perform an ecological assessment of subaqueous soils in the Lower Lakes to provide a better understanding of the health and potential impacts of recovered ASS. This information can be used to assess the health of these environments and future management of these systems. The overall objective of the project is to perform temporal and spatial ecotoxicological assessment of the surface water, pore water and sediments at selected study areas in the Lower Lakes. Four study areas represented by four study sites were selected: 1) Point Sturt North sampled in 2013 2) Point Sturt North sampled in 2014, 3) Dog Lake sampled in 2013, and 4) Boggy Creek sampled in 2014.

The specific objectives are as follows:

1. Undertake chemical characterisation of surface water, pore water and sediments at four sites within four study areas in the Lower lakes.
2. Assess the ecotoxicological risk of surface water, pore water and sediments at four sites within four study areas in the Lower lakes.
3. Assess the risks posed to benthic organisms based on analytical and biological assessment of sediment core profiles collected at the four selected study areas.
4. Assess the potential cause(s) for any observed sediment toxicity at the four sites in the four selected study areas.

This interim document reports data collected during the 2014 sampling year at the Point Sturt North and Boggy Creek sites representing two of the four sites. These two sites were selected because a previous study found subaqueous soils at Point Sturt North pose a medium acidification hazard and subaqueous soils at Boggy Creek pose a high acidification hazard (Baker and Shand 2014).

2 Interim report on Ecotoxicological work on surface water, pore water and whole sediment samples from two sites

2.1 Sample Sites

In February 2014, Point Sturt North was re-sampled and one additional site, Boggy Creek was also selected for the monitoring study (Figure 1). Study area LF02 was located on the north eastern side of Point Sturt on the south western side of Lake Alexandrina and the study area LF15 was located in Boggy Creek, a tributary of Holmes Creek that forms the eastern boundary of Hindmarsh Island (Figure 1).



Figure 1 Study areas selected for monitoring from the previous acid sulfate soils monitoring sites are Boggy Creek (LF15) and Point Sturt North (LF02) (circled in red).

Where possible, the sites sampled for this project were positioned within a few metres of former sampling sites that were established as part of ASS monitoring in Lakes Alexandrina and Albert (Baker *et al.* 2010;

Baker *et al.* 2011; Fitzpatrick *et al.* 2010b; Fitzpatrick *et al.* 2008a; Fitzpatrick *et al.* 2008b; Fitzpatrick *et al.* 2009; Fitzpatrick *et al.* 2008c). A Global Positioning System (GPS) was used to re-locate sample sites. Soil profile sampling was carried out by observable soil horizon and was achieved using spades and a range of augers (n=4). Sampling was confined within surface layers (< 1.0 m) to encompass the materials most likely to be influenced by oxidation.

At each site, GPS co-ordinates and site descriptions were recorded and photographs of the site were taken at photographic points that had been established in previous studies (See Baker *et al.* 2013a; Baker *et al.* 2013b). Cores were stored in ice for transportation to the laboratory and in the laboratory, each core was photographed with a length scale and soil horizons were sub sampled (See Baker *et al.* 2013a; Baker *et al.* 2013b). Soil material was described and physical properties such as colour, consistency, structure and texture were recorded following McDonald *et al.* (1990) (See Baker *et al.* 2013a; Baker *et al.* 2013b). The presence of odours associated with 'sulfidic' conditions (e.g., H₂S – rotten egg gas and methyl thiols) as well as oxidising odours (SO₂) were also recorded. Representative subsamples were collected and placed in plastic jars for acid-base accounting, electrical conductivity (EC) and pH measurements. Additional subsamples were collected in chip trays for morphological study and incubation experiments according to the methods described by Baker *et al.* (2010, 2011, 2013a, 2013b).

Sediment cores sampled from Point Sturt North were collected up to 67 cm in depth with four distinct layers, descriptions of which are provided in Table 1. Boggy Creek sediment cores had five distinct layers and were sampled up to 62 cm in depth, with descriptions provided in Table 2.

Table 1 Description of subaqueous profile of soils at the Point Sturt North sampling site

SAMPLE NAME	DEPTH RANGE (cm)	SITE DESCRIPTION
PS2.1	0-12	Grey (5Y 5/1) olive grey (5Y 5/2) clayey medium sand with black particulate and some dark grey organic matter at surface and few reddish brown mottles associated with medium roots; few fine roots; two cores with clayey material at surface; gradual boundary.
PS2.2	12-25	Greyish brown (2.5YR 5/2) clayey or loamy medium sand with weak, diffuse, coarse mottles of slightly yellowish grey colour and distinct yellowish mottles associated with rare coarse roots; few coarse reddish brown mottles; clear boundary.
PS2.3	25-42	Grey grading to dark grey (5Y 5/1 to 4/1) loamy to clayey sand with 20 to 30% diffuse yellowish brown mottles (10YR 5/8 to 6/8) mottles; dark organic accumulation in top 3.5 cm, few fine living roots; sharp boundary.
PS2.4	42-67	Greenish grey (5GY 6/1) clay with some fine sand and shell layers (two cores only; chip tray and 70 ml bottle)

Table 2 Description of subaqueous profile of soils at the Boggy Creek sampling site

SAMPLE NAME	DEPTH RANGE (cm)	SITE DESCRIPTION
BC15.1	0-3	Black (2.5Y 2/1) sandy peat with common coarse organic material with some clay towards the base and sandy at top; clear boundary.
BC15.2	3-13	Dark grey (5Y 4/1) loamy sand, occasional black mottles in top with occasional fine rootlets.
BC15.3	13-27	Olive grey (5Y 5/2) medium sandy loam with occasional inclusions of grey, very clayey material; prominent pale yellow (5Y 7/3) jarosite mottles (pH 4.5) following sub-vertical old root channels with haematite in centre, sharp, irregular boundary.

BC15.4	27-47	Dark grey (5Y 4/1) clayey medium sand with frequent clay laminations. Occasional shell fragments and fine rootlets. No sulfidic smell noted.
BC15.5	47-62	Dark grey (5Y 4/1) slightly clayey coarse sand. Occasional very soft clayey sand lenses. Occasional shell fragments and very fine rootlets.

2.2 Experimental design

Detailed ecotoxicological assessment was carried out on water and sub aqueous soil samples collected at Point Sturt North and Boggy Creek in 2014. Surface water, pore water and whole sediment samples were collected from these two sites and a suite of bioassays and chemical analyses were conducted. A brief overview of the experimental design is provided as Figure 2.

Surface water (Bioassays)	
1.	Microbial assessment (MARA)
2.	Algae/duckweed
3.	<i>Ceriodaphnia dubia</i> (waterflea)
4.	<i>Paratya australiensis</i> (freshwater shrimp) survival and oxidative stress
5.	Native fish (Golden perch or Murray cod larvae)
Sediment (top layer)	
	Depth (cm)
1.	Whole-sediment ecotox - Midge - <i>Chironomus tepperi</i> 10
2.	Pore-water - MARA and <i>Ceriodaphnia dubia</i>
Sediment (depth 2)	
1.	Whole-sediment ecotox - Midge - <i>Chironomus tepperi</i> 20
2.	Pore-water-MARA and <i>Ceriodaphnia dubia</i> 30
Sediment (depth 3)	
1.	Whole-sediment ecotox - Midge - <i>Chironomus tepperi</i> 40
2.	Pore-water-MARA and <i>Ceriodaphnia dubia</i> 50
Sediment (depth 4)	
1.	Whole-sediment ecotox - Midge - <i>Chironomus tepperi</i> 60
2.	Pore-water-MARA and <i>Ceriodaphnia dubia</i>

Figure 2 Overview of ecotoxicological assessment

2.3 Methodology

2.3.1 SURFACE WATER SAMPLE COLLECTION

Surface water grab samples (n=8) were collected from Boggy Creek and Point Sturt North. All water samples were collected in acid-washed plastic containers and transported to the CSIRO Adelaide laboratory, where they were stored at 4°C. Low temperatures are expected to inhibit microbial degradation, chemical transformations, and loss of any highly volatile organic substances. On arrival, pH, EC and DO measurements were performed for each sample using a TPS 90-FL electronic water quality meter.

2.3.2 PORE WATER SAMPLE COLLECTION

Whole subaqueous sub-layers were homogenized in containers and subsampled for ecotoxicity assessment and pore water extraction. Samples for ecotoxicity testing were stored at 4°C until testing.

Whole sediment samples from different depths were transferred into 50 mL centrifuge tubes and centrifuged for 25 min. at 3500 rpm to collect pore water samples. The pore water samples were immediately stored at 4°C and diluted with synthetic water for preparing dilutions to run ecotoxicological bioassays.

2.3.3 TOXICITY TESTS

All surface water and pore water toxicity tests were carried out on unfiltered water from Point Sturt North and Boggy Creek sites and were serially diluted with synthetic water to prepare 100 to 0.1% dilutions for various ecotoxicological tests (where 100% is undiluted surface or pore water).

Microbial assay for risk assessment (MARA)

The assay uses a selection of taxonomically diverse microbial species lyophilised in a microplate. Ten prokaryotic species and a eukaryote (yeast) constitute the biological indicators of toxicity assessment. The growth of the organisms exposed to a dilution series of the test sample is determined with the reduction of tetrazolium red (TZR). A scanned image of the microplate obtained using a flatbed scanner is analysed using purpose-built software. In order to provide a comprehensive and optimal assessment utilising the significant feature of the MARA as a multi-species test, a determination referred to as the Microbial Toxic Concentration (MTC) was calculated.

Lemna growth inhibition test

Lemna tests with the duckweed *L. minor* were performed according to the standard OECD Test Guideline 221 (2002). Bioassays were carried out in 400 ml beakers with an outer diameter of 80 mm filled with 150-ml medium. Inoculum for each beaker was 12 fronds. Only plants with two or three fronds were chosen. Six control replicates and three treatment replicates were used. Tests were carried out in an incubator at 25 ± 2 °C, with fluorescent tubes mounted on the top. Light intensity was adjusted at 100 $\mu\text{Es}^{-1}\text{m}^{-2}$. Test duration was 7 days (168 h). Test vessels were placed randomly in the incubator to minimise the influence of spatial differences in light intensity or temperature. Test solutions were renewed on days 3 and 5 to ensure that test conditions remained constant. The test was terminated after seven days exposure and total numbers of fronds were counted in each test vessel.

Cladoceran immobilisation and reproduction

Toxicity tests with the waterflea, *Ceriodaphnia dubia*, measured both acute (immobilisation) and chronic (reproduction) toxicity of the surface water and pore waters. Cultures of *C. dubia* are maintained at CSIRO, Adelaide in demineralised water (DMW).

The acute bioassay measuring immobilisation of *C. dubia* over 48 h followed the OECD guideline 202 (OECD 2004) with minor modifications as summarised in Table 3. Surface water and pore water samples (n=4) were diluted with synthetic water to achieve concentrations of 0.15 to 100% (where 100% is undiluted surface or pore water).

Reproduction of *C. dubia* was assessed over 8 days and based on the OECD Test Guideline 211 (2012) used for *Daphnia magna*, and is summarised in Table 4. Surface water and pore water samples (n=4) were diluted with MHW to achieve concentrations of 0.1 to 100% (undiluted). The pH, dissolved oxygen (DO), EC and temperature were measured at the beginning and end of the bioassay, and when test solutions were renewed. A control consisting of MHW and the reference toxicant, copper, were also tested for quality assurance purposes.

Table 3 Summary of the test conditions for the acute *Ceriodaphnia dubia* immobilisation bioassay

TEST PARAMETER	TEST CONDITION
Test type	Static, non- renewal
Test duration	48 h
Temperature	25 ± 1 °C
Light quality	Cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	50 mL vial
Test solution volume	25 mL
Age of test organisms	Less than 24 h old
No. of organisms per replicate	5
No. Of replicates per treatment	3
No. Of organisms per treatment	15
Feeding regime	None
Dilution water	Moderately hard water (MHW)
Test concentrations	5-6
Control treatments	MHW
Endpoint	Immobilisation
Test acceptability criteria	≥90% survival in controls. Reference toxicant EC50 within Cusum chart control limits

Table 4 Summary of the test conditions for the chronic *Ceriodaphnia dubia* reproduction bioassay

TEST PARAMETER	TEST CONDITION
Test type	Semi-static
Test duration	8 d
Temperature	25 ± 1°C
Light quality	Cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	50 mL beaker
Test solution volume	25 mL
Renewal of test solutions	Every 24 h
Age of test organisms	Less than 24 h old
No. of organisms per replicate	1
No. of replicates per treatment	10
No. of organisms per treatment	10
Feeding regime	Fed <i>Pseudokirchneriella subcapitata</i> on daily basis
Dilution water	Moderately hard water (MHW)
Test concentrations	4
Control treatments	MHW
Endpoint	Number of neonates over three broods
Test acceptability criteria	≥ 80% survival of original daphnids in the control treatment. Reference toxicant EC50 within Cusum chart control limits

Shrimp Survival

This acute test measured the survival of *Paratya australiensis* shrimp over a 96 h exposure to surface waters from two sites. The test is described in Kumar *et al.* (2010, Table 5). After 96 h, oxidative stress in *P. australiensis* was also assessed.

The freshwater shrimp *Paratya australiensis* were obtained from Aquablue Seafood, NSW, and acclimated in 60 L aquariums and fed twice daily (fish wafers and Hikari Tropical® sinking wafers) for at least two weeks prior to use in toxicity tests.

One litre of each test concentration (12.5-100% surface water) was prepared in a 3 L borosilicate glass beaker (n= 3). A summary of the test conditions is provided in Table 5. Death was assumed when animals lost orientation and there was no movement of the legs or scaphognathite. The pH, EC and DO were measured in each treatment at the beginning and end of the test and when test solutions were renewed.

Table 5 Summary of the test conditions for the shrimp *Paratya australiensis* survival bioassay

TEST PARAMETER	TEST CONDITION
Test type	Semi-static
Test duration	96 h
Temperature	23 ± 1°C
Light quality	Cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	1000 mL beaker
Test solution volume	800 mL
Renewal of test solutions	Once (48 h)
Age/size of test organisms	1-4 cm
No. Of organisms per replicate	10
No. Of replicates per treatment	3
No. Of organisms per treatment	30
Feeding regime	Shrimp not fed during exposure period
Test chamber cleaning	Not required
Test chamber aeration	Aeration provided
Dilution water	Moderately hard water (MHW, 230mg CaCO ₃ /L)
Test concentrations	4
Control treatments	MHW
Endpoint	Survival - movement observed
Test acceptability criteria	≥90% survival in controls; Dissolved oxygen > 60%

Oxidative stress

Contaminant exposure induces oxidative stress in an organism either by generating reactive oxygen species (ROS) or interfering with the antioxidant defense mechanism (Kavitha & Venkateswara Rao 2008). The ROS including superoxide anion radicals, hydrogen peroxide (H₂O₂) and hydroxyl radicals, are highly reactive, thus damaging biological molecules leading to lipid peroxidation (Livingstone 2001). Among commonly used biomarkers, antioxidant enzymes try to compensate or to avoid oxidative damages. Catalase (CAT) and glutathione peroxidase (GPx) are involved in the detoxification of superoxide anion radical (O₂⁻), H₂O₂ and lipid hydroperoxides (Guemouri *et al.*, 1991). Glutathione-S-transferase (GST) is a group of multifunctional enzymes involved in the detoxification of both reactive intermediates and oxygen radicals (Smith and Litwack, 1980). Under normal conditions, ROS are eliminated by antioxidant enzymes such as catalase (CAT; EC.1.11.1.6), which decomposes H₂O₂ into water and O₂ molecules (Diguiseppi & Fridovich 1984). An intoxicated organism may recover by the use of detoxification enzymes such as glutathione-S-transferase (GST; EC.2.5.1.18), which catalyses the conjugation of the thiol moiety of reduced glutathione with a variety of compounds bearing electrophilic centres. Both antioxidant and detoxification enzyme activities have been used as biomarkers for environmental assessment.

Glutathione reductase (GR) catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in presence of β-Nicotinamide adenine dinucleotide phosphate reduced (NADPH). The principle of

this assay is based on the increase in absorbance caused by the reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to 3-thio-6-nitrobenzoate (TNB) at 412 nm (colorimetric assay) according to Smith *et al.* (1988). The extinction coefficient of TNB is $14.15 \text{ mM}^{-1}\text{cm}^{-1}$. GR activity was expressed in mU mg^{-1} protein. CAT activity was measured at 240 nm by determining the decay of hydrogen peroxide levels following Beers and Sizer (1952). One unit of CAT activity is defined as the amount of enzyme that catalyses the degradation of $1 \mu\text{mol}$ of H_2O_2 per min. and specific activity corresponding to μmol transformation of substrate (H_2O_2) per minute per milligram protein. CAT activity was expressed in U mg^{-1} protein. GST activity was assayed at 340 nm by measuring the increase in absorbance using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate according to Habig *et al.* (1974). One unit of GST activity was defined as the formation of $1 \mu\text{mol}$ of conjugated product per minute. The extinction coefficient $9.6 \text{ mM}^{-1}\text{cm}^{-1}$ of CDNB was used for the calculation. Glutathione-S-transferase (GST) activity was expressed in mU mg^{-1} protein. Protein content was determined according to Bradford (1976) using bovine serum albumin as standard. Absorbance was recorded at 595 nm.

Fish survival, growth and malformations

This sub-chronic toxicity test measures the number of imbalanced (loss of ability to balance) fry of aquacultured Murray cod fish (*Maccullochella peelii*), after exposure to surface water for 7 d. Growth and observations of malformations were also measured to identify the effect of surface water on fish early life development. The toxicity test was based on the methods of OECD Guideline 204 (1984) and summarised in Table 6. Fertilised eggs of *M. peelii* were obtained from a NSW aquaculture facility. Post hatch 2 day larval fish (≤ 48 h old) were used in the toxicity tests.

Five concentrations (three replicates each) of each surface water sample was prepared by dilution with MHW (25-100%). Controls consisting of each treatment were prepared in triplicate and ten fish fry were randomly added to each test vessel. A summary of the test conditions is provided in Table 6. Water quality parameters (pH, EC and DO) were also measured. The test was terminated after 7 days. Fish were euthanized by the addition of MS222 (ethyl 3-aminobenzoate methanesulfonate, Sigma) and immediately fixed in 10% buffered formalin.

Table 6 Summary of test conditions for the fish *Maccullochella peelii* (aquacultured) survival test

TEST PARAMETER	TEST CONDITION
Test type	Static non-renewal
Test duration	7 d
Temperature	23 ± 1°C
Light quality	Cool-white fluorescent tube lighting
Light intensity	800 ± 160 Lux
Photoperiod	16 h light : 8 h dark
Test chamber size	500 mL
Test solution volume	400 mL
Renewal of test solutions	Day 2, 4 and 6
Age of test organisms	2 weeks post hatch
No. of organisms per replicate	10
No. of replicates per treatment	3
No. of organisms per treatment	30
Feeding regime	Fish not fed during exposure period
Test chamber cleaning	Not required
Test chamber aeration	Aeration provided
Dilution water	Synthetic water
Test concentrations	4-5
Endpoint	Survival (Imbalance – loss of swimming ability)
Test acceptability criterion	≥ 90% balanced fish fry in the controls

Midge survival and larval development – sediment

The acute and chronic toxicity of sediment to the midge, *Chironomus tepperi* was assessed. Survival and growth of midge larvae after 6-7 days and larval development over 12 days was measured. Test methods are summarised in Table 5. Sediment core samples at different depths from Point Sturt North and Boggy Creek were used for whole sediment toxicity assessment.

Larvae from aquaria-raised midges were used for the toxicity tests. Five days prior to testing, egg masses were collected from cultures maintained at CSIRO, Adelaide, and placed in 1 L beakers (2 egg masses/beaker) with 800 mL of moderately hard water (MHW: 220 – 300 µS/cm, pH 6.9 to 7.9, DO >60%) containing 7.5 g of artificial substrate (shredded tissue). Over the next 5 days, egg masses in these beakers were aerated continuously, fed twice with ground fish flakes (4 g/100 mL), and incubated under constant temperature conditions (23 ± 1°C) with a 16:8 h light:dark photo period using cool-white fluorescent lamps (10-20 µmol photons/s/m²). Five-day-old larvae were used for testing. The cultures were considered suitable for use in toxicity tests if they provided a constant supply of larvae, if the larvae were healthy and behaved normally, and if mortality was ≤ 10%. Test conditions are summarised in Table 7.

For the growth bioassay, ten 5-d old midge larvae were added to beakers containing ca. 140 g (wet weight) of 2 mm sieved sediment and 400 mL MHW (or River Murray water), with 4 replicates per treatment. Each beaker was incubated under the conditions described above. After 6 d, and prior to pupation, midge larvae

from each replicate were removed, pooled and their wet weight recorded. Larvae were then freeze dried and their dry weight recorded. Survival of the midge larvae was also determined.

Larval development (that is, emergence from sediment) was determined after ten 5 d old midge larvae were added to beakers containing ca. 140 g (wet weight) of 2 mm sieved sediment and 400 mL MHW, with 4 replicates per treatment. Beakers were incubated for 12-14 d at 23°C (16:8 h light:dark) and the number of emerging adult *C. tepperi*, and their sex, was measured daily.

The pH and EC of the surface waters were measured at the beginning and end of the bioassay, while DO and temperature in the test solutions were measured daily.

The 5 d growth and survival and, 14 d larval development test endpoints were observed. The highest concentration of sample tested, causing no significant toxicity (NOEC), and the lowest concentration of test material, causing significant toxicity (LOEC), were determined by the Steels Many-One Rank test. The test was acceptable if there was ≥80% survival in the controls.

A reference toxicant test, copper, was also carried out using *C. tepperi* larvae from the same batch of cultures used in the sediment bioassay.

Table 7 Summary of the test conditions for the midge *Chironomus tepperi* bioassays

TEST PARAMETER	TEST CONDITION
Test type	Static non-renewal
Test duration	Survival and growth: 5 d Larval development: 12-14 d
Temperature	23 ± 1°C
Light quality	Cool-white fluorescent tube lighting
Light intensity	10-20 µmol photons s ⁻¹ m ⁻²
Photoperiod	16 h light : 8 h dark
Test chamber size	500 mL
Test solution volume	140 g sediment plus 400 mL MHW
Age of test organisms	2 nd Instar larvae, 5 days
No. of organisms per replicate	10
No. of replicates per treatment	8
No. of organisms per treatment	80
Feeding regime	Midges fed during exposure period
Test chamber aeration	Aeration provided
Dilution water/overlying water	Moderately hard water
Endpoint	5 day: Survival and growth 12-14 days: larval development (emergence) and sex ratio
Test acceptability criterion	≥80% survival in controls; Reference toxicant LC50 within Cusum limits

2.4 Ecotoxicological assessment- Results

2.4.1 PHASE 1: DIRECT TOXICITY ASSESSMENT OF SURFACE WATER SAMPLES

Results from ecotoxicological bioassays on surface waters are summarised in Tables 8-13.

Table 8 Microbial assessment of surface water samples

Concentrations (% dilution)	POINT STURT NORTH	BOGGY CREEK
	*Growth relative to control (%)	
Control (0)	100	100
3.1	95	93
6.3	96	95
13	96	94
25	96	96
50	95	92
100 (undiluted surface water sample)	95	93

*mean value is based on eight replicates/concentration

Table 9 *Lemna* Bioassay – Frond numbers after 7 days exposure

CONCENTRATIONS	FROND NUMBERS	
	BOGGY CREEK	POINT STURT NORTH
100%	71±7*	67±13*
50%	74±18	63±8*
25%	84±7	62±7
12.5%	92±10	72±10
Control	92±5	82±6

Data represented as Mean ± SD

*Significantly different from controls.

Survival of shrimp after 96 h exposure in the shrimp bioassay toxicity test provided 100% survival rates for both sites (Point Sturt North and Boggy Creek) across all concentrations and replicates. Data is therefore not tabulated. The results from the ecotoxicological assessment on surface waters are summarised in Table 14. No microbial toxicity was detected. Waterfleas during acute and chronic exposure did not exhibit any observable toxicity. Shrimp survival was also not impacted during exposure to surface water from Boggy Creek and Point Sturt North. However, shrimp (oxidative stress enzymes, superoxide dismutase [SOD] activity) and fish larvae (growth) exhibited low toxicity when exposed to the undiluted surface water from Boggy Creek and Point Sturt North surface water samples. The toxicity was removed completely at 50% dilution of the two surface water samples. Low to moderate toxicity to duckweed (*Lemna* sp.) was observed.

Table 10 *Ceriodaphnia* acute bioassays – Surface water toxicity

BOGGY CREEK SURFACE WATER CONCENTRATION	NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
	A	B	C	A	B	C			pH	DO (%)	Conductivity (μ S/cm)
Control	5	4	5	0	1	0	14	1	8.08	95.3	202.3
12.50%	5	4	5	0	1	0	14	1	8.12	94.1	262
25.0%	5	5	5	0	0	0	15	0	8.2	94.9	315
50.0%	5	5	5	0	0	0	15	0	8.34	96.9	443
100.0%	5	5	5	0	0	0	15	0	8.54	100.4	682

POINT STURT NORTH SURFACE WATER CONCENTRATION	NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
	A	B	C	A	B	C			pH	DO (%)	Conductivity (μ S/cm)
Control	5	5	5	0	0	0	15	0	8.36	95.2	198.6
12.50%	5	5	5	0	0	0	15	0	8.29	95.6	284
25.0%	5	5	5	0	0	0	15	0	8.28	94.7	358
50.0%	5	5	5	0	0	0	15	0	8.34	95.8	540
100.0%	5	5	5	0	0	0	15	0	8.51	97.8	867

Table 11 *Ceriodaphnia* Chronic bioassays – Surface water toxicity

CONCENTRATIONS (%)	BOGGY CREEK NUMBER OF YOUNG ONES OVER THREE BROODS	POINT STURT NORTH NUMBER OF YOUNG ONES OVER THREE BROODS
Control	24.7±2.8	24.6±2.45
100	28.0±2.7	27±3.86
50	25.5±4.0	24.8±6.21
25	24.2±3.4	22.5±4.64

Data represented as Mean ± SD

Table 12 Shrimp oxidative stress after 96 h exposure to surface water from Boggy Creek and Point Sturt North

SAMPLE	GR (nmol/min/mg protein)	GST (mU/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Control	7.56±1.42	20.92±0.21	42.37±4.21	6.15±0.49
Boggy Creek 50%	6.32±1.09	22.60±3.04	48.35±3.47	6.00±0.39
Boggy Creek 100%	8.48±0.92	21.68±2.44	44.56±6.98	4.64±0.34*
Pt Sturt North 50%	9.76±1.89	20.86±1.68	46.50±4.09	5.66±0.52
Pt Sturt North 100%	9.08±1.41	23.11±2.28	45.16±3.69	4.52±0.54*

Data represented as Mean ± SD

Table 13 Summary of ecotoxicological assessment of surface water samples

Dates sampled	Sites	Microbial	Waterflea	Shrimp	Fish larvae	<i>Lemna</i>
Feb 2014	Point Sturt North	NT	NT	LT	LT	T
Feb 2014	Boggy Creek	NT	NT	LT	LT	LT
Laboratory control water	Synthetic water	NT	NT	NT	NT	NT

NT: No toxicity NOEC 100-90%	LT: Low toxicity NOEC 89-50%	T: Moderate to high toxicity NOEC 49-10%	HT- very high toxicity NOEC <10%
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2.4.2 PHASE 2: DIRECT TOXICITY ASSESSMENT OF PORE WATER SAMPLES

Pore water samples were collected from the core sub-layers for the ecotoxicological and chemical assessments. Results from ecotoxicological bioassays on pore waters are summarised in Tables 14-18.

Table 14 *Ceriodaphnia* acute bioassays – pore water from subaqueous soils collected at 0-3 cm and 3-13 cm depth at Boggy Creek

BOGGY CREEK PORE WATER – (0-3 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	5	5	5	0	0	0	15	0	8.25	96.8	140.5		
12.50%	5	5	5	0	0	0	15	0	8.23	95.7	320		
25.0%	5	5	4	0	0	1	14	1	8.4	95.5	488		
50.0%	5	5	5	0	0	0	15	0	8.74	95	877		
100.0%	5	5	4	0	0	1	14	1	8.92	94.5	1513		
BOGGY CREEK PORE WATER –(3-13 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	5	5	5	0	0	0	15	0	8.39	95.8	143		
12.50%	5	5	5	0	0	0	15	0	8.45	95	563		
25.0%	5	4	5	0	1	0	14	1	8.27	94.6	959		
50.0%	4	2	5	1	3	0	11	4	8.49	94.2	1824		
100.0%	1	0	1	4	5	4	2	13	8.58	93.1	3140		

Figures highlighted in red are significantly different from controls

Table 15 *Ceriodaphnia* acute bioassays – pore water from subaqueous soils collected at 13-27 cm and 27-47 cm depth at Boggy Creek

BOGGY CREEK PORE WATER – (13-27 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	5	4	4	5	4	4		13	2		8.27	93.3	202.2
12.50%	5	5	4	5	5	4		14	1		8.17	95.1	438
25.0%	4	4	4	4	4	4		12	3		8.06	93.8	687
50.0%	3	2	3	3	2	3		8	7		7.93	93.6	1145
100.0%	1	2	0	1	2	0		3	12		7.07	93.6	2084
BOGGY CREEK PORE WATER – (27-47 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	5	5	4	0	0	1		14	1		7.85	94	200.9
12.50%	1	3	1	4	2	4		5	10		7.89	94.5	317
25.0%	1	1	1	4	4	4		3	12		7.91	93.9	420
50.0%	0	0	0	5	5	5		0	15		7.85	93.8	616
100.0%	0	0	0	5	5	5		0	15		7.73	94.5	1029

Figures highlighted in red are significantly different from controls

Table 16 *Ceriodaphnia* acute bioassays – pore water from subaqueous soils collected at 0-12 cm and 12-25 cm depth at Point Sturt North

POINT STURT NORTH PORE WATER – (0-12 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	4	5	5	1	0	0		14	1		7.55	94.4	212.5
12.50%	4	5	4	1	0	1		13	2		7.59	93.4	289
25.0%	5	5	5	0	0	0		15	0		7.62	94.5	361
50.0%	4	3	4	1	2	1		11	4		7.53	95.6	514
100.0%	4	4	3	1	1	2		11	4		6.65	95.8	819
POINT STURT NORTH PORE WATER – (12-25 cm depth)			NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
CONCENTRATION	A	B	C	A	B	C					pH	DO (%)	Conductivity (µS/cm)
Control	4	5	4	1	0	1		13	2		8.66	97	214.7
12.50%	4	4	5	1	1	0		13	2		8.1	93.9	353
25.0%	4	5	5	1	0	0		14	1		7.71	93.5	474
50.0%	0	0	0	5	5	5		0	15		3.77	94.8	822
100.0%	0	0	0	5	5	5		0	15		3.04	71.8	1866

Figures highlighted in red are significantly different from controls

Table 17 *Ceriodaphnia* acute bioassays – pore water from subaqueous soils collected at 25-22 cm and 42-67 cm depth at Point Sturt North

POINT STURT NORTH PORE WATER – (25-42 cm depth)	NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
	A	B	C	A	B	C			pH	DO (%)	Conductivity (µS/cm)
Control	5	5	4	0	0	1	14	1	8.38	95.2	200.6
0.78%	5	3	5	0	2	0	13	2	8.4	94.7	238
1.6%	1	2	3	4	3	2	6	9	8.28	94.5	268
3.1%	2	2	3	3	3	2	7	8	8.22	94.4	323
6.3%	2	3	4	3	2	1	9	6	8.14	95.1	474
POINT STURT NORTH PORE WATER – (42-67 cm depth)	NUMBER ALIVE			NUMBER DEAD			TOTAL ALIVE	TOTAL DEAD	WATER QUALITY		
	A	B	C	A	B	C			pH	DO (%)	Conductivity (µS/cm)
Control	5	4	5	0	1	0	14	1	7.67	97.1	215.5
12.50%	5	5	5	0	0	0	15	0	7.96	95.9	812
25.0%	5	5	5	0	0	0	15	0	8.15	94.8	1396
50.0%	5	5	5	0	0	0	15	0	8.4	93.5	2490
100.0%	5	5	5	0	0	0	15	0	8.53	93.9	4210

Figures highlighted in red are significantly different from controls

Moderate to high microbial toxicity at all depths, except 13-27 cm, at Boggy Creek were observed. Pore water from surface sediments showed no acute toxicity to waterfleas, while moderate to high toxicity was observed when waterfleas were exposed to pore water from deeper subaqueous soils at Boggy Creek (Table 18).

Pore waters collected from sediments at 0-25 cm depth at Point Sturt North exhibited no to low microbial toxicity. The two deeper subaqueous soils (25- 67 cm) showed moderate to high microbial toxicity (Table 18). Pore water from surface sediments showed no acute toxicity to waterfleas. Pore water from the deepest subaqueous layer (42-67 cm depth) at Point Sturt North exhibited no acute toxicity to waterfleas but low toxicity was evident during chronic exposures. This low chronic toxicity was completely removed at 50% dilution of the pore water.

Table 18 Summary of ecotoxicological assessment of pore water samples collected from surface and subaqueous soils.

Date Sampled	Pore Water samples	Microbial	Waterflea Acute	Waterflea Chronic
Boggy Creek				
Feb 2014	0-3 cm depth	T	NT	LT
	3-13 cm depth	T	T	HT
	13-27 cm depth	LT	T	HT
	27-47 cm depth	T	T	HT
Point Sturt North				
Feb 2014	0-12 cm depth	NT	NT	LT
	12-25 cm depth	LT	T	HT
	25-42 cm depth	T	HT	HT
	42-67 cm depth	T	NT	LT
River water				
Laboratory control water		NT	NT	NT

NT: No toxicity NOEC >100-90%	LT: Low toxicity NOEC 89-50%	T: Moderate to high toxicity NOEC 49-10%	HT- very high toxicity NOEC <10%
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2.4.3 PHASE 3: WHOLE SEDIMENT TOXICITY ASSESSMENT

Whole sediment toxicity assessment was carried out on five distinct sediment layers from Boggy Creek (up to 62 cm depth) and four different layers (up to 67 cm depth) from Point Sturt North. The results are summarised in Tables 19-20. The major outcomes were:

Boggy Creek

- Midge larvae survival was not affected in soil sub-layers at Boggy Creek.
- Growth was impacted in midge larvae exposed to sediments from Boggy Creek.
- % emergence of midge larvae was impacted when exposed to soil sub-layers from 0-3, 3-13, 27-47 and 47-62 cm depths.
- Sex ratios were only skewed in midge larvae exposed to the soil sub layer 27-47 cm from Boggy Creek.

Point Sturt North

- Midge larvae survival was not affected in soil sub-layers at Point Sturt North.
- Growth was impacted in midge larvae exposed to sediments from 12-25 and 25-42 cm depths.
- % emergence of midge larvae was impacted in soil sub-layers collected from 25-67 cm depths.
- Sex ratios were not skewed in midge larvae exposed to the soil sub layers from Point Sturt North.

Table 19 Survival and growth of midge larvae exposed to surface and subaqueous soils

	DEPTH RANGE (CM)	% ALIVE	AVERAGE LENGTH ± ST DEV	% EMERGENCE	% MALE AND FEMALES	
					MALE	FEMALE
River Murray (reference site)	0-10	90	14.84 ± 1.92	87.5	51	49
Boggy Creek 15.1	0-3	100	15.03 ± 1.38	60	63	38
Boggy Creek 15.2	3-13	90	14.88 ± 1.91	65	54	46
Boggy Creek 15.3	13-27	90	14.56 ± 1.52	92.5	49	51
Boggy Creek 15.4	27-47	100	14.90 ± 1.63	75	63	37
Boggy Creek 15.5	47-62	92.5	15.05 ± 1.56	67.5	48	52
Pt Sturt Nth 2.1	0-12	97.5	14.00 ± 1.65	87.5	60	40
Pt Sturt Nth 2.2	12-25	95	13.89 ± 1.86	85	47	53
Pt Sturt Nth 2.3	25-42	92.5	13.89 ± 2.12	77.5	58	42
Pt Sturt Nth 2.4	42-67	92.5	13.13 ± 2.06	52.5	52	48

Data represented as Mean ± SD

Figures highlighted in red are significantly different from the reference site

3 Interim report- Chemical characterisation of surface water, pore water, and surface and subaqueous soils

3.1 Laboratory Chemical Analysis Methods

3.1.1 SURFACE WATER AND PORE WATER ANALYSES

Surface waters were collected as per Section 2.3.1. The samples were filtered through 0.45 µm filters and divided into two subsamples. One subsample was used for the measurement of pH, EC and DO, and major anions and the second acidified to pH~2 and kept at 4°C until elemental analysis by ICP-AES and ICP-MS.

Soil sub-layers from core profiles were transferred into 50 mL centrifuge tubes and centrifuged for 25 min at 3500 rpm on a Sorvall R3C3 Plus centrifuge. The supernatant pore waters were then removed and filtered using 0.45 µm syringe filters (Millex GV Durapore PVDF) into 50 mL tubes for water quality analysis including DO, pH, EC using a Hach HQd water quality meter and Eh using a TPS WP81 meter and Ionode IJ64 Redox electrode. A sub sample of pore water was acidified as for surface waters for elemental analysis by ICP-AES and ICP-MS.

The pore water samples were analysed for: (i) pH, EC and DO, (ii) alkalinity/acidity, (iii) TOC, (iv) major anions (Cl, NO₃, Ammonia, PO₄, SO₄), (iv) major cations (Al, Fe, Mn, Na, K, Ca, Mg) and (v) trace elements (As, Cd, Co, Cr, Cu, Ni, Zn). Various instrumental methods were used for surface water and pore water analyses as shown in Table 20.

Table 20 Instrumental methods used for analyses of surface water and pore water samples

ANALYTE	METHOD
Dissolved metals by ICP-AES	Dissolved metals were measured by ICP-AES (CIROS, SPECTRO). The sample is converted to an aerosol and transported into the plasma. Atoms and ions of the plasma are excited and emit light at characteristic wavelengths. The light emitted by the sample passes through the entrance slit of the spectrometer. The different wavelengths are measured and converted to a signal and quantified by comparison with standards.
Dissolved metals by ICP-MS	Dissolved metals were measured by ICP-MS (Agilent 7500 CE). Analyte species originating in a liquid are nebulised by a Micromist nebuliser and a cooled double-pass spray chamber. The ions are detected by an electron multiplier. The ions are quantified by comparison with prepared standards.
Alkalinity and Acidity as calcium carbonate	APHA 21st ed., 2320 B This procedure determines alkalinity by both manual measurement and automated measurement (PC Titrate) using pH 4.5 for indicating the total alkalinity end-point. Acidity is determined by titration with a standardised alkali to an end-point pH of 8.3.
Major anions - filtered Chloride	APHA 21st ed., 4500 Cl - B. Automated Silver Nitrate titration. APHA 21st ed., 3120; USEPA SW 846 - 6010 The ICP-AES technique ionises filtered sample atoms emitting a characteristic spectrum. This spectrum is then compared against matrix matched standards for quantification.
Nitrite and nitrate as N	APHA 21st ed., 4500 NO ₃ ⁻ I. Nitrate is reduced to nitrite by way of a cadmium reduction column followed by quantification by flow injection analyser (FIA). Nitrite is determined separately by direct colourimetry and result for Nitrate calculated as the difference between the two results.
Reactive phosphorus – filtered	APHA 21st ed., 4500 P-E. Water samples are filtered through a 0.45µm filter prior to analysis. Ammonium molybdate and potassium antimonyl tartrate reacts in acid medium with orthophosphate to form a heteropoly acid -phosphomolybdic acid - which is reduced to intensely coloured molybdenum blue by ascorbic acid. Quantification is achieved by FIA.
Total organic carbon (TOC)	APHA 21st ed., 5310 B, The automated total organic carbon (TOC) analyser determines Total and Inorganic Carbon by IR cell. TOC is calculated as the difference.
Moisture content	A gravimetric procedure based on weight loss over a 12-24 h drying period at 110±5°C.
Paste pH, conductivity	Paste pH (USEPA 600/2-78-054): pH determined on a saturated paste by ISE. Electrical Conductivity of Saturated Paste (USEPA 600/2-78-054) - conductivity determined on a saturated paste by ISE.

3.1.2 ELEMENTAL ANALYSES IN SUBAQUEOUS SOILS

Strong acid microwave digestion

Total metal analyses of soil sub-layers occurred following acid digestion using US EPA method 3051A (revised version 2007) microwave assisted acid digestion of sediments, sludges, soils and oils (US Environmental Protection Agency, Washington, DC.) The dried sample was digested in a microwave oven (MARS CEM) using a mixture of concentrated nitric acid and hydrochloric acid (3:1 (v/v) respectively). Approximately 0.25 g dry soil was weighed into Teflon digest vessels with 2.5 mL HCl and 7.5 mL HNO₃ and left overnight to cold digest. After cold digestion, the microwave vessels were sealed and microwave digested using the following time and temperature program: ramp to 110°C in 10 min., ramp to 180°C in 10 min. and maintain temperature at 180°C for 60 min. After cooling, the digest solutions were filtered (0.45 µm filter paper) and analysed for total elements by ICP-AES and ICP-MS. The digest solutions were analysed for a wide range of elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se and Zn) by ICP-AES and ICP-MS using the method described in Table 20.

A few refractory sample matrix compounds, such as quartz, silicates, titanium dioxide, alumina, and other oxides may not have been totally digested using this strong acid digestion procedure. In this study, elemental concentrations using microwave strong acid digestion are considered the total pool of elements that may be bioavailable or mobile in the environment.

1M hydrochloric acid extraction

The potential bioavailable or mobile pool of elements in soil sub-layers was assessed using 1M HCL (REFS). The concentration of elements in 1M HCL extracts will (in general) be lower than total elemental concentrations determined using strong acid digestion/extraction because elements are often present in fixed pools associated with organic matter, complexes and precipitates not readily mobilised by weak acids.

Hydrochloric acid (1M, 40 mL) was added to 1 g field wet soil (± 0.1 g) in a 50 mL centrifuge tube and extracted for 4 hours on an end over shaker. Samples were centrifuged at 3500 rpm for 30 min and the supernatants removed. The samples were filtered using Millex Nylon 0.45 μ m syringe filters and analysed for a range of elements (As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se and Zn) by ICP-AES and ICP-MS.

3.1.3 ACID-BASE ACCOUNTING ANALYSES

Acid-base accounting (ABA)

Acid-base accounting (ABA) is used to assess both the potential of a soil material to produce acidity from sulfide oxidation and also its ability to neutralise any acid formed (e.g. Sullivan *et al.* 2001; Sullivan *et al.* 2002b). The standard ABA applicable to acid sulfate soil is as described in Ahern *et al.* (2004) and summarised here. The equation below shows the calculation of Net Acidity (NA).

$$\text{Net Acidity} = \text{Potential Sulfidic Acidity} + \text{Existing Acidity} - \text{ANC}^*/\text{Fineness Factor}$$

*ANC = Acid Neutralising Capacity

The components in this ABA are further discussed below and by Ahern *et al.* (2004).

Potential Sulfidic Acidity (PSA)

The potential sulfidic acidity is most easily and accurately determined by assessing the chromium reducible sulfur. This method was developed specifically for analysing acid sulfate soil materials (Sullivan *et al.* 2000) to assess their potential sulfidic acidity (PSA) also known as the 'acid generation potential' (AGP). The method is also described in Ahern *et al.* (2004), which includes the chromium reducible sulfur method (SCR or CRS: Method Code 22B) and its conversion to PSA.

Existing Acidity

Existing acidity is the sum of the actual acidity and the retained acidity (Ahern *et al.* 2004). Titratable actual acidity (TAA) is a measure of the actual acidity in acid sulfate soil material that has already oxidised. TAA measures the sum of both soluble and exchangeable acidity in acid sulfate soil material and non-acid sulfate soil material. The retained acidity (RA) is the acidity 'stored' in minerals such as jarosite, schwertmannite and other hydroxysulfate minerals. Although these minerals may be stable under acidic conditions, they can release acidity to the environment when these conditions change. The methods for determining both TAA and RA are given by Ahern *et al.* (2004).

Acid Neutralising Capacity (ANC)

Soils with pH_{KCl} values > 6.5 may potentially have ANC in the form of (usually) carbonate minerals, principally of calcium, magnesium and sodium. The carbonate minerals present are estimated by titration, and alkalinity present is expressed in CaCO_3 equivalents. By accepted definition (Ahern *et al.* 2004), any acid sulfate soil material with a $\text{pH}_{\text{KCl}} < 6.5$ has a zero ANC. The methods for determining ANC are given by Ahern *et al.* (2004). Soil terms and abbreviations are listed in Table 21.

Table 21 Explanation of acid sulfate soil terms and abbreviations

VARIABLE	ABBREVIATION	EXPLANATION
Soil pH	pH _w	Soil is mixed with deionised water at a 1:1 (v:v) ratio, allowed to equilibrate for a short period of time, and pH is then measured.
Chromium-reducible sulfur	CRS	Sulfide measured by iodometric titration after acidic chromous chloride reduction.
Titrateable Actual Acidity	TAA	Acidity titration to pH 6.5 with standardised NaOH on 1:40, suspension in 1 M potassium chloride.
Retained Acidity	RA	The 'less available' fraction of the existing acidity (not measured by TAA) that may be released slowly into the environment by hydrolysis of relatively insoluble sulfate salts (e.g. jarosite and natrojarosite).
Acid Neutralisation Capacity	ANC	Measurement of a soil's ability to neutralise or buffer added acid and conventionally expressed as equivalent CaCO ₃ .
Net Acidity	NA	Soil acidity calculated as: Net Acidity = potential sulfuric acidity + existing acidity - (acid neutralising capacity/fineness factor).

3.2 Chemical characterisation: Results

Results from pore water analyses are summarised in Tables 22-24. The pH in pore waters was below the lower limit trigger value for slightly disturbed ecosystems in the 0-42 cm sub-layers at Point Sturt North and 13-47 cm sub-layers at Boggy Creek (Table 22). The EC in pore waters ranged from 828 to 4320 $\mu\text{S}/\text{cm}$ at Point Sturt North and 1579-15190 $\mu\text{S}/\text{cm}$ at Boggy Creek (Table 22). The EC in pore water was higher than the upper default trigger value for EC (salinity) generally found in lakes and wetlands (ANZECC/ARMCANZ, 2000). In general, the EC in soil profiles at both sites increased with depth. This increase is consistent with an increase in the concentration of major ions such as Na, K, and Mg that in general increase with depth. This is most likely related to downward advective flow of lake water following refilling of the Lakes at the end of the drought (Shand *et al.* 2012).

Table 22 Physico-chemical analyses of pore water samples collected from surface and subaqueous soil samples

SITE	SAMPLE ID	DEPTH	pH	ALKALINITY	ACIDITY	DO	EC	NH ₄ -N	NO _x -N	NO ₂ -N	PO ₄ -P	NO ₃ ⁻	SO ₄ ⁼
		cm		(M _{EQ} /L)	(M _{EQ} /L)	(%SAT)	($\mu\text{S}/\text{cm}$)	$\mu\text{g}/\text{L}$	$\mu\text{g}/\text{L}$	$\mu\text{g}/\text{L}$	$\mu\text{g}/\text{L}$	$\mu\text{g}/\text{L}$	mg/L
Point Sturt North	2.1	0-12	5.67	0.184	n/a	105.9	828	1522	316.8	15.64	LOD	1800	180
	2.2	12-25	3.72	n/a	137.8	102.5	1531	4354	1045	36.37	37.62	15000	530
	2.3	25-42	5.32	0.146	n/a	105.4	2250	3780	347.9	27.39	LOD	3000	1000
	2.4	42-67	7.62	4.49	n/a	104.4	4320	367.6	566.1	384.1	14.86	LOD	1900
Boggy Creek	15.1	0-3	7.4	6.2	n/a	104	1579	6555	97.56	36.25	38.15	LOD	55
	15.2	3-13	6.77	3.73	n/a	98.8	3230	13955	373.7	37.82	29.05	1500	580
	15.3	13-27	5.82	0.837	n/a	98.4	6600	21985	74.81	55.22	30.7	LOD	1900
	15.4	27-47	5.32	0.366	n/a	97	11360	29000	0.9314	11.67	28.75	1100	4000
	15.5	47-62	6.67	8.06	n/a	95.5	15190	23015	90.25	28.38	13.37	LOD	3400

Figures highlighted in red are above trigger values

Table 23 Major cations of pore water samples collected from surface and subaqueous soil samples

SITE	SAMPLE ID	DEPTH	Ca	K	Mg	Na	S	NPOC	TN	Al	Fe	Mn
		cm	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	mg/L	mg/L
Point Sturt North	2.1	0-12	38.3	19.2	13.9	103	51	8633	2596	5.5	LOD	0.56
	2.2	12-25	57	37.7	42.4	145	181	20470	8410	317.9	58.5	2.31
	2.3	25-42	164	62.6	166	402	565	27630	9216	0.9	30.7	5.91
	2.4	42-67	94.8	40.6	116	813	549	24710	3871	41.2	<0.4	0.29
Boggy Creek	15.1	0-3	59.1	14.1	38.3	196	8.82	25670	8710	20.1	1.630	1.89
	15.2	3-13	129	30.7	72.5	412	170	43420	18600	4.4	0.933	2.09
	15.3	13-27	304	55.9	210	836	589	38740	25360	2.9	107	6.80
	15.4	27-47	481	105	429	1510	1220	50920	32640	13	365	14.3
	15.5	47-62	482	132	549	2380	988	26620	26980	2.4	24.5	8.160

Table 24 Trace elements of pore water samples collected from surface and subaqueous soil samples

	SAMPLE ID	DEPTH	Co	Cu	Mo	Ni	Pb	Sr	U	Zn	As	Ag
		cm	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Point Sturt North	2.1	0-12	0.52	0.5	0.06	10.5	LOD	279	LOD	5.3	3.9	0.04
	2.2	12-25	4.3	8.5	LOD	49.6	0.7	411	1.91	110.1	11.8	LOD
	2.3	25-42	8.6	0.5	0.6	162.5	LOD	1320	0.07	17.2	2.48	LOD
	2.4	42-67	0.3	0.7	29	1.6	0.1	1120	3.17	1.9	6.93	LOD
Boggy Creek	15.1	0-3	0.62	4.1	0.5	1.4	0.62	514	0.32	6.3	5.19	0.08
	15.2	3-13	0.81	0.9	0.96	2	LOD	1010	0.78	1.7	3.26	0.05
	15.3	13-27	0.8	0.8	0.8	2.7	LOD	2650	0.19	4.9	5.26	0.5
	15.4	27-47	3.9	4.1	0.5	465	LOD	4710	0.08	27	13.7	0.5
	15.5	47-62	0.3	1.1	5.2	59.5	LOD	5780	5.03	2.9	3.32	0.76

Figures highlighted in red are above trigger values

Table 25 Water quality guideline values

	Al	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Fe	Mn
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L
WQG (95%PC; TV ~30 g CaCO ₃ /L) ^a	55	0.05	13	0.2	1.4	3.3	1.4	11	3.4	8	NV	1.9
WQG (hardness=60) ^b	55	0.05	13	0.36	1.4	5.9	2.5	20	8.2	14	NV	1.9

^a WQG (95%PC) = ANZECC/ARMCANZ (2000) WQG trigger value (TV) for 95% species protection applicable to freshwaters of hardness 30 mg CaCO₃/L. Values provided are without hardness correction.

As(V) = 13 µg/L / As(III) = 24 µg/L, Cr(VI) = 1 µg/L / Cr(III) = 3.3 µg/L.

NV = no guideline value. ^b Hardness-adjusted WQGs for Ag, Cd, Cr, Cu, Ni, Pb and Zn applicable to fresh waters (Appendix D). TV = no hardness adjustment applicable and trigger value applies.

The water quality guideline values (ANZECC/ARMCANZ, 2000) are listed in Table 25. The pore water concentrations for all elements other than lead, strontium and uranium, were frequently found to be above trigger values in sub-layers of the core profile samples from the two sites (Table 23 and 24). There was no relationship observed between trace element concentrations in pore waters and depth at the two sites (Tables 23 and 24).

The distribution of redox sensitive elements such as Fe and Mn in pore water can be used to give an indication of the redox and/or acidic conditions in subaqueous soils. During a change to reducing conditions Mn(III,IV) is typically reduced prior to Fe(III) to release Mn(II) and Fe(II), respectively. In general, pore water Fe concentrations showed a negative correlation with pH and a positive correlation with SO₄. Taking into account that strongly oxidised sulfuric materials were present prior to refilling, it appears likely that the results indicate that dissolution of Fe hydroxysulfate minerals has contributed to the dissolved Fe and SO₄ in pore water (Shand *et al.* 2012).

Elemental concentrations in whole sediments and 0.1M HCL extracts were found in the majority of cases to be below their guideline values (Tables 26-28). These findings suggest that even though total metal concentrations in whole soil layers are below those considered to impact environmental health, the metal concentrations in pore waters (which may be the most bioavailable fraction) may still be present at concentrations that could be harmful for aquatic organisms.

Table 26 Total elemental concentrations in surface and subaqueous soil samples collected from two Lower Lake sites

SITE	SAMPLE ID	DEPTH	Al	As	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
		cm	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Point Sturt North	2.1	0-12	751	0.598	0.717	0.0996	0.193	<LOQ	0.398	992	25.9	LOD	0.797	0.0976	1.59
	2.2	12-25	725	0.598	0.279	<0.02	0.227	<LOQ	0.797	1090	14.9	LOD	0.797	0.129	1.59
	2.3	25-42	3930	1.15	0.229	0.0573	1.24	4.77	2.48	3720	53.1	3.05	1.91	0.464	6.3
	2.4	42-67	23300	3.92	0.392	<0.02	3.73	23.1	6.27	18200	106	12.2	5.88	0.176	18.6
Boggy Creek	15.1	0-3	26700	10	0.449	0.0612	13.5	39.6	30.6	32000	353	30.4	18	1.82	72.2
	15.2	3-13	21000	5.2	0.3	<0.02	6.9	26.8	20.8	24200	133	17.2	15	1.78	47
	15.3	13-27	5300	1.81	0.361	0.0602	1.96	9.04	5.02	5620	43.8	4.42	4.42	0.351	13.3
	15.4	27-47	7150	2.17	0.157	<0.02	2.01	10	4.72	6710	47	4.72	4.13	0.374	14
	15.5	47-62	4590	2.64	0.224	<0.02	2.58	7.32	3.46	5690	99.6	4.27	2.85	0.343	11

Figures highlighted in red are above trigger values

Table 27 Total elemental concentrations in 0.1M HCl extracts of surface and subaqueous soil samples collected from two Lower Lakes sites

SITE	SAMPLE ID	DEPTH cm	Al mg/kg	As mg/kg	Ag mg/kg	Cd mg/kg	Co mg/kg	Cr mg/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Ni mg/kg	Pb mg/kg	U mg/kg	Zn mg/kg
Point Sturt North	2.1	0-12	27	0.013	0.0029	0.0038	0.029	0.07	0.147	342	2.0	0.029	0.25	0.010	0.220
	2.2	12-25	37	0.0168	<LOQ	LOQ	0.018	0.05	0.158	303	2.0	0.025	0.36	0.086	0.246
	2.3	25-42	204	0.332	<LOQ	0.0041	0.324	0.21	0.129	223	8.3	0.447	0.33	0.489	0.864
	2.4	42-67	1370	0.317	<LOQ	0.0042	0.220	1.57	0.461	598	21	0.866	1.98	0.063	0.733
Boggy Creek	15.1	0-3	1787	0.739	0.0027	0.023	0.694	1.823	5.415	6294	135	2.928	7.14	0.126	8.900
	15.2	3-13	2386	0.486	<LOQ	0.019	1.621	1.965	3.764	8742	97	2.942	7.10	0.118	13.061
	15.3	13-27	782	0.400	<LOQ	0.0032	0.316	0.756	1.957	2524	34	0.903	3.70	0.086	3.575
	15.4	27-47	510	0.457	0.0017	0.0039	0.195	0.471	1.089	1710	26	0.464	4.57	0.121	2.136
	15.5	47-62	492	0.319	<LOQ	0.008	0.128	0.873	0.031	506	26	0.229	0.49	0.071	1.927
LOQ			<8	<0.016	<0.0016	<0.0032	<0.004	<0.008	<0.032	<8	<8	<0.016	<0.004	<0.001	<0.031

Table 28 Whole Sediment guideline values

SITE	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sn	V	Zn	pH
	µg/g or mg/kg													
Trigger value (TV) ^a	NV	20	1.5	NV	80	65	NV	NV	21	50	5	NV	200	NA
ISQG-High ^b	NV	70	10	NV	370	270	NV	NV	52	220	70	NV	410	NA

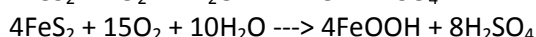
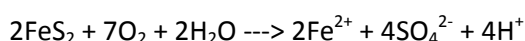
^a **Trigger value (TV)** = ANZECC/ARMCANZ (2000) SQG-low trigger value (TV) for 95% species protection.

^b **ISQG-High** = ANZECC/ARMCANZ (2000) SQG-high trigger value (TV) for 95% species protection.

NV = no guideline value.

Sulfide minerals are generally stable under reducing conditions. However, when they become exposed to the atmosphere, the acidity produced from sulfide oxidation can impact on water quality, crop production, and corrode concrete and steel structures (Dent 1986). In addition to the acidification of sediments, soils and waters, a reduction in water quality may result from occurrences such as: low dissolved oxygen levels (Burton *et al.* 2006; Sammut *et al.* 1993; Sullivan *et al.* 2002a), high concentrations of aluminium and iron (Ferguson and Eyre 1999; Ward *et al.* 2002) and the release of other potentially toxic metals (Burton *et al.* 2008a; Preda and Cox 2001; Sullivan *et al.* 2008; Sundstrom *et al.* 2002).

In nature, a number of oxidation reactions of sulfide minerals (principally pyrite: FeS₂ which produce acidity) may occur, including:



A range of secondary minerals, such as jarosite, sideronatrite and schwertmannite may also form, which act as stores of acidity i.e. they may produce acidity upon dissolution (rewetting).

The total amount of non-organic reduced-S (or reduced inorganic sulfur – RIS), contained mainly within sulfide minerals (S_{CR}), is determined by the Cr-reducible S technique (Ahern *et al.* 2004). The total amount of acid generated, assuming complete oxidation, can be quantified, usually in mol H⁺ tonne⁻¹, or taking into account the bulk density, as mol H⁺ m⁻³. However, shielding of sulfide minerals, e.g. by iron (oxy) hydroxides, may limit sulfide oxidation, in effect decreasing the amount of potential acid available for reaction. As well as potential acidity, the amount of acidity already present in the soil can be quantified as titratable actual acidity (TAA). In sulfuric materials, retained acidity may form a major component of stored acid (e.g. stored in mineral phases such as jarosite). The sum of acidity generated by S_{CR}, TAA and retained acidity represents the acid generating potential (AGP) of the sample. As well as taking into account the total acid potential of the sample, acid generated post-sampling and prior to analysis is included as part of the total potential of the sample.

S_{CR} concentration results varied widely across the two study areas, as well as within individual soil profiles (Tables 29 and 30). The soil samples tested exceeded the Australian (coastal) action criteria or trigger values for the preparation of an ASS management plan. The trigger values are texture-dependent, as coarser-grained soils are often more prone to acidification, owing to them typically containing larger amounts of quartz sand or relatively unreactive aluminosilicate minerals such as K-feldspar.

Table 29 Acid volatile sulfur (AVS) and titratable actual acidity (TAA) in surface and subaqueous soil samples collected from two Lower Lake sites

SAMPLE SITE	DEPTH	TEXTURE	MOISTURE CONTENT		ACID VOLATILE SULFUR (AVS)		TITRATABLE ACTUAL ACIDITY (TAA)		
			(% moisture of total wet weight)	(g moisture / g of oven dry soil)	(% SAV WW)	(% SAV DW)	pH _{KCL}	(mole H ⁺ /tonne) (to pH 6.5)	
Point Sturt North	2.1	0-12	Coarse	22.4	0.3	0.003	0.004	7.20	0
	2.2	12-25	Coarse	16.7	0.2	0.002	0.002	6.13	4
	2.3	25-42	Medium	26.4	0.4	0.000	0.000	5.05	11
	2.4	42-67	Fine	24.7	0.3	0.001	0.002	8.89	0
Boggy Creek	15.1	0-3	Fine	69.3	2.3	0.086	0.279	6.18	10
	15.2	3-13	Fine	56.5	1.3	0.087	0.199	5.69	15
	15.3	13-27	Medium	39.1	0.6	0.014	0.024	5.39	18
	15.4	27-47	Medium	36.8	0.6	0.007	4.98	4.98	15
	15.5	47-62	Medium	37.3	0.6	0.001	8.91	8.91	0

Figures highlighted in red are above trigger values

Table 30 ASS in surface and subaqueous soil samples collected from two Lower Lake sites

SAMPLE SITE	DEPTH	REDUCED INORGANIC SULFUR (% CHROMIUM REDUCIBLE S)		TOTAL ORGANIC CARBON	ACID NEUTRALISING CAPACITY (ANC _{BT})		NET ACIDITY CHROMIUM SUITE (mole H ⁺ /tonne)	LIME CALCULATION CHROMIUM SUITE (kg CaCO ₃ /tonne DW)
		*%Scr	(mole H ⁺ /tonne)	(% C)	(%CaCO ₃)	(mole H ⁺ /tonne)	(BASED ON %Scr)	(INCLUDES 1.5 SAFETY FACTOR WHEN LIMING RATE IS +VE)
Point Sturt North	2.1	0.023	14	0.16	0.00	0	14	1.1
	2.2	0.020	12	0.08	..	0	16	1.2
	2.3	0.141	88	0.23	..	0	98	7.4
	2.4	0.328	205	0.23	3.18	635	-219	-11.0
Boggy Creek	15.1	0.968	604	7.54	..	0	613	46.0
	15.2	0.729	455	7.36	..	0	470	35.3
	15.3	0.089	56	1.41	..	0	73	5.5
	15.4	0.065	41	0.83	..	0	55	4.2
	15.5	0.312	195	0.50	3.00	599	-205	-10.3

Figures highlighted in red are above trigger values

Prior to the drought, median soil pH in the Lower Lakes was circumneutral and displayed a narrow range. Drought caused median and interquartile pH ranges to decrease significantly. However, soil acidification along the margins of the Lakes was very variable and largely related to heterogeneity of the soil types and ASS parameters as well as geomorphological controls. Following inundation, in late 2010, median pH increased significantly (Baker and Shand (2014)). However, many of the soils remained acidic up to the end of the monitoring period (2013) and a number of hot spots were identified in some parts of the lakes (e.g. Dog Lake, Boggy Lake, Campbell Park, Finniss River and the northern side of Lake Albert) where high Net Acidity (NA) and incubation pH indicated high acidification hazard potential.

Based on the present study conducted at Point Sturt North and Boggy Creek in February 2014, and the monitoring conducted in the Lower lakes over the last seven years, recovery has been observed in the upper soil layers but the middle layers remain acidic. According to Baker and Shand (2014) this could be due to:

- Higher labile organic matter in surface soils which support reduction processes and the consequent generation of alkalinity.
- Infiltration during refilling of fresh surface water generating a downward flux of acidity and contaminants from surface layers.
- The common occurrence of more sandy sediments at the surface.
- The soil pH levels having not returned to pre-drought levels and the acidification hazard remains high around much of the Lower Lakes. Contaminants in deeper sediments could undergo upward flux and increase risk to the biota inhabiting the upper sections at ASS impacted sites.

LF02 – Point Sturt North: Soil profiles sampled comprised sulfuric and hypersulfidic subaqueous soil with medium acidification hazard. At 25-42 cm depth, net acidity was relatively high (maximum of 100 moles H^+ /tonne). Acidification potentials were generally high throughout the profiles. The lower portion of the profile (42-67 cm) had negative net acidity, low acidification potential and very high levels of ANC (Tables 29 and 30).

LF15 – Boggy Creek: Soil profiles sampled comprised hypersulfidic subaqueous soils with medium acidification hazard. The upper portion of the profile (above 27 cm) had positive net acidity, high acidification potential and no ANC was present. The net acidity was relatively high with a maximum of 613 moles H^+ /tonne at 0-3 cm and 470 H^+ /tonne at the depth of 3-13 cm. The lower portion of the profile (47-62 cm) had negative net acidity, low acidification potential and very high levels of ANC (Tables 29 and 30).

4 Conclusions

4.1 Ecotoxicological assessment

Surface water

- Surface water from Boggy Creek and Point Sturt North generally was not associated with reproduction impairment in *Ceriodaphnia dubia* waterfleas.
- Low toxicity was observed when shrimp and fish larvae were exposed to surface waters from Boggy Creek and Point Sturt North.
- In general, elemental concentrations in surface waters were below those considered to be of risk to aquatic organisms.

Pore water

- Pore water collected from deeper sections of the sediment profile from Boggy Creek (3-13, 13-27 and 27-47 cm) and Point Sturt North (12-25, and 25-42 cm) were severely toxic to water fleas during both acute and chronic exposures. Microbial toxicity of the sediments varied from low to moderate.
- Metal concentrations in pore water from deeper cores were above their guideline trigger values at both sites. Combinations of Al, Co, Mn, Ni, Cu, Zn and As at low pH and high EC could be contributing to this toxicity.

Whole sediments

- At Boggy Creek, midge larvae survival was not affected during 5-day exposure. Percentage emergence of midge larvae was impacted when exposed to sediments from the upper four layers. However, exposure to the layer at 47-67 cm did not impact midge emergence. Sex ratios were skewed in midge larvae exposed to the sediment layers from 0-3 and 27-47 cm depth at Boggy Creek.
- At Point Sturt North, midge larvae survival was not affected. Both the growth and emergence of midge larvae were impacted when exposed to sediments from 12-25 and 25-42 cm depths. Sex ratios were not skewed in midge larvae exposed to any sediments.

The ecotoxicological assessment of surface water, pore water and sediments at different depths at the two sites, four years after inundation, confirms that the contaminants generated at the ASS impacted sites, at deeper sediment depths, are potentially severely toxic to aquatic organisms. If this monitoring was undertaken during or straight after the water had returned, the surface sediments may also have posed this risk level to aquatic organisms.

4.2 Implications

- In sub aqueous soils, under acidic conditions, a combination of stressors such as pH, conductivity and metals may adversely affect the growth and reproduction in the aquatic organisms inhabiting sites where ASS are present.
- Sediments at deeper profiles could cause an upward flux of contaminants that may pose a moderate to high level risk to the biota inhabiting Lower Lakes sites where ASS are present.

5 Recommendations

- Further ecotoxicological monitoring is recommended to assess the spatial and temporal variation in the toxicity at selected Lower Lakes sites to address seasonal changes in partitioning of contaminants and their bioavailability in subaqueous soils.
- The development of rapid monitoring tools and modelling approaches should be considered. They would utilise chemical, physical and microbial parameters to enable assessment of sediment health and impacts of stress-induced changes.
- Mesocosm studies involving drying and wetting of sediments should be included in future monitoring studies so as to better integrate chemical and ecotoxicological investigations.
- In the present study, a mixture of metals including Al, Cu, Zn, As, Mn and Co were above ANZECC/ARMCANZ guideline values in the pore water collected from the sediments at some sites. As the potential for multiple chemical exposure increases, the question raised is whether the toxicity of mixtures of chemicals is simply additive or whether there is potentiation of toxicity. The general consensus has been that chemicals interact by concentration addition, however past studies have demonstrated that concentration addition of the components of a mixture does not always reflect the overall interaction of a mixture. Risk assessment procedures should account for mixtures of contaminants present in a given system.
- Pore water Al had the highest hazard quotient. Thus, it becomes obvious that Al is a significant hazard associated with ASS. Unfortunately, there is a notable shortage of literature on the biological response of the aquatic biota to Al released from ASS. The locations and soil characteristics of ASS are well defined throughout the literature. Similarly, Al is recognised as a highly toxic element when bioavailable. However, Al forms a range of chemical species, little is known about speciation, bioavailability and toxicity when it comes to a system dominated by ASS. The current Al guideline is applicable at pH <6.6. The ANZECC/ARMCANZ water quality guidelines require review for aluminium, particularly in relation to deriving guideline value(s) for aluminium toxicity in lower pH water. The sediment guidelines for aluminium should also be reviewed.

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Appendix- 1- Project Activities 2013-2014

ACTIVITY ID	DESCRIPTION	SERVICE AND DELIVERABLE	STATUS
2.1	Project management: Coordinate staff and resources to facilitate efficient and timely delivery of project goals. Attend meetings and communicate project findings to stakeholders.	Completion of field work at two sites, compile fieldwork database and transfer samples to laboratories for ecotoxicological assessment of two sites.	Completed in Feb 2014
2.2	Fieldwork / sampling: Conduct subaqueous soil sampling in Feb 2014 at TWO additional study sites around the margins of the Lower Lakes. This will comprise collection of 8 soil cores (4 at each site). Surface water samples will also be collected at these two sites in Feb 2013 for ecotoxicological assessment (activity 1.3) and chemical characterization (Activity 1.4). All field collected samples will be stored appropriately and transferred to the laboratory for ecotoxicological assessment.	Interim report on ecotoxicological work on surface water and pore water and whole sediment samples from two sites. This includes ecotoxicological testing using 5 species for surface water, three species for pore water and whole sediment bioassays using midge larvae at 4 depths for two sites.	Draft report Submitted to DEWNR in August 2014 and data summarised in Section 2 of this report.
2.3	Effect assessment: Surface water, pore water and sediment ecotox work based will be carried out on at least 4 soils cores at two sites in 2014	Complete physio-chemical analyses of surface water, pore water and sediment samples. Access to analytical data on sediments from 2013 Spatial and Temporal Monitoring of Recovery in the Lower Lakes Project.	Data checked and lodged in database and data summarised in Section 3 of this report
2.4	Exposure assessment: surface water and pore water (at four depths at each site) from two sites will be analysed for (i) alkalinity/acidity (ii) organic carbon, (iii) the major anions (Cl, NO ₃ , Ammonia, PO ₄ , SO ₄), (iv) the major cations Na, K, Ca, Mg, (v) the dissolved trace metals or metalloids Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Zn. Soil analyses will comprise chiptray ageing, peroxide pH, soil pH, CRS, ANC, AVS, SNAS and soluble sulfate analyses for each site at four depths.	Submission of draft report: Field and laboratory data will be provided in tables and summarised in a brief final report (approximately 30 pages, including appendices). The report will only include data collected during the 2013-2014 sampling.	Draft report Submitted to DEWNR in August 2014 and data summarised in Section 3 of this report.
2.5	Data management: Data management will involve creation of a database that will contain all field data, ecotoxicological data collected from two sites.	Presented findings at the Ecotoxicology Project Advisory Group Meeting and refined research strategies and promoted research findings to stakeholders and scientific community	Two Presentations at DEWNR- July 2014 and October 2014 and Wetland Day presentation at Coorong and Lakes Environment Forum on 30 Jan 2015
2.6	Data summary and report writing: Field and laboratory data will be provided in tables and summarised in a brief final report (approximately 30 pages, excluding appendices).	Submission of revised satisfactory final report following review through CSIRO E-publish system (DEWNR to be involved in review process).	Summarised in Sections 3 and 4 of this report. Completed- 30 March 2016

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