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

Interim report (Milestones 2.2 and 2.4)

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Interim report for:

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Executive Summary

Drought from 2007 to mid 2010 caused large expanses of previously inundated sediments and subaqueous soils to be exposed around the margins of Lakes Albert and Alexandrina in South Australia. This exposed acid sulfate soil (ASS) materials that became progressively oxidised to greater and greater depths in the soil profiles. The resultant formation of sulfuric materials (pH < 4) produced significant water quality and ecological problems.

The main focus of earlier field monitoring and research of ASS environments in the Lakes Alexandrina and Albert has been on measuring and analysing the physico-chemical parameters at the two lakes. Based on the seven years of monitoring it has been confirmed that 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 has in many areas returned to circumneutral pH that have 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 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 reports present ecotoxicity and chemical data collected during the 2014 sampling year at the Point Sturt North and Boggy Creek sites. Point Sturt North and Boggy Creek sites were selected for surface water and sediment sampling in 2014. Surface water samples from the two sites were evaluated using a microbial assay for risk assessment (MARA), a duckweed (*Lemna* sp.), a waterflea (*Ceriodaphnia dubia)*, a freshwater shrimp (*Paratya australiensis)* and an embryo-larval stages of golden perch or Murray cod (*Maccullochella peelii).* 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). *W*hole sediment bioassays were conducted on each of these sediment sub-layers at different depths to assess their contribution towards toxicity. Laboratory cultured, second-instrar 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 *C.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, (ivi) the major anions (Cl, NO_3, Ammonia, PO_4, SO_4,), (v) the major cations (Al, Fe, Mn, Na, K, Ca, Mg), and (vi) trace elements ((As, Cd, Co, Cr, Cu, Ni, Zn).

Conclusions

Surface water ecotoxicological assessment

- Surface water from Boggy Creek and Point Sturt North sites generally did not exhibit reproduction impairment in the exposed *Ceriodahnia dubia*.
- Low toxicity to shrimp and fish larvae were observed when exposed to surface waters from Boggy Creek and Point Sturt North sites.
- In general, elemental concentrations in surface waters were below those considered to be of concern to aquatic organisms.

Pore-water ecotoxicological assessment

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

Whole sediment ecotoxicological assessment

- At Boggy Creek site**,** midge larvae survival was not affected during 5-day exposure. Percentage emergence of midge larvae was impacted when exposed to sediments from 0-3, 3-13, 13-27 and 27-47 cm depths. However, exposure to deeper sediment layer at 47-67 cm did not impact midge emergence. Sex ratios was skewed in midge larvae exposed to the sediment layers from 0-3 and 27-47 cm depth at Boggy Creek site
- At Point Sturt North site, midge larvae survival was not affected. Growth was impacted in midge larvae exposed to sediments from 12-25 and 25-42 cm depths. Percentage emergence of midge larvae was also impacted in sediments collected from 12-25 and 25-42 cm depths. Sex ratios were not skewed in midge larvae exposed to the sediment at all depths.

The ecotoxicological assessment of surface water, pore water and sediments at different depths at the two sites confirms that the contaminants generated at the ASS impacted sites at deeper sediment depths if bioavailable, could be severely toxic to aquatic organisms.

Implications

- A combination of stressors such as pH, conductivity and metals may adversely affect the growth and reproduction in the aquatic organisms inhabiting ASS impacted sites.
- Sediments at deeper profiles could cause flux of contaminants that may pose a moderate to high level risk to the biota inhabiting ASS impacted sites.

Recommendations

- Ecotoxicological monitoring studies are recommended to assess the spatial and temporal variation in the toxicity at selected sites.
- Development of rapid monitoring tools and modelling approaches that utilise chemical, physical and microbial parameters to enable assessment of sediment health and impact of stress-induced changes should be considered .
- Mesocosm studies involving drying and wetting of sediments should be included in future monitoring studies involving the integration of chemical and ecotoxicological investigations.
- Risk assessment procedures should account for mixtures of contaminants present in a given system.
- 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 the south east Australia including the Murray-Darling Basin. The combination of decreasing water levels and gently sloping near-shore lake beds caused large expanses of previously inundated sediments and subaqueous soils to be exposed to the atmosphere. With continued lowering of water levels, acid sulfate soil (ASS) materials became progressively oxidised to greater and greater depths in the soil profiles. The resultant formation of sulfuric materials (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 sulfuric materials that had formed in the previously dried margins of the Lower Lakes. The main focus of earlier field monitoring and research of Acid Sulfate Soil (ASS) environments in the Lakes Alexandrina and Albert has been on 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 projects have 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, Baker and Shand (2014) have 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, seventeen study areas have been monitored (physical and chemical properties), providing good spatial coverage of the recovery of ASS around the Lower Lakes (Figure 1). The surface of subaqueous soils has in many areas returned to circumneutral pH that have 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 an ecotoxicological assessment of the surface water, pore-water and sediments at four sites in four selected study areas in the Lower Lakes.

The specific objectives are as follows:

1. 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 risk posed to benthic organisms based on analytical and biological assessment of core profile sediments 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 reports data collected during the 2014 sampling year at the Point Sturt North and Boggy Creek sites. These two sites were included in this project because previous studies found subaqueous soils at Point Sturt North to pose a medium acidification hazard and subaqueous soils at Boggy Creek considered to pose a high acidification hazard (Baker and Shand 2014).

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

2.1 Sample Sites

In Feb 2014, Point Sturt South 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. LF 2 and LF 15 represent Boggy Creek and Point Sturt North sites

Where possible, the sites sampled for this project were positioned within a few metres of former sampling sites that had been established as part of studies of ASS in Lake Alexandrina and Lake 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 auger types (n=4). Sampling was relatively shallow (< 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. 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. 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 recorded following McDonald *et al.* (1990) (See Baker *et al.* 2013a; Baker *et al.* 2013b). The presence of 'sulfidic' smells (e.g., H_2S – rotten egg gas and methyl thiols) as well as oxidising odours (SO_2) were recorded. Representative sub-samples were 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 with four distinct layers (Table 1). Boggy creek sediment core had five distinct layers and was sampled up to 62 cm in depth. The site description is 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 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 Pont Sturt North and Boggy Creek sites 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 Figure 2.

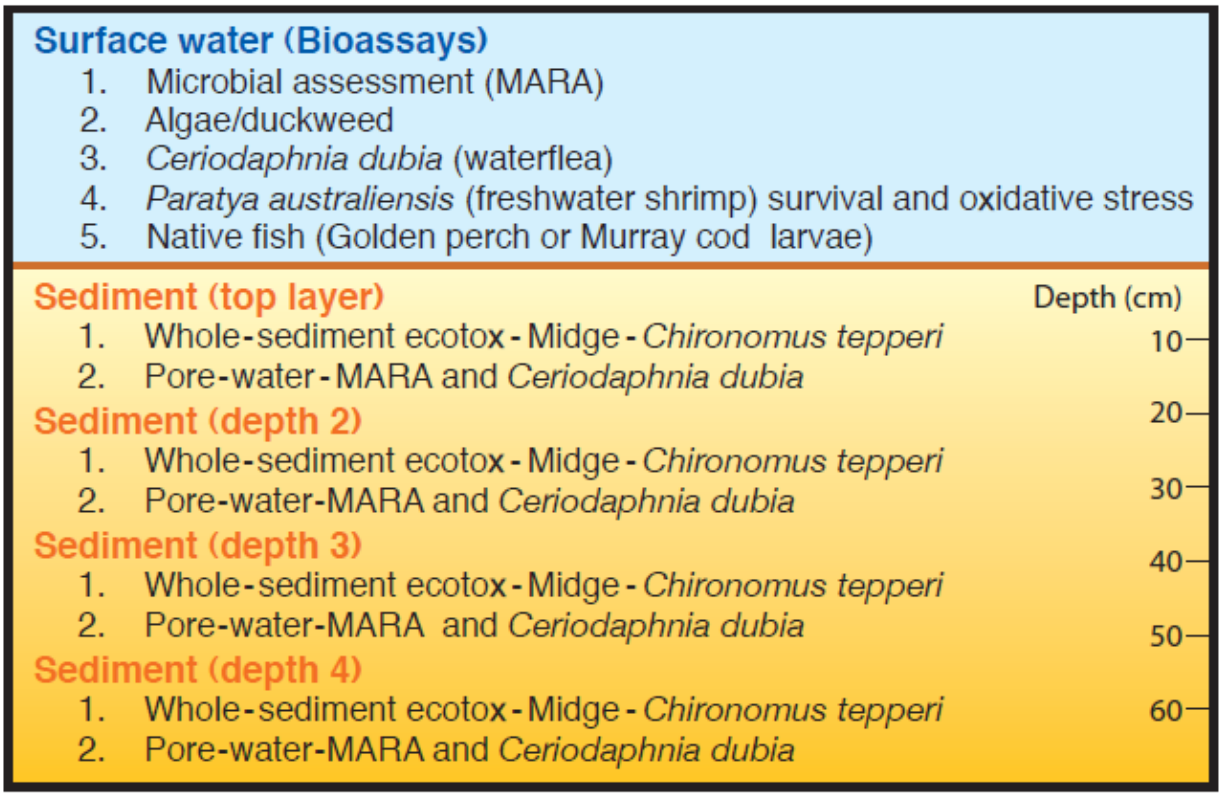

Figure 2 Overview of ecotoxicological assessment

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 sites. 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 sub-sampled 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 50mL centrifuge tubes and centrifuged for 25 min at 3500rpm 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 test uses a selection of taxonomically diverse range of 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.

Cladoceran immobilisation and reproduction tests

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 being maintained at CSIRO, Adelaide in demineralised water (DMW).

The acute bioassay measuring immobilisation of *C. dubia* over 48 h follows the OECD guideline 202 (OECD 2004) with minor modifications (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). Each concentration was dispensed, in triplicate, into 20-mL glass vials (containing 18 mL test solution). Moderately hard water (MHW) was also prepared in triplicate for use as control treatments. Five *C. dubia* neonates (<24 h old) were added to each vial and incubated at 25 ± 1˚C (16:8 h light:dark) using cool white fluorescent lamps. After 48 h, the number of alive and immobilised (dead) neonates was counted. Test solutions were not renewed (i.e. a static test) during the 48 h exposure.

Reproduction of *C. dubia* was assessed over 8 days and is summarised in Table 4 and based on the OECD Test Guideline 211 (2012) used for *Daphnia magna*. Surface water and pore-water samples (n=4) were diluted with MHW to achieve concentrations of 0.1 to 100% (undiluted). Tests were carried out in 50-mL beakers containing 25 mL of test solution with ten replicates per treatment. Control treatment was also prepared with MHW. Ten neonates (< 24 h old) were added to each beaker and incubated at 25 ± 1˚C with a photoperiod of 16:8 light:dark cycle. Daphnids were fed a microalgal mixture of *Pseudokirchneriella subcapitata* on daily basis. During the 8-d test duration, test solutions were renewed every day. After 8 days, the number of surviving daphnids and the total number of young generated over three broods were counted. 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 condition 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 criterion	≥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	200 mL beaker
Test solution volume	100 mL
Renewal of test solutions Age of test organisms	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 *Pseudokirchneriella subcapitata* on daily basis
Dilution water	Moderately hard water (MHW)
Test concentrations	4
Control treatments	MHW
Endpoint	Number of neonates over three broods
Test acceptability criterion	≥ 80% survival of original daphnids in the control treatment. Reference toxicant EC50 within Cusum chart control limits

Shrimp Survival

This acute test measures 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 feed 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 3 L borosilicate glass beakers (n= 3). The test was undertaken in 1-L borosilicate glass beakers containing 800 mL of test solution with each concentration prepared in triplicate. MHW control was also prepared in triplicate. The shrimps were isolated at random and transferred to the test solutions using a fish net. Ten shrimps were added to each test vessel and incubated at a temperature of 23 ± 1°C on a 16 h light and 8 h dark cycle for 96 h. Each test vessel was examined at 48 h and 96 h for shrimp mortality. In addition, test solutions were renewed at 48 h. 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.

Greater than or equal to 90% survival in the control is required to achieve minimum acceptability.

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 criterion	≥90% survival in controls; Dissolved oxygen > 60%

Oxidative stress response

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 (H_2O_2) 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 8uthanized (GPx) are involved in the detoxification of superoxide anion radical (O_2^-), H_2O_2 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 H_2O_2 into water and O_2 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). 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 mM-1cm-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 µmol 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 µmol of conjugated product per minute. The extinction coefficient 9.6 $mM^{-1}cm^{-1}$ 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 tests

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 NSW aquaculture facility. Post hatch 2- day larval fish (≤48 h old) were used in the toxicity tests.

Toxicity tests were undertaken in 350-mL beakers containing 100 mL test solution. 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. Test vessels were incubated at 23 ± 1˚C on a 16 h light: 8h dark light cycle. Test solutions were renewed at 48, 96 and 144 h by replacing the test solution with freshly diluted surface water and the number of surviving fish counted. After 2, 4 and 7 days, dead fish were removed from test vessels and preserved for growth and malformations measurements. Water quality parameters (pH, EC and DO) were also measured. The test was terminated after 7 days. Fish were 9uthanized by the addition of MS222 (ethyl 3-aminobenzoate methanesulfonate, Sigma) and immediately fixed in 10% buffered formalin. The test was acceptable if there was ≥90% balanced fish fry in the controls.

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 and 4
Age of test organisms	Fry
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 test – sediment toxicity test

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 was measured over 12 days and test methods are summarised in Table 5. Sediment core samples at different depths from the point Sturt North and the 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/m^2). 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 (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	140g sediment plus 400 mL MHW
Age of test organisms	2nd 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 (MHW, 230mg $CaCO_3$/L)
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

	POINT STURT NORTH	BOGGY CREEK
CONCENTRATIONS (%)	AVERAGE GROWTH (%)	
0	100	100
3.1	95	93
6.3	96	95
13	96	94
25	96	96
50	95	92
100	95	93

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

	FROND NUMBERS	
CONCENTRATIONS	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

*Significantly different from controls

Table 10 Shrimp bioassay – Survival after 96 h exposures

		% SURVIVAL	
CONCENTRATIONS	REPLICATES	BOGGY CREEK	POINT STURT NORTH
100%	1	100	100
	2	100	100
	3	100	100
50%	1	100	100
	2	100	100
	3	100	100
25%	1	100	100
	2	100	100
	3	100	100
12.5%	1	100	100
	2	100	100
	3	100	100
Control	1	100	100
	2	100	100
	3	100	100

Table 11 *Ceriodaphnia* acute bioassays – Surface water toxicity

BOGGY CREEK SURFACE WATER	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	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	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	8.36	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 12 *Ceriodaphnia* Chronic bioassays – Surface water toxicity

CONCENTRATIONS (%)	BOGGY CREEK	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

Table 13 Shrimp oxidative stress after 96 h exposures to surface water from Boggy Creek and Point Sturt North sites

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*

The results from the ecotoxicological assessment on surface waters are summarised in Table 14.

No microbial toxicity and low to moderate toxicity to duckweed was observed. Water fleas during 48 h acute and 7-8 day chronic exposures did not exhibit any observable toxicity. Shrimp survival was also not impacted during 96 h exposures to the surface waters from Boggy Creek and Point Sturt North sites. 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.

Table 14 Summary of ecotoxicological assessment of surface water samples

Surface water Dates sampled	Sites	Microbial	*Lemna*	Water flea	Shrimp	Fish larvae
	Point Sturt North	**NT**	**T**	**NT**	**NT**	**LT**
	Boggy Creek	**NT**	**LT**	**NT**	**LT**	**LT**
	Synthetic water	**NT**	**NT**	**NT**	**NT**	**NT**

NT: No toxicity NOEC 100-90%	LT: Low toxicity NOEC 89-49%	T: Moderate to high toxicity NOEC 50-10%	HT- very high toxicity NOEC <10%

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 15-19. Results from the *Ceriodaphnia* and microbial bioassays are summarised in Tables 16-19.

Table 15 *Ceriodaphnia* acute bioassays – pore water from sediments collected at 0-3 cm and 3-13 cm depth at Boggy Creek site

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 16 *Ceriodaphnia* acute bioassays – pore water from sediments collected at 13-27 cm and 27-47 cm depth at Boggy Creek site

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 17 *Ceriodaphnia* acute bioassays – pore water from sediments collected at 0-12 cm and 12-25 cm depth at Point Sturt North site

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 18 *Ceriodaphnia* acute bioassays – pore water from sediments collected at 25-22 cm and 42-67 cm depth at Point Sturt North site

POINT STURT NORTH PORE WATER – –(25-42 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	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		
CONCENTRATION	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

Pore waters collected from sediments at 0-25 cm depth at Point Sturt site exhibited no to low microbial toxicity. However, the two deeper layers (25- 67 cm) showed moderate to high microbial toxicity (Table 19). In general, pore water from surface sediments showed no acute toxicty to waterfleas. Moderate to high toxicity was observed when water fleas were exposed to pore water from deeper sediment layers at Boggy Creek site (Table 19). In contrast, pore water from the deepest sediment layer (42-67 cm depth) at Point Sturt Site exhibited no acute toxicity to water fleas during 48 h exposures but low toxicity was evident during 8 day chronic exposures. This low chronic toxicity was completely removed at 50% dilution of the pore water.

Table 19 Summary of ecotoxicological assessment of pore water samples

Water samples	Microbial	Water flea Acute	Water flea Chronic
Boggy Creek (0-3 cm depth)	T	NT	LT
Boggy Creek (3-13 cm depth)	T	T	HT
Boggy Creek (13-27 cm depth)	LT	T	HT
Boggy Creek- (27-47 cm depth)	T	T	HT
Point Sturt North- (0-12 cm depth)	NT	NT	LT
Point Sturt North (12-25 cm depth)	LT	T	HT
Point Sturt North (25-42 cm depth)	T	HT	HT
Point Sturt North (42-67 cm depth)	T	NT	LT
River water	NT	NT	NT

NT: No toxicity NOEC >100-90%	LT: Low toxicity NOEC 89-49%	T: Moderate to high toxicity NOEC 50-10%	HT- very high toxicity NOEC <10%

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 site. The results are summarised in Tables 20-21.

Point Sturt North site

- Midge larvae survival was not affected in soil sub-layers at Point Sturt North site
- 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 site.

Boggy Creek site

- Midge larvae survival was not affected in soil sub-layers at Boggy Creek site
- 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 site.

Table 20 Survival and growth of midge larvae

	DEPTH RANGE (CM)	% ALIVE	AVERAGE LENGTH	ST DEV
River Murray	0-10	90	14.84	1.92
Boggy Creek 15.1	0-3	100	15.03	1.38
Boggy Creek 15.2	3-13	90	14.88	1.91
Boggy Creek 15.3	13-27	90	14.56	1.52
Boggy Creek 15.4	27-47	100	14.90	1.63
Boggy Creek 15.5	47-62	92.5	15.05	1.56
Pt Sturt Nth 2.1	0-12	97.5	14.00	1.65
Pt Sturt Nth 2.2	12-25	95	13.89	1.86
Pt Sturt Nth 2.3	25-42	92.5	13.89	2.12
Pt Sturt Nth 2.4	42-67	92.5	13.13	2.06

Table 21 Survival and growth of midge larvae

			% MALE AND FEMALES	
	DEPTH RANGE (cm)	% EMERGENCE	MALE	FEMALE
River Murray (reference site)	0-10	87.5	51	49
Boggy Creek 15.1	0-3	**60**	63	38
Boggy Creek 15.2	3-13	**65**	54	46
Boggy Creek 15.3	13-27	92.5	49	51
Boggy Creek 15.4	27-47	**75**	**63**	**37**
Boggy Creek 15.5	47-62	**67.5**	48	52
Pt Sturt Nth 2.1	0-12	87.5	60	40
Pt Sturt Nth 2.2	12-25	85	47	53
Pt Sturt Nth 2.3	25-42	**77.5**	58	42
Pt Sturt Nth 2.4	42-67	**52.5**	52	48

Figures highlighted in red are significantly different from the reference site

3 Interim report- Chemical characterization of surface water, pore-water and whole sediment samples

3.1 Laboratory Chemical Analysis Methods

3.1.1 SURFACE WATER AND PORE-WATER ANALYSES

Surface waters were collected as previously described for ecotoxicity testing. The samples were filtered through 0.45 µm filters and divided into two subsamples. One subsample was as previously described 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 50mL centrifuge tubes and centrifuged for 25 min at 3500rpm 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 50mL tubes for water quality analysis including DO, pH, EC using Hach HQd water quality meter and Eh using 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, NO_3, Ammonia, PO_4, SO_4,), (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 22.

Table 22 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	APHA 21st ed., 4500 Cl - B. Automated Silver Nitrate titration.
Chloride	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 0.45um filter prior to analysis. Ammonium molybdate and potassium antimonyl tartrate reacts in acid medium with othophosphate 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) analyzer 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 ELEMETAL 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 0.25g dry soil was weighed into Teflon digest vessels with 2.5mL HCl and 7.5mL HNO_3 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°Cin 10min, ramp to 180oC in 10 min and maintain temperature at 180°C for 60 min. After cooling, the digest solutions were 0.45 µm filtered and analyzed for total elements by ICP-AES and ICP-MS. The digest solution 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 method described in Table 22.

A few refractory sample matrix compounds, such as quartz, silicates, titanium dioxide, alumina, and other oxides may not be 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.

40 mL of 1M hydrochloric acid (HCl) was added to 1g field wet soil (±0.1 g) in a 50mL 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 µm syringe filters and analyzed 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).

Net Acidity = Potential Sulfidic Acidity + Existing Acidity – ANC*/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 CaCO3 equivalents. By accepted definition (Ahern et al. 2004), any acid sulfate soil material with a pH_{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 23.

Table 23 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.
Titratable 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 24-26. The pH in pore-waters was found to 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 24). The EC in pore-waters ranged from 828 to 4320 µS/cm at the Point Sturt and 1579-15190 µS/cm at the Boggy Creek site (Table 24). The EC in pore-water was higher than the upper default trigger value for EC (salinity) generally found in lakes and wetlands (ANZECC, 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 24 Physio-chemical analyses of pore water 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)	(µS/cm)	µg/L	µg/L	µg/L	µg/L	µg/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 25 Physio-chemical analyses of pore water samples.

SITE	SAMPLE ID	DEPTH	Ca	K	Mg	Na	S	NPOC	TN	Al	As	Ag
		cm	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Point Sturt North	2.1	0-12	38.3	19.2	13.9	103	51	8633	2596	5.5	3.9	0.04
	2.2	12-25	57	37.7	42.4	145	181	20470	8410	317.9	11.8	LOD
	2.3	25-42	164	62.6	166	402	565	27630	9216	0.9	2.48	LOD
	2.4	42-67	94.8	40.6	116	813	549	24710	3871	41.2	6.93	LOD
Boggy Creek	15.1	0-3	59.1	14.1	38.3	196	8.82	25670	8710	20.1	5.19	0.08
	15.2	3-13	129	30.7	72.5	412	170	43420	18600	4.4	3.26	0.05
	15.3	13-27	304	55.9	210	836	589	38740	25360	2.9	5.26	0.5
	15.4	27-47	481	105	429	1510	1220	50920	32640	13	13.7	0.5
	15.5	47-62	482	132	549	2380	988	26620	26980	2.4	3.32	0.76

Table 26 Physio-chemical analyses of pore water samples

	SAMPLE ID	DEPTH	Co	Cu	Fe	Mn	Mo	Ni	Pb	Sr	U	Zn
		cm	µg/L	µg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Point Sturt North	2.1	0-12	0.52	0.5	LOD	0.56	0.06	10.5	LOD	279	LOD	5.3
	2.2	12-25	4.3	8.5	58.5	2.31	LOD	49.6	0.7	411	1.91	110.1
	2.3	25-42	8.6	0.5	30.7	5.91	0.6	162.5	LOD	1320	0.07	17.2
	2.4	42-67	0.3	0.7	<0.4	0.29	29	1.6	0.1	1120	3.17	1.9
Boggy Creek	15.1	0-3	0.62	4.1	1.630	1.89	0.5	1.4	0.62	514	0.32	6.3
	15.2	3-13	0.81	0.9	0.933	2.09	0.96	2	LOD	1010	0.78	1.7
	15.3	13-27	0.8	0.8	107	6.80	0.8	2.7	LOD	2650	0.19	4.9
	15.4	27-47	3.9	4.1	365	14.3	0.5	465	LOD	4710	0.08	27
	15.5	47-62	0.3	1.1	24.5	8.160	5.2	59.5	LOD	5780	5.03	2.9

Figures highlighted in red are above trigger values

Table 27 Water quality guideline values

SITE	Al	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Fe	Mn
					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 $CaCO_3$/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. **Blue** when >WQG trigger 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 pore-water concentrations for all elements, except lead, strontium and uranium, were found to be above trigger values in sub-layers core profile samples (except Point Sturt North) from the two sites (Table 25 and 26) (ANZECC, 2000). There was no relationship observed between trace element concentrations in pore-waters and depth at the two sites (Table 25 and 26). The water quality guideline values are listed in Table 27.

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)) typically is reduced prior to Fe(III) to release Mn(II) and Fe(II), respectively. In general, pore-water Fe concentrations show a negative correlation with pH and positive correlation with SO_4. Taking into account the presence of strongly oxidised sulfuric materials prior to refilling, it appears likely that the dissolution of Fe hydroxysulfate minerals has contributed to the dissolved Fe and SO_4 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 (Table 28-30). This 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 (most bioavailable fraction) may still be present at concentrations that could impact the health of aquatic organisms.

Table 28 Total elemental concentrations in whole sediment samples

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 29 Total elemental concentrations in 0.1M HCl extracts of sediments

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	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 30 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. **Blue** when >SQG trigger value.
[b] **ISQG-High** = ANZECC/ARMCANZ (2000) SQG-high trigger value (TV) for 95% species protection. Red when > SQG-high value.
NV = no guideline value.

Sulfide minerals are generally stable under reducing conditions, however, on exposure 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 both ground and waters, a reduction in water quality may result from 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: FeS_2) may occur which produce acidity, including:

$2FeS_2 + 7O_2 + 2H_2O$ ---> $2Fe^{2+} + 4SO_4^{2-} + 4H^+$
$4FeS_2 + 15O_2 + 10H_2O$ ---> $4FeOOH + 8H_2SO_4$

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 total potential of the sample.

S_{CR} concentrations vary widely across the two study areas as well as within individual soil profiles (Tables 31 and 32). 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, since they typically comprise larger amounts of quartz sand or relatively unreactive aluminosilicate minerals such as K-feldspar.

Table 31 Sediment analyses for AVS and TAA

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

Table 32 Sediment analyses for ASS

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 %Scrs)	(INCLUDES 1.5 SAFETY FACTOR WHEN LIMING RATE IS +VE)
Point Sturt North	2.1	0-12	0.023	14	0.16	0.00	0	14	1.1
	2.2	12-25	0.020	12	0.08	..	0	16	1.2
	2.3	25-42	0.141	88	0.23	..	0	**98**	7.4
	2.4	42-67	0.328	205	0.23	3.18	635	-219	-11.0
Boggy Creek	15.1	0-3	0.968	604	7.54	..	0	**613**	46.0
	15.2	3-13	0.729	455	7.36	..	0	**470**	35.3
	15.3	13-27	0.089	56	1.41	..	0	**73**	5.5
	15.4	27-47	0.065	41	0.83	..	0	**55**	4.2
	15.5	47-62	0.312	195	0.50	3.00	599	-205	-10.3

Based on the present study conducted at point Sturt North and Boggy Creek, and the monitoring conducted in the Lower lakes over the last seven years, the recovery has been observed in the upper soil layers but the middle layers remained 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.
- As the soil pH levels have not returned to pre-drought levels and the acidification hazard remains high around much of the Lower Lakes. Sediments at deeper cores could cause flux of contaminants and may pose a risk to the biota inhabiting ASS impacted sites.

LF02 – Point Sturt North: Soil profiles sampled comprised sulfuric and hypersulfidic subaqueous soil with medium acidification hazard. At25-42 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 32 and 33).

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 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 32 and 33).

4 Conclusions

Surface water ecotoxicological assessment

- Surface water from Boggy Creek and Point Sturt North sites generally did not exhibit reproduction impairment in the exposed *Ceriodahnia dubia*.
- Low toxicity to shrimp and fish larvae were observed when exposed to surface waters from Boggy Creek and Point Sturt North sites.
- In general, elemental concentrations in surface waters were below those considered to be of concern to aquatic organisms.

Pore-water ecotoxicological assessment

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

Whole sediment ecotoxicological assessment

- At Boggy Creek site**,** midge larvae survival was not affected during 5-day exposure. Percentage emergence of midge larvae was impacted when exposed to sediments from 0-3, 3-13, 13-27 and 27-47 cm depths. However, exposure to deeper sediment 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 site.
- At Point Sturt North site, midge larvae survival was not affected. Growth was impacted in midge larvae exposed to sediments from 12-25 and 25-42 cm depths. Percentage emergence of midge larvae was also impacted in sediments collected from 12-25 and 25-42 cm depths. Sex ratios were not skewed in midge larvae exposed to the sediment at all depths.

The ecotoxicological assessment of surface water, pore water and sediments at different depths at the two sites confirms that the contaminants generated at the ASS impacted sites at deeper sediment depths if bioavailable, could be severely toxic to aquatic organisms.

Implications

- A combination of stressors such as pH, conductivity and metals may adversely affect the growth and reproduction in the aquatic organisms inhabiting ASS impacted sites.
- Sediments at deeper profiles could cause flux of contaminants that may pose a moderate to high level risk to the biota inhabiting ASS impacted sites.

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Appendix- 1- Project Activities 2013-2014

ACTIVITIES	DESCRIPTION
Activity 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.
Activity 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.
Activity 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
Activity 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.
Activity 2.5	**Data management:** Data management will involve creation of a database that will contain all field data, ecotoxicological data collected from two sites.
Activity 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).

ID	SERVICE AND DELIVERABLE	STATUS
2.1	Completion of field work at two sites, compile fieldwork database and transfer samples to laboratories for ecotoxicological assessment of two sites.	Completed
2.2	Interim report on ecotoxicolgical 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.	Completed
2.3	Complete physio-chemical analyses of surface water, pore-water and sediment samples. Access to analytical data on sediments form 2013 Spatial and Temporal Monitoring of Recovery in the Lower Lakes Project.	Completed
2.4	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.	Completed
2.5	Presented findings at the Ecotoxicology Project Advisory Group Meeting and refined research strategies and promoted research findings to stakeholders and scientific community	Completed
2.6	Submission of revised satisfactory final report following review through CSIRO E-publish system (DEWNR to be involved in review process).	Completed

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