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Highly treated mine waters may require major ion addition before environmental release

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HIGHLIGHTS

- A treated waste water product was assessed using ecotoxicology and TIE methods.
- Water quality of the distillate was markedly improved but residual toxicity remained.
- Major ion deficiency was identified as the primary cause of effects to *Hydra* sp.
- Residual metal was measured at concentrations that may have contributed to toxicity.
- Treated waters may have inadvertent environmental effects that need to be managed.

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ABSTRACT

Mining operations often use passive and/or active water treatments to improve water quality prior to environmental release. Key considerations in choosing a treatment process include the extent to which the water quality is actually improved, and the potential residual environmental risks of the release of such water. However, there are few published studies concerning the environmental impacts of treated waste waters. This study used toxicity identification evaluation (TIE) methods to quantify and identify the "toxic" constituents of a highly-treated water (distillate) produced by brine concentration of a mining process water. Exposure of five freshwater species (*Chlorella* sp., *Lemna aequinoctialis*, *Hydra viridissima*, *Moinodaphnia macleayi* and *Mogurnda mogurnda*) to a concentration range of the distillate (0, 25, 50 and 100%) found that it was toxic to *H. viridissima* (50–100% effect when exposed to 100% distillate). TIE tests demonstrated that the effect wasn't due to residual ammonia (~1 mg L^{-1} N) or trace organics, and unlikely to be due to manganese (Mn; 130–230 μg L^{-1}). Conversely, addition of 0.2 and 0.5 mg L^{-1} calcium improved the growth rate of *H. viridissima* by 61 and 66%, respectively, while addition of calcium, sodium and potassium (0.5, 1.0 and 0.4 mg L^{-1}, respectively) to levels comparable to that in the local aquatic environment resulted in 100% recovery. Further assessment on the likelihood of residual metal toxicity indicated that Mn concentrations in the distillate were at levels that could inhibit the growth of *H. viridissima*. Ultimately, the results demonstrated that ion deficiency should be considered as a potential stressor in risk/impact assessments of the discharge of treated wastewaters, and these may need to be supplemented with the deficient ions to reduce environmental impacts. The findings have highlighted the need for water managers to consider the possibility of unintended environmental risks from the discharge of highly-treated wastewaters.

1. Introduction

Depending on the resource being exploited, mining operations can produce gigalitres of saline, acidic, turbid and/or metal contaminated mine and process waters (Lottermoser, 2010). These waters represent a major economic and potential environmental liability and need to be managed appropriately (Mudd, 2007). Hence, there are numerous examples of mining operations that use passive and/or active water treatment methods to improve water quality prior to environmental release (Allen, 2008; Banks et al., 1997; Butler et al., 2011; Driussi and Jansz, 2006; Masarczyk et al., 1989). Some costly active treatment processes, such as reverse osmosis and distillation, are capable of producing high-purity waters that contain constituents that are near or below analytical detection limits and have very low electrical conductivity (EC) (Lottermoser, 2010). However, where such highly-treated waters are discharged to the environment, it should not be assumed that they will be environmentally benign. The potential for highly treated waters to impact the environment may still exist due to residual toxicity caused by toxicants that were not effectively removed by the process, and/or a lack of essential ions. For example, a reverse osmosis (RO) treated sewage water was determined to pose an unacceptable environmental risk,

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due to ion deficiencies, if the EC of the water was <120 μS cm^{-1} (Griffith and Biddulph, 2010). Consequently, waste water managers need to consider unintended risks such as those associated with ion deficiency.

Key considerations in choosing an appropriate treatment process include the intended end use of the treated water, the extent to which the treatment process improves water quality, and the residual risks of the use or release to the environment of such water. The residual (or acquired) toxicity of a treated water can be assessed using traditional ecotoxicological protocols, while toxicity identification evaluation (TIE) may be able to identify the toxic constituents of the water (Mirenda and Hall, 1992). Toxicity identification evaluations involve specific manipulations (e.g. pH adjustment, EDTA additions, sodium thiosulfate additions, and C18 solid phase extraction) of a whole effluent in order to change the amount and/or speciation/bioavailability of potential toxic constituents. The subsequent level of toxicity of the manipulated water relative to the unmanipulated water provides information on the likely toxic constituents. TIE methods have been commonly used to identify toxic constituents of treated sewage (Adams et al., 2008; Bailey et al., 2000) but, to our knowledge, they have never been used to assess the toxicity of a highly-treated mine water product.

The present study assessed, and identified the causes of, the residual/acquired toxicity of a distillate produced from brine concentration (also referred to as falling film evaporation) of process water sourced from the tailings storage facility at the Ranger uranium mine. The mine is surrounded by Kakadu National Park, which has a World Heritage listing for both its natural and cultural values, and Ramsar listing for its wetlands of international importance.

Brine concentration involves the cascading of heated process water down a falling film tube bundle, with the vaporised fraction then being compressed to heat the falling film tubes and finally condensed into a purified distillate product. The objectives of the study were to: (i) detect and quantify any residual toxicity of the distillate and, (ii) in the event effects were observed, to identify the toxic constituent(s) of the distillate using TIE methods.

2. Methods

2.1. General laboratory procedures

All plastics and glassware were washed by soaking in 5% (v/v) nitric acid for 24 h before being washed with a non-phosphate detergent (Gallay Clean A powder, Gallay Scientific, Burwood, Australia) in a laboratory dishwasher operated with reverse osmosis/deionised water (Elix, Millipore, Molshiem, France). All reagents used were of analytical grade and stock solutions were made up in high purity water (18 MΩ, Milli-Q Element, Millipore, Molshiem, France).

2.2. Test waters

The distillate was produced from Ranger uranium mine process water by a pilot-scale brine concentrator, which used a falling film evaporation process. Two separate batches of the distillate were collected from the brine concentrator for toxicity testing. The first batch was a 20 L composite sample collected from 11 to 17 July 2011, and was used for the initial screening toxicity tests involving three species (see Section 2.4). The second batch was a 20 L grab sample collected on 10 August 2011, and was used for the remainder of the toxicity and TIE tests. This sample was collected as a grab because the pilot plant project was due to be terminated. Both batch samples were collected in acid-washed high-density polyethylene containers and immediately air-freighted at 4 °C to the Environmental Research Institute of the Supervising Scientist laboratory.

On receipt of the samples, the distillate was immediately sub-sampled for physico-chemical analyses. Specifically, pH (SenTix41 probe, WTW, Weilheim, Germany), dissolved oxygen (DO; CellOx 325 probe, WTW), electrical conductivity (EC; TetraCon 325 probe, WTW) and dissolved organic carbon (DOC; TOC-V CSH, Shimadzu, Kyoto, Japan) were measured in-house. Additional sub-samples were analysed by external laboratories for alkalinity (APHA2320B), total and filtered (<0.45 μm) metals (inductively coupled plasma-mass spectrometry (ICP-MS) full scan), nitrate, phosphate, ammonia (colourimetric methods, EPA 353.2, EPA 365.1 and EPA 350.1), and volatile and semi-volatile organic compounds (gas chromatography–mass spectrometry (GC–MS) scan).

2.3. Test diluent

Natural Magela Creek water (MCW) obtained from Bowerbird Billabong (latitude 12° 46′ 15″, longitude 133° 02′ 20″) was used as the control treatment and for dilution of the distillate samples in all tests. The water was collected and transported to the laboratory in 20 L acid-washed plastic containers, and stored at 4 ± 1 °C prior to filtration through a 3.0 μm pore size filter (Sartopure PP2 depth filter MidiCaps, Sartorius, Göttingen, Germany). Filtration of the natural water was required to remove wild organisms that could confound the results of the toxicity tests, e.g. species that prey on the test subjects. Throughout the testing period, the MCW had a pH of 6.2–6.8 units, an EC of 15–20 μS cm^{-1} and DO of >90% saturation.

Diluent water was sub-sampled and analysed for the same physico-chemical parameters described for the distillate, and a more limited metal and major ion suite (i.e. totals only; Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na and SO_4 (analysed as S and converted); referred to as the standard metal and major ion suite hereafter).

2.4. Toxicity test species and methods

The toxicity of the distillate was assessed using five Australian tropical freshwater species: the unicellular green alga (*Chlorella* sp.); the duckweed (*Lemna aequinoctialis*); the green hydra (*Hydra viridissima*); the cladoceran (*Moinodaphnia macleayi*); and the Northern trout gudgeon (*Mogurnda mogurnda*). All the organisms were originally isolated from the soft surface waters in Kakadu National Park and have been cultured continuously at the Environmental Research Institute of the Supervising Scientist over many years (10–25 years depending on the species). The optimised and standardised protocols for the toxicity tests are described in full in Riethmuller et al. (2003; http://www.environment.gov.au/ssd/publications/ssr/173.html). Key details of each test are provided in Table 1. For the *L. aequinoctialis* and *Chlorella* sp. tests, nutrients (nitrate and phosphate) were added at the minimum concentrations that would sustain acceptable growth (see Table 1). The MCW used in the *Chlorella* sp. tests also had 1 mM HEPES buffer added to maintain a stable pH.

Initial toxicity screening of the first batch of the distillate was conducted with a limited range of dilutions of the distillate using three species which had previously displayed sensitivity to process water permeate produced by a microfiltration/reverse osmosis treatment process (van Dam et al., 2011). Specifically, *Chlorella* sp. (72-h cell division rate), *H. viridissima* (96-h population growth rate) and *M. macleayi* (3-brood reproduction) were exposed to the MCW control and three dilutions of the distillate in MCW (i.e. 0, 25, 50 and 100% distillate).

The toxicity of the second batch of the distillate was assessed using the same three species used for the first batch of the distillate (*Chlorella* sp., *H. viridissima* and *M. macleayi*), although only at 0 (MCW control) and 100% distillate concentrations, in order to assess the inter-batch reproducibility of the test methods. Further toxicity testing was also conducted on the second batch of the distillate with two different species, *L. aequinoctialis* (96-h growth rate) and *M. mogurnda* (96-h larval survival) using the same concentration range, 0, 25, 50 and 100% distillate.

Table 1
Details of toxicity tests for the five Australian tropical freshwater species used to assess the toxicity of a brine concentrator distillate. Full details of the methods are provided in Riethmuller et al. (2003).

Species (common name)	Test duration and endpoint	Control response acceptability criterion	Temperature, light intensity, photoperiod	Feeding/nutrition	No. replicates (individuals per replicate)	Test volume (mL)	Static/ daily renewals
Chlorella sp. (unicellular green alga)	72-h population growth rate	1.4 ± 0.3 Mean growth rate doublings day^{-1}; % CV[a] <20%	29 ± 1 °C 100–150 μmol m^{-2} s^{-1} 12:12 h	14.5 mg L^{-1} NO_3 0.14 mg L^{-1} PO_4	3 (3×10^4 cells mL^{-1})	50	Static
Lemna aequinoctialis (tropical duckweed)	96-h growth rate	Mean surface area growth rate (k, mm^2 day^{-1}) ≥0.40; % CV <20%	29 ± 1 °C 100–150 μmol m^{-2} s^{-1} 12:12 h	3 mg L^{-1} NO_3 0.3 mg L^{-1} PO_4	3 (4 plants with 3 fronds)	100	Static
Hydra viridissima (green hydra)	72-h population growth rate	Mean population growth rate (k, day^{-1}) ≥0.27; % CV <20%	27 ± 1 °C 30–100 μmol m^{-2} s^{-1} 12:12 h	3–4 *Artemia* nauplii day^{-1}	3 (10)	30	Daily renewals
Moinodaphnia macleayi (cladoceran)	3 brood (120–144 h) reproduction	Mean adult survival ≥80%; mean neonates per adult ≥30	27 ± 1 °C 30–100 μmol m^{-2} s^{-1} 12:12 h	30 μl FFV[b] and 6×10^6 cells of *Chlorella* sp. d^{-1}	10 (1)	30	Daily renewals
Mogurnda mogurnda (Northern trout gudgeon)	96-h survival	Mean larval survival ≥80%; % CV <20%	27 ± 1 °C 30–100 μmol m^{-2} s^{-1} 12:12 h	Nil	3 (10)	30	Daily renewals

[a] % CV: percent co-efficient of variation.
[b] FFV: fermented food with vitamins is an organic and bacterial suspension prepared by a method described in Riethmuller et al. (2003).

2.5. Toxicity identification evaluation (TIE) tests

In order to identify the toxic constituents of the distillate, a limited range of TIE toxicity tests were conducted using, *H. viridissima*. While the addition of major ions is not a standard component of the TIE methods (Mirenda and Hall, 1992), it needed to be done for our work to specifically identify if the adverse effects were due to a lack of essential ions in the high purity distillate.

All six TIE tests used the standard *H. viridissima* protocol described in Section 2.4, modified as described below (Table 2). All TIE tests included a control water (MCW or synthetic soft water, SSW) and the distillate that were treated as described below, as well as the untreated control water (MCW and/or SSW) and the distillate.

2.5.1. Effect of pH

The pH of the distillate and MCW was decreased from 6.5 to pH 5.5 using 1 M hydrochloric acid (HCl), and increased to pH 8.0 using 1 M sodium hydroxide (NaOH). For the duration of the toxicity test, pH adjustments were made each day before renewal of the test solutions (see Table S1 for pH measurements and adjustments).

Sub-samples of the treatments were analysed for a standard metal and major ion suite (Table S2). In order to confirm that pH manipulation of the controls and the distillate resulted in concentrations of the added anions, the controls (pH 6.5) and HCl treated solutions (pH 5.5) were also analysed for Cl^- (Table S2), while the controls (pH 6.5) and NaOH treated solutions (pH 8.0) were analysed for alkalinity (Table S3).

2.5.2. Effect of EDTA addition

Aliquots of an EDTA stock solution were added to both MCW and the distillate to produce working concentrations of 0, 2.8, 5.5 and 11 mg L^{-1} EDTA (BDH, Kilsyth, NSW, Australia). The highest concentration of 11 mg L^{-1} EDTA was based on a 1:1 molar ratio with the sum of the concentrations of the major divalent cations and a Mn concentration of 130 μg L^{-1}, which was the concentration measured in the first batch of the distillate. Sub-samples of the treatments were analysed for the "bioavailable" Mn fraction using the Chelex® assay (Greenberg and Kingston, 1983; Tables 6 and S4).

2.5.3. Solid phase extraction (SPE) with carbon 18 (C18)-based resin

Both MCW and the distillate were subjected to SPE by drawing 1 L through 1000 mg of C18 resin (Restek, Bellefonte, PA, USA) using a vacuum pump at a flow rate of ~5 mL min^{-1}. The fraction collected after passing through the column was designated "filtrate". The column was kept wet and the fraction retained on the C18 resin was eluted using 3 mL of pure methanol (BDH, Kilsyth, NSW, Australia). The eluate was added to MCW and tested for toxicity. A control of MCW with methanol was also included.

A sub-sample of the distillate filtrate was analysed for volatile and semi-volatile organics via a GC/MS scan (Table S5). The GC/MS scanning method used in this project is not a definitive method of organic compound identification. The retention peaks and mass spectra of the detected compounds are matched to a library and estimates of concentrations are inferred from the closest internal standard.

2.5.4. Ammonia stripping

The day prior to the TIE test, 1 L of both the MCW and the distillate were increased to pH 11 using 10 M NaOH. The pH was checked and re-adjusted throughout the day to counter any pH drift. The samples were covered and aerated overnight with air delivered by an aquarium pump aerator at room temperature. The pH of the MCW and the distillate were readjusted to pH 6.5 before the initiation of the TIE test.

Table 2
Toxicity identification evaluation toxicity tests using *Hydra viridissima*.

TIE test	Test solution manipulation	Reason for manipulation
Graduated pH	MCW and distillate adjusted to pHs (nominal) 5.5 and 7.5	Differentially alters speciation and toxicity of chemicals
EDTA[a] addition	0, 2.8, 5.5 and 11.0 mg L^{-1} EDTA added to MCW and distillate	EDTA binding reduces cationic metal bioavailability and toxicity
Ammonia stripping	MCW and distillate adjusted to pH (nominal) 11 and aerated for 18 h. pH re-adjusted to 6.5 prior to testing.	Removes toxicity due to ammonia
C18 solid phase extraction (SPE)	MCW and distillate post-C18 column water tested. Eluate of distillate tested in MCW	Tests for toxicity of organic compounds
Calcium addition	0, 0.2, 0.5 mg L^{-1} calcium concentrations tested in synthetic soft water (SSW) and distillate	Reintroduction of an essential element
Major ion addition	0, 50 and 100% proportions (compared to SSW[b]) of sodium, calcium and potassium added to SSW and distillate	Reintroduction of essential elements

[a] Ethylenediamine tetraacetic acid.
[b] Synthetic soft water contains 0.5, 1.0 and 0.4 mg L^{-1} of calcium, sodium and potassium, respectively.

Ammonia content was assessed using a colourimetric NH_3 test kit (Merck, Darmstadt, Germany). Sub-samples of the treatments were analysed for the standard metal and major ion suite (Table S6).

2.5.5. Major ion modifications

In the first major ion modification TIE, SSW and the distillate were prepared with nominal concentrations of 0.0, 0.2 and 0.5 mg L^{-1} Ca, which are equivalent to 0, 50 and 100% of the Ca concentration present in the standard SSW. An untreated MCW control was included as a QA/QC control because *H. viridissima* growth rates are known to be slightly lower in SSW.

In a second major ion modification TIE, SSW and the distillate were prepared with nominal concentrations of 0.0, 0.2 and 0.5 mg L^{-1} Ca, 0.0, 0.5 and 1.0 mg L^{-1} Na and 0.0, 0.2 and 0.4 mg L^{-1} K, which are equivalent to 0, 50 and 100% of the standard SSW concentrations. An untreated MCW control was included as a QA/QC control for reasons described above. Magnesium was present in the distillate at environmentally relevant concentrations (0.6 mg L^{-1}) so it was not necessary to add this for major ion TIE assessment.

Sub-samples of the treatments were analysed for the standard metal and major ion suite (Tables S7 and S8).

2.5.6. Effect of Mn in low major ion waters

Although not strictly a TIE test, the effects of Mn in SSW with very low major ion concentrations were assessed as an additional line of evidence, to determine the likelihood that Mn was contributing to the toxicity of the distillate. Three types of SSW were prepared with Ca, Na and K at concentrations equivalent to 0, 50 and 100% of the standard SSW concentrations (as described above). These waters were then spiked with manganese sulfate to measured concentrations of <17, 130 and 230 μg L^{-1} Mn to produce 9 treatments (3 Mn concentrations × 3 major ion concentrations).

Sub-samples of the treatments were analysed for the standard metal and major ion suite. Additionally, dissolved (0.1 μm filtered) and total Mn concentrations were measured in all treatments (Table S9).

2.6. Quality control

2.6.1. Chemistry

Chemical analyses were conducted on all TIEs and toxicity tests where metals and major ions were added to test solutions in order to confirm the accuracy of the added concentrations. All concentrations reported are measured concentrations. For each test, blanks and procedural blanks (i.e. ultra-pure water that has been exposed to all components of the test system) were analysed for the standard metal and major ion suite. Chemistry data for the blanks and procedural blanks were initially assessed by searching for analyte concentrations higher than the detection limits. Where these concentrations were greater than 1 μg L^{-1} and above background levels of the control water (Table S10), duplicate procedural blank samples were re-analysed and/or the control water concentrations were compared to those in tests without a blank contamination, to determine if the contamination was limited to the one sample bottle or experienced throughout the test. The likelihood that contamination may have confounded the toxicity test results was investigated on a case-by-case basis, in the event that any elevated concentrations were detected in the blanks.

2.6.2. General water quality

For each test, data were considered acceptable if: the recorded temperature of the incubator remained within the prescribed limits (see Table 1); the recorded pH was within ±1 unit of values at test commencement; the EC for each test solution was within 10% (or 5 μS cm^{-1} for samples with low conductivity) of the values at test commencement; and the DO concentration was greater than 70% throughout the test. The occurrence of any significant water quality changes outside of these prescribed ranges was investigated on a case-by-case basis.

2.6.3. Control responses

Tests were considered valid if the organisms in the QC treatment (i.e. those in the MCW or SSW control) met the criteria listed in Table 1.

2.7. Statistics

For the toxicity tests, linear interpolation or non-linear regression (2-parameter log-logistic) analysis was used to determine inhibitory concentrations (ICs) that reduced endpoint responses by 10% and 50% (i.e. IC10 and IC50) relative to the control responses. Because the *M. mogurnda* test represents an acute exposure and measures lethality, a more conservative 5% effect/lethal concentration was estimated instead of a 10% effect/lethal concentration. All statistical analyses for the full dilution toxicity tests were undertaken using CETIS™ (V 5.0.23, TidePool Scientific).

For the two major ion addition TIEs and the Mn toxicity test, two-way analysis of variance (ANOVA) and Tukey's *post-hoc* tests ($\alpha = 0.05$) were performed using water type and major ion or Mn concentration as the two factors. Prior to ANOVA, the assumptions of normality and homoscedasticity were tested (SigmaPlot 11.0, Systat software, Germany). Non-compliance with normality was not considered to be consequential to the analyses because sample sizes were the same across groups and the datasets had equal variances (Zar, 1984). For the remaining TIEs, ANOVA results did not prove informative and are not reported. Rather, as recommended by the USEPA protocols (Norberg-King et al., 1991), judgements on significance of the improvements or reductions in the *H. viridissima* population growth rate were made based on observed effects and experience and knowledge of the speciation of the key chemicals of concern.

3. Results

3.1. Quality control

All TIE and toxicity tests met the criteria for control performance except for the first *M. macleayi* test, which produced an average of 25 neonates per adult. However, the data from this test were accepted because the number of neonates produced was only marginally below the acceptability criterion of 30 neonates per adult, as well as the reproductive output of the second cladoceran test (32 neonates per adult) and all adults survived and displayed no signs of poor health.

Chemical analyses of the blank and procedural blank samples showed that all tests were free from confounding metal contaminants (Table S10). Hence, all tests reported here were of acceptable quality. Chemical analyses of manipulated TIE waters showed that the same amounts of prescribed modifying chemicals (e.g. acids and bases) were added to both the controls and the distillate, and hence, were not a confounding factor in the TIEs. Nominal concentrations of spiked metals and major ions were within 10% of measured concentrations (Tables S3–S10). However, there were two notable exceptions, where Na concentrations were higher in the 0% SSW control of both the major ion addition TIE and the Mn toxicity test (see Sections 3.4.5 and 3.4.6).

3.2. Distillate chemistry

The distillation of the process water reduced all major ions, ammonia and trace metals to near detection limits (Table 3). Trace amounts of ammonia were measured at 0.7–0.8 mg L^{-1} NH_3–N, while Mn and U were detected at 130–230 and 1.1–1.5 μg L^{-1}, respectively. All major cations were reduced to near or below detection limits, except for Mg, which was measured at 0.4–0.6 mg L^{-1}. Some

Table 3
Composition of the process water pre- and post-treatment with the brine concentrator.

Analyte	Detection limit	Process water (feed)[a]	First distillate batch	Second distillate batch
pH	0.1	4.1–4.5	5.8	6.7
Electrical conductivity (μS cm^{-1})	1.0	20,900–29,700	17	12
DOC (mg L^{-1})	0.1	<1.0–6.0	0.6	NM[c]
Calcium (mg L^{-1})	0.1	300–341	0.1	<0.1
Magnesium (mg L^{-1})	0.1	3607–4123	0.6	0.4
Sodium (mg L^{-1})	0.1	73–107	<0.1	<0.1
Potassium (mg L^{-1})	0.1	67–115	<0.1	<0.1
Bicarbonate (mg L^{-1} $CaCO_3$)	1.0	<1.0	7.0	6.0
Ammonia (mg L^{-1} N)	5.0×10^{-3}	550–756	0.7	0.8
Manganese (mg L^{-1})	1.0×10^{-4}	1367–1551	0.23	0.13
Uranium (μg L^{-1})	1.0×10^{-3}	9600–25 300	1.1	1.5
Decane (μg L^{-1})	1.0	Not detected	NM[c]	2.0[d]
Phenol, 3,5-bis (1,1-dimethylethyl) (μg L^{-1})[b]	1.0	Not detected	NM[c]	5.8[d]
Phenol, 2,4-bis (1,1-dimethylethyl) (μg L^{-1})[b]	1.0	Not detected	NM[c]	12.0[d]
1,2-Benzenedicarboxylic acid, butyl (μg L^{-1})[b]	1.0	Not detected	NM[c]	9.9[d]

[a] Value ranges based on numerous composite samples of the feed taken from 10 July to 9 August 2011 (data provided by Energy Resources of Australia Ltd).
[b] Known to leach from plastics.
[c] NM: not measured.
[d] Not a definitive measurement. Concentration was estimated from the closest internal standard.

organic compounds that were not detected in the feed water were detected at low μg L^{-1} concentrations in the distillate (Table 3).

3.3. Toxicity test results

The toxicity test results showed that the distillate was of low toxicity to four of the five organisms tested (Table 4; Fig. 1). However, the population growth rate of *H. viridissima* was reduced by ~50% following exposure to an undiluted (100%) sample of the first batch of the distillate (Fig. 1). The second batch of the distillate was found to be higher in toxicity to *H. viridissima*, with a full toxic effect observed following exposure to the 100% distillate (Table 4). In contrast, the second batch of the distillate appeared to be of lower toxicity to *M. macleayi* and *Chlorella* sp. A toxicity estimate for *M. mogurnda* could not be calculated due to limitations of the dataset (a low number of treatments and variation within treatments). This resulted in concentration–response models not being able to be used to fit the dataset.

3.4. Toxicity identification evaluation (TIE) results

3.4.1. Graduated pH

The pH of the modified MCW and the distillate was relatively stable at pH 5.5 compared to pH 7.5 and required less adjustment during the test (Table S1). The growth rate of *H. viridissima* at pH 5.5 appeared to be no different to that in the unmodified distillate, whereas at pH 7.5, growth rate was markedly higher than the unmodified distillate (Table 5). The pH adjustment of the MCW and the distillate did not introduce confounding metals or major ions but, interestingly, increasing the pH to 7.5 appeared to reduce the concentration of Mn by 32% and 40% for the unfiltered and 0.45 μm filtered fractions, respectively (Table S3).

3.4.2. EDTA addition

Addition of 5.5 and 11 mg L^{-1} EDTA to the MCW control and the distillate resulted in no *H. viridissima* growth in either water type. The growth rate of *H. viridissima* in MCW with 2.8 mg L^{-1} EDTA was 55% of the 0 mg L^{-1} EDTA control, but there was no growth of *H. viridissima* in the distillate with 2.8 mg L^{-1} EDTA (Table 5). Performance of the Chelex® assay on sub-samples of the EDTA spiked test solutions indicated that the Mn was effectively completely complexed with the EDTA and, thus, did not bind to the Chelex®-100 resin (Table 6).

3.4.3. Ammonia stripping

Treating the distillate by increasing the pH to >9 and aerating for ~23 h effectively reduced the ammonia concentration to below detection limits (Table 6). However, *H. viridissima* still did not grow in the ammonia-stripped distillate, whilst the growth rate in the control treated in the same manner was approximately half that of that in the untreated MCW control (Table 5).

3.4.4. Solid phase extraction (SPE) with carbon 18 (C18)

Gas chromatography–mass spectrometry scans identified different volatile organic compounds (VOCs) and semi-volatile organic compounds (sVOCs) in both the distillate and filtrate but all concentrations were in the low μg L^{-1} range (Tables 6 and S6). The growth rates of *H. viridissima* exposed to the filtrates from the control and the distillate C18-SPE treatments were no different from the untreated control and the distillate. Moreover, addition of the eluate from the distillate SPE treatment to MCW did not increase the toxicity of the water.

3.4.5. Major ion modifications

The addition of 0.2 and 0.5 mg L^{-1} Ca to the distillate resulted in significant ($P<0.001$) 61% and 66% growth rate recoveries of *H. viridissima* relative to the SSW control, respectively. Chemical analysis of the test solutions showed that the Ca concentrations in the corresponding SSW and the distillate treatments matched accurately (Table 6). The addition of

Table 4
Toxicity of the pilot brine concentrator distillate.

Species	Endpoint	IC_{10} or IC_5[a] (95% confidence limits)	Percentage effect relative to the control (±CV %[b]) following exposure to 100% distillate	
			1st batch	2nd batch
Chlorella sp. (unicellular alga)	72-h cell division rate	79 (N.C.)[c]	11 ± 2	0 ± 0
Lemna aequinoctialis (duckweed)	96-h growth rate	>100 (N.C.)[d]	N.T.[e]	0 ± 0
Hydra viridissima (green hydra)	96-h population growth rate	30 (N.C. — 77)	53 ± 5	100 ± 0
Moinodaphnia macleayi (cladoceran)	3 brood (6 day) reproduction	72 (50–100)	13 ± 6	6 ± 13
Mogurnda mogurnda (fish)	96-h survival	N.C.[d]	N.T.	7 ± 25

[a] Inhibitory concentrations (IC) are expressed as percentage of distillate that causes a 10% effect (IC_{10}) or, in the case of *M. mogurnda*, a 5% effect (IC_5).
[b] Percent coefficient of variation.
[c] N.C. = not calculable.
[d] Derived from test conducted on the 2nd batch of distillate.
[e] N.T. = not tested.

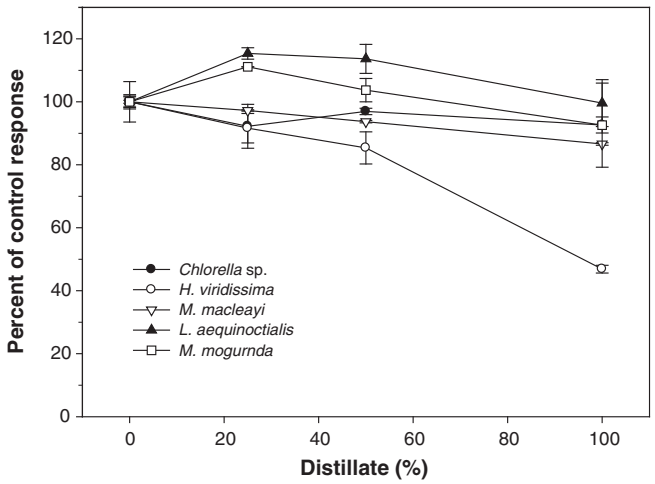

Fig. 1. Concentration–response plots for the toxicity of the pilot brine concentrator distillate to five freshwater species (distillate batch 1 for *Chlorella* sp., *Hydra viridissima* and *Moinodaphnia macleayi*; distillate batch 2 for *Lemna aequinoctialis* and *Morgurnda mogurnda*). Magela Creek Water control responses were; *Chlorella* sp. = 1.7 ± 0.3 doubling day^{-1}; *H. viridissima* = 0.35 ± 0.01 day^{-1}; *M. macleayi* = 25 ± 0.5 neonates $adult^{-1}$; *L. aequinoctialis* = 0.35 ± 0.01 day^{-1}; *M. mogurnda* = 90 ± 5% survival.

major cations (Ca, Na and K) at concentrations of 50 and 100% of those present in SSW showed significant ($P<0.001$) 100% and 96% growth rate recoveries relative to the SSW control, respectively (Table 5). Furthermore, there was no statistical difference ($P=0.293$) between the SSW and the distillate water types if the major ions were at the same concentrations in the SSW and the distillate.

3.4.6. Effect of Mn in low major ion waters

Manganese reduced the growth rate of *H. viridissima* relative to the relevant SSW type control in all SSW types. The effect was most noticeable in the SSW with half the standard Na, K and Ca concentrations, where the growth rates were reduced by 9 and 20% in the 130 and 250 μg L^{-1} Mn treatments, respectively (Fig. 2). The growth rates of *H. viridissima* in the 250 μg L^{-1} Mn treatments were significantly lower than the controls, although there was no interaction between major ion concentration and Mn toxicity ($P=0.76$). However, it should be noted that the measured concentrations of Na in the no Ca, K and Na group were higher than expected and similar to the half concentrations of Ca, K and Na group (Table S9).

4. Discussion

The undiluted distillate was generally of low toxicity across the range of species tested, but caused a 50–100% reduction in the population growth rate of *H. viridissima*. In many contexts, treated waters displaying such limited toxicity to most species tested would not require further scrutiny and may be deemed acceptable for discharge to the environment. However, where the protection of very high-value environments is required, such as is the case in the present study, a more stringent level of assessment and management is needed. In particular, specific information about the cause(s) of toxicity will provide additional understanding and enable better targeting of management options to ensure environmental protection.

Changing the pH of an effluent can change the speciation of toxicants, which subsequently can change their bioavailability and toxicity. For example, decreasing the pH can increase the proportion of toxic free metal ions, while increasing the pH can increase the proportion of toxic ammonia in the solution. Increasing the pH of the distillate to 7.5 decreased its toxicity and improved the growth of the *H. viridissima* relative to the pH 6.5 control. This indicated that the toxicity was not due to ammonia, as the higher pH would have resulted in greater toxicity due to a higher proportion of the toxic unionised ammonia (NH_3) ions (Table 5). The improved *H. viridissima* growth rate at pH 7.5 may have been due to one or more of several reasons, including: (i) the addition of essential sodium ions in the form of sodium hydroxide, which was used to increase the pH; (ii) an improved physiological/metabolic function of the *H. viridissima* at pH 7.5; and/or (iii) reduced toxicity of Mn (or metals in general) due to a reduction in the proportion of bioavailable free Mn ions compared to the lower pHs of 5.5 and 6.5. Adding weight to the third reason was that dissolved Mn concentrations

Table 5
Results of toxicity identification evaluation toxicity tests using *Hydra viridissima*.

TIE test	Treatment[a]	Control growth rate (mean day^{-1} ± SE)	Distillate growth rate (mean day^{-1} ± SE)	Distillate compared to control (mean % ± SE)
TIEs with Magela Creek Water as the control water				
Graduated pH	pH unadjusted (~6.5)	0.33 ± 0.00	0.04 ± 0.02	11 ± 6
	Daily pH adjusted to ~5.5	0.34 ± 0.02	0.00 ± 0.00	0.0
	Daily pH adjusted to ~7.5	0.34 ± 0.01	0.16 ± 0.03	50 ± 1
EDTA addition	0 mg L−1 EDTA added	0.31 ± 0.02	0.04 ± 0.04	12 ± 12
	2.8 mg L^{-1} EDTA added	0.17 ± 0.01	0.00 ± 0.00	0.0
	5.5 mg L^{-1} EDTA added	0.00 ± 0.00	0.00 ± 0.00	0.0
	11 mg L^{-1} EDTA added	0.00 ± 0.00	0.00 ± 0.00	0.0
Ammonia stripping	Unadjusted	0.32 ± 0.02	0.00 ± 0.00	0.0
	pH increased and aerated "NH_3 stripped"	0.16 ± 0.01	0.00 ± 0.00	0.0
C18 SPE[b]	Untreated	0.28 ± 0.01	0.14 ± 0.02	50 ± 6
	Filtrate "Organic stripped"	0.29 ± 0.01	0.16 ± 0.00	59 ± 1
	Eluate added to MCW	0.27 ± 0.01	N.A.[c]	N.A.
	MCW with ethanol	0.27 ± 0.00	N.A.[c]	94 ± 3[d]
TIEs with synthetic soft water as the control water[e]				
Calcium modification	0.0 mg L^{-1} Ca	0.14 ± 0.00	0.00 ± 0.00	0.0
	0.2 mg L^{-1} Ca	0.27 ± 0.01	0.16 ± 0.00	61 ± 2
	0.5 mg L^{-1} Ca	0.28 ± 0.00	0.18 ± 0.01	66 ± 5
Major ion modification	0.0 mg L^{-1} Ca, Na and K	0.04 ± 0.06	0.13 ± 0.01	353 ± 23[f]
	0.2, 0.5 and 0.2 mg L^{-1} Ca, Na and K	0.29 ± 0.02	0.29 ± 0.01	100 ± 4
	0.5, 1.0 and 0.4 mg L^{-1} Ca, Na and K	0.34 ± 0.01	0.32 ± 0.02	96 ± 5

[a] Control treatments were either Magela Creek Water or synthetic soft water that were treated the same as the distillate.
[b] Solid phase extraction.
[c] Not applicable because the eluate is transferred into MCW and there is no treated distillate equivalent.
[d] Result shows the growth rate of the MCW with eluate group compared to the MCW with the ethanol group.
[e] Standard synthetic soft water contains 0.5, 1.0 and 0.4 mg L-1 Ca, Na and K.
[f] Growth rate in the distillate was three times higher compared to SSW with no major ions, but was still significantly lower than the distillate with major ions added.

Table 6
Key analytes or physico-chemical measurements from toxicity identification evaluations.[a]

TIE test	Treatment[a]	Control water key analyte(s)	Distillate key analyte(s)
		pH range	
Graduated pH	pH unadjusted (~6.5)	6.2–6.4	6.1–6.5
	Daily pH adjusted to ~5.5	5.3–5.8	5.4–6.2
	Daily pH adjusted to ~7.5	6.9–8.1	7.1–8.5
		Free manganese (Mn, μg L^{-1})[b]	
EDTA addition	0 mg L^{-1} EDTA added	1.0	110
	2.8 mg L^{-1} EDTA added	<0.1	<0.1
	5.5 mg L^{-1} EDTA added	<0.1	<0.1
	11 mg L^{-1} EDTA added	<0.1	<0.1
		Ammonia (mg L^{-1} N)	
Ammonia stripping	Unadjusted	<LOQ[c]	1.0
	pH increased and aerated "NH_3 stripped"	<LOQ	<0.1
		Volatile or semi-volatile organic compounds (μg L^{-1})[d]	
C18 SPE	Untreated	N.A.	<LOQ[e]
	Filtrate "organic stripped"	N.A.	1.0 and 1.4[f]
		Calcium (mg L^{-1})	
Calcium modification	0.0 mg L^{-1} Ca	<0.1	<0.1
	0.2 mg L^{-1} Ca	0.2	0.2
	0.5 mg L^{-1} Ca	0.4	0.4
		Ca, Na and K (mg L^{-1}), respectively	
Major ion modification	0.0 mg L^{-1} Ca, Na and K	<0.1, 0.6, <0.1	<0.1, <0.1, 0.1
	0.2, 0.5 and 0.2 mg L^{-1} Ca, Na and K	0.2, 0.7, 0.2	0.2, 0.5, 0.3
	0.5, 1.0 and 0.4 mg L^{-1} Ca, Na and K	0.4, 0.9, 0.4	0.4,1.0, 0.5

[a] See Supplementary data for measurements of a larger suite of elements.
[b] Measured by Chelex® assay.
[c] Limit of quantification.
[d] Concentrations estimated by GC–MS scan.
[e] No peaks detected.
[f] 2-Furancarboxaldehyde, 5-methyl- and 2,5-heptadien-4-one, 2,6-dimethyl-, respectively.

decreased by 40% in the pH 7.5 distillate. It is difficult to determine the cause of this loss because the speciation of Mn in natural waters is complicated (Hart et al., 1992; Laxen et al., 1984). However, it may be due to the formation of colloidal Mn at the higher pH, resulting in the precipitation of this metal.

Unionised ammonia (NH_3) is volatile compared to the ionised ammonium ion (NH_4^+). The proportion of ammonia ions increases with increasing pH. Consequently, raising the pH of the distillate combined with vigorous aeration effectively removed the ammonia from the distillate. Removal of ammonia from the distillate confirmed the results from the graduated pH TIE. Specifically, the distillate's toxicity was unchanged following the treatment, which indicated that ammonia was not the primary toxicant (Table 5).

Ethylene diamine tetraacetic acid (EDTA) is a strong chelator of divalent cations such as Mn, which was the key elevated metal in the distillate. Hence, the addition of EDTA may reduce the bioavailability and toxicity of these cations. In contrast to the findings from the graduated pH TIE test, the addition of EDTA to the distillate indicated that toxicity due to Mn was unlikely. Only the lowest concentration of EDTA (2.8 mg L^{-1}) provided informative results, as the higher EDTA concentrations resulted in no growth of the *H. viridissima* in MCW (Table 5). The lack of growth at 5.5 and 11 mg L^{-1} EDTA was probably due to the binding of essential cations, such as Ca^{2+}, due to excess EDTA being added to the distillate and MCW. The calculation of the amount of EDTA needed for complete binding of all the Mn was based on the first batch of the distillate, which contained 230 μg L^{-1} Mn, almost double the concentration in the second batch (i.e. 130 μg L^{-1} Mn). Nevertheless, the addition of 2.8 mg L^{-1} EDTA resulted in a *H. viridissima* population growth of 0.17 day^{-1} in the MCW control, but no growth in the corresponding distillate treatment. Thus, if the toxic effect of the distillate was due to Mn then it would have been expected that the growth in the 2.8 mg L^{-1} EDTA treated distillate would be similar to the MCW control. Furthermore, the results from the Chelex® assay also supported the conclusion that Mn was not the toxic component (Table 6). The fraction that was able to bind to the Chelex®-100 resin was effectively reduced to below detection limits, but the toxicity of the distillate was not reduced. This indicated that Mn was not present at a toxic concentration in the second batch, which had a concentration of 130 μg L^{-1}.

Although the total organic content of the distillate was low (~1 mg L TOC), a GC–MS scan of the first batch indicated that VOCs and sVOCs might be present. However, in this context, it is important to note that the sub-sampling of the distillate for organic compounds was not ideal, in that plastic was used instead of glass, and some of the detected compounds are known to leach from plastics. Nevertheless, the decane that was measured at 2 μg L^{-1} may have been misidentified nonane, because they are aliphatic hydrocarbons with 10 and 9 carbons, respectively. Nonane is a major component of the industrial solvent, Shellsol, which was identified as an organic chemical of relevance due to its use in the U extraction process and occasional presence in process water. No VOCs and sVOCs were detected in the batch of the distillate used for

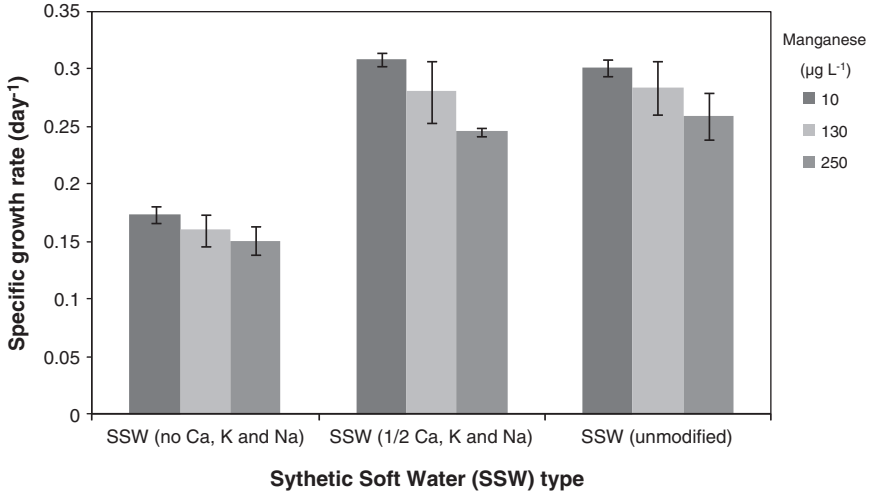

Fig. 2. Effect of manganese on *Hydra viridissima* in modified synthetic soft waters (SSW). Data represent the mean ± se ($n = 3$).

the TIE studies. Two organics that matched 2-furancarboxaldehyde, 5-methyl- and 2,5-heptadien-4-one, 2,6-dimethyl were detected at very low concentrations <1.5 μg L^{-1} in the distillate that has passed through the C18-SPE column (filtrate). The source of these compounds was unclear but they may have been acquired during the SPE treatment. However, their presence in the filtrate did not change the toxicity of the water. Furthermore, no toxicity was observed in the MCW containing the eluate (Table 5). Hence, all results suggested that the toxicity of the distillate was not due to the presence of trace amounts of the organic compounds.

In light of the above negative findings, the issue of major ion deficiency was specifically investigated as a potential cause of the effect on *H. viridissima*. Firstly, Ca addition was investigated given its importance for nematocyst function and other physiological processes in *Hydra* (Gitter et al., 1994; Kawaii et al., 1999). It is also a well-known ameliorator of metal and major ion toxicity (Markich and Jeffree, 1994; van Dam et al., 2010). Hence, the very low concentration of Ca in the distillate (Table 3) was initially targeted as a cause of the adverse effects of the distillate. The addition of 0.2 and 0.5 mg L^{-1} Ca to the distillate resulted in a significant recovery, suggesting Ca deficiency as a major reason for the effect of the distillate on *H. viridissima*. However, it was also noted that the distillate contained concentrations of Na and K that were below the detection limits (Table 3). Thus, it was hypothesised that the addition of the Ca, Na and K up to concentrations that were consistent with those in MCW would improve the condition of the distillate for *H. viridissima*. Addition of these three cations to the distillate resulted in a full recovery of *H. viridissima* and there was no significant difference between the SSW and the distillate water types if the major ions were at the same concentrations. This strongly indicated that the majority of the adverse effect from the distillate on *H. viridissima* was due to major ion deficiency rather than a chemical toxicity. Interestingly, a similar finding has been previously reported for RO-treated sewage water that was produced by a pilot-scale plant and had a low EC of 20–40 μS cm^{-1} (Griffith and Biddulph, 2010). They reported that the cladoceran, *Ceriodaphnia dubia*, and the fish, *Macquaria novemaculeata*, required an EC of 120 μS cm^{-1} for normal reproduction and growth, respectively. However, a noteworthy observation from the present study is that the composition of specific ions that contribute to the EC of the water may be important in the effects exerted on an organism. Specifically, adding just Ca to the distillate resulted in a substantial (66%) recovery of *H. viridissima* but full recovery was not observed unless Na and K were also added.

Despite the substantive removal of adverse effect by the replacement of major cations, the concentrations of Mn in the distillate (130–230 μg L^{-1}) remained a concern as they were higher than the IC_{10} of 70 μg L^{-1} previously reported for *H. viridissima* in circumneutral pH (6.0–7.0) soft waters (Harford et al., 2009). Additionally, the lack of major ions in the distillate had the potential to exacerbate Mn toxicity (Peters et al., 2011). Therefore, the effects of Mn in the presence of reduced concentrations of major ions were examined using modified SSW (i.e. with 0, 50 and 100% of the major ion concentrations in unmodified SSW). Manganese at 250 μg L^{-1} significantly reduced the growth rate of *H. viridissima* relative to the relevant SSW type control. The differences in major cation concentration of the modified SSWs did not affect Mn toxicity. This is likely due to the fact that the concentrations of the major cations were too low, even in the highest cation concentration treatment, to enable significant competition for binding sites with Mn, and subsequent amelioration of Mn toxicity. Thus, despite the recognised issue with deficiencies of major ions in the distillate, a specific toxic response to Mn was identified in a water type with physico-chemical characteristics similar to those of the distillate. It is noteworthy that a recent draft document has recommended an environmental quality standard for Mn in the European Union of 62–123 μg L^{-1} (Peters et al., 2010). Consequently, it is clear that further investigation of Mn toxicity is warranted to better understand the potential for toxicity under various physico-chemical conditions.

5. Conclusions

In this work the application of a staged TIE procedure has shown that a lack of major ions was the primary factor causing the reduced growth rate of *H. viridissima* exposed to a brine concentrator distillate. Additional focussed toxicity tests also indicated that the highest concentrations of Mn (>200 μg L^{-1}) that were measured in the distillate may have contributed.

There is a surprising paucity of published literature concerning the potential impact of treated waters on ecosystems, despite some industries producing gigalitres of highly-treated waters (e.g. the coal seam gas industry; RPS, 2011), of which a significant volume will require discharge into the environment. The present study is a clear example of a mine water treatment process producing a product that was too low in major ion concentrations to be able to sustain certain freshwater species. We have found only one other published study (RO-treated sewage water) that has previously reported a similar result (Griffith and Biddulph, 2010). Thus, ion deficiency needs to be considered as a potential stressor in risk/impact assessments of the discharge of treated waste waters.

A TIE approach provides a practical tool for assessing this effect and can inform water managers and treatment engineers on the appropriate post-treatment that may be required to limit impacts. In this context our personal experience has been that personnel working in water management typically consider highly-treated waters to be of sufficient quality for direct discharge into natural surface waters. Whilst this may be the case in some circumstances, additional or alternative management actions may be required where the environmental risk of such direct discharges has been assessed, through chemical analyses and toxicity tests, to be unacceptably high. Management actions to reduce risks of ion deficiency could include the supplementation of the treated waters with deficient ions (in the case of the distillate assessed in the present study, Ca, Na and K) prior to their discharge to the off-site aquatic environment. This could be achieved actively by direct addition of relevant salts, or passively by passing the distillate through a wetland system/watercourse prior to discharge. However, while the conditioning of the distillate through a wetland/watercourse is likely to improve water quality (by increasing major ion concentrations and, potentially, reducing dissolved metal concentrations), the risk of exhausting the system's capacity to sustainably contribute the required loading of salt may need to be considered if large volumes are to be flushed through the system.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.10.054.

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