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Humic substances increase survivorship of the freshwater shrimp Caridina sp. D to acid mine drainage.

--Manuscript Draft--

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Abstract:	Humic substances (HS) are known to decrease the toxicity of heavy metals to aquatic organisms, and it has been suggested that they can provide buffering protection in low pH conditions. Despite this, little is known about the ability for HS to increase survivorship to acid mine drainage (AMD). In this study, the ability of HS to increase survivorship of the freshwater shrimp (Caridina sp. D sensu Page, Choy & Hughes, 2005) to acid mine drainage was investigated, using test waters collected from the Mount Morgan open pit in Central Queensland and the addition of Aldrich humic acid (AHA). The AMD water from Mount Morgan open pit is highly acidic (pH 2.67) as well as being contaminated with heavy metals (1780 mg/L Al; 101 mg/L Cu; 173 mg/L Mn; 51.8 mg/L Zn and 51.8 mg/L Fe). Freshwater shrimp were exposed to dilutions in the range of 0.5 - 5 % AMD water, with and without the addition of 10 or 20 mg/L AHA treatments. In the absence of HS, all shrimp died in the 2.5% AMD treatment. By contrast, addition of HS increased survivorship in the 2.5% AMD by up to 66%, as well as significantly decreasing the concentration of dissolved Cu, Co, Cd and Zn. The decreased toxicity of AMD in the presence of HS is likely to be due to the complexation and the precipitation of heavy metals with the HS; it is also possible that HS caused changes to the physiological condition of the shrimp, thus increasing their survival. These results are valuable in contributing to an improved understanding of potential role of HS in ameliorating the toxicity of AMD environments.
Response to Reviewers:	RESPONSE TO REVIEWER #1 COMMENTS: Reviewer #1: This is a well-written paper that clearly demonstrates the protective effect of HA against AMD toxicity in a freshwater shrimp. The following issues however do need to be addressed: 1.Elaborate on how HA reduced the concentrations of DISSOLVED metals. Dissolved organic carbon (DOC)/HA is envisaged to bind dissolved metals making them unavailable for uptake and toxicity. The DOC-bound/complexed metals remain in

solution, and are measurable as part of dissolved metals concentration. However the free/bioavailable metals species decrease in presence of DOC resulting in less toxicity. Free metal species concentration can be measured by specialized techniques or calculated using speciation programs such as WHAM. In this study, the reduction in dissolved metals concentrations suggests precipitation as alluded to on page 8 line 2 and not complexation as discussed on page 9 lines 39-44. Authors therefore need to clarify the role of HA in metals precipitation and complexation, and the effects of these processes on AMD free and dissolved metals concentrations. The fact that individual dissolved metals concentrations decreased by different extents also needs to be discussed.

RESPONSE: The discussion into the role of HA in metal precipitation and complexation has been expanded and a paragraph into the fact that individual dissolved metals decreased by different extents has also been added (see page 10 lin1 - page 11 line 12)

Shrimp survival to AMD substantially increased in the presence of both 10mg/L and 20 mg/L of AHA. Decreases in toxicity of heavy metals to invertebrates and fish have been shown to occur in the presence of HS (Dobranskyte et al. 2006,Gillis et al. 2010,Schwartz et al. 2004). The decrease in toxicity of heavy metals in the presence of DOC such as HS has been suggested to be due to the complexation of metals by DOC. Heavy metals once in contact with HS, are bound to the carbon skeleton through heteroatom's, with the carboxylic and phenolic groups playing major parts by forming complex compounds with the heavy metals (Sanjay, Srivastava et al. 1995; Pehlivan and Arslan 2006). This complexation with HS causes a shift in the speciation of different metals from a free/bioavailable form such as $Al3+$ to one that is less available thereby decreasing the metals toxicity (Steinberg 2003). Our findings suggest that the decrease in toxicity of the mine water to the shrimp is likely to be due to the complexation and precipitation of heavy metals with HS, with a significant decrease in Cu, Cd, Co, and Zn being recorded in treatments containing HS. At high metal ion to HS ratios, the colloid charge of HS becomes neutralised by complete saturation with metal ion, this causes the agglomeration of HS/metal ions which then precipitate out of solution (El-Eswed and Khalili 2006; Suteerapataranon, Bouby et al. 2006). This precipitate can then be filtered out or may settle out of solution and become part of the sediments (Suteerapataranon, Bouby et al. 2006). In the case of this study the precipitate was filtered out when samples were collected for the determination of dissolved metal concentrations, leading to the decrease in the amount of dissolved metals in solution.

The dissolved metals were shown to decrease by different extents in treatments with HS, with Cu displaying a higher sorption affinity for HS than the other metals. In the 10 mg/L treatment the removal of metals through precipitation compared to treatment without AHA follows the following order Cu (67.5%) > Zn (18.7%) > Cd (12%) > Co (6.3%) > Mn (5.7%) >Ni (5.5%). In the 20 mg/L treatment the precipitation of metals followed a similar pattern Cu (80.4%) > Zn (41.7%) > Cd (19%) > Co (9.5%) > Ni (11.1%) > Mn (5.7%), however, the amount of Mn removed from solution was shown to not change between the 10 and 20 mg/L solutions. Other studies exploring the ability of HS to remove metals from wastewater or AMD have also shown similar absorption affinities (Pahlman and Khalafalla 1988; Brown, Gill et al. 2000; Bogush and Voronin 2010). Competition between the metals for binding sites on HS often leads to dissolved metals being decreased by different extents. Metals of high ionic potential (ratio of charge to ionic radius) and higher charge are attracted more strongly to HS and are likely to be bound first, out competing the other metals for binding sites (Brown, Gill et al. 2000). The pH of solution can also influence the binding of different metals to HS, with decreases in pH below 5 generally leading to decreases in heavy metal binding (Liu and Gonzalez 2000).

2.Line 17: Does the control water contain MOPS? Did MOPS affect metals speciation and toxicity? Was a control for MOPS run?

RESPONSE: A paragraph outlining why MOPS was deemed appropriate for use in trials has been added (See page 5 line 21 – page 6 line 4).

THE SENTENCE IN THE PAPER: Test waters (treatments and controls) were prepared by diluting the AMD with, filtered (0.7µm, GF/F) Dee River water (collected upstream of the mine) containing 0.75g/l MOPS (3-N morpholinopropanesulfonic acid) buffer. Pilot trials showed MOPS to be non-toxic to the freshwater shrimp Caridina sp. D. Previous studies have also shown MOPS to be non toxic to other freshwater species (De Schamphelaere, Heijerick et al. 2004; Rendal, Trapp et al. 2012). MOPS has previously been shown to not affect metal speciation (Kandegedara and Rorabacher 1999) and has been used by a number of authors in experiments involving toxicity of heavy metals to freshwater organisms (Deleebeeck, De Schamphelaere et al. 2007; Clifford and McGeer 2010; Mager, Brix et al. 2011).

3. It is not clear how many organisms were used in the test chambers. In line 19, is n=54 test organisms or test chambers?

RESPONE: The number of organisms used in test chambers has been clarified (see page 6 line 23-24).

Each treatment in each trial was replicated three times with 4 shrimp in each chamber, giving a total of 12 shrimp exposed to each treatment and a total n = 54 test vessels.

REFERENCES

Bogush, A. A. and V. G. Voronin (2010). "Application of a Peat-humic Agent for Treatment of Acid Mine Drainage." Mine Water and the Environment: 1-6.
Brown, P. A., S. A. Gill, et al. (2000). "Metal removal from wastewater using peat." Water Research 34(16): 3907-3916.
Clifford, M. and J. C. McGeer (2010). "Development of a biotic ligand model to predict the acute toxicity of cadmium to Daphnia pulex." Aquatic Toxicology 98(1): 1-7.
De Schamphelaere, K. A. C., D. G. Heijerick, et al. (2004). "Comparison of the effect of different pH buffering techniques on the toxicity of copper and zinc to Daphnia Magna and Pseudokirchneriella Subcapitata." Ecotoxicology 13(7): 697-705.
Deleebeeck, N. M. E., K. A. C. De Schamphelaere, et al. (2007). "A bioavailability model predicting the toxicity of nickel to rainbow trout (Oncorhynchus mykiss) and fathead minnow (Pimephales promelas) in synthetic and natural waters." Ecotoxicology and Environmental Safety 67(1): 1-13.
El-Eswed, B. and F. Khalili (2006). "Adsorption of Cu(II) and Ni(II) on solid humic acid from the Azraq area, Jordan." Journal of Colloid and Interface Science 299(2): 497-503.
Kandegedara, A. and D. B. Rorabacher (1999). "Noncomplexing Tertiary Amines as “Better” Buffers Covering the Range of pH 3−11. Temperature Dependence of Their Acid Dissociation Constants." Analytical Chemistry 71(15): 3140-3144.
Liu, A. and R. D. Gonzalez (2000). "Modeling adsorption of Copper (II), Cadmium (II) and Lead (II) on purified Humic Acid." Langmuir 16(3902-3909).
Mager, E. M., K. V. Brix, et al. (2011). "Effects of water chemistry on the chronic toxicity of lead to the cladoceran, Ceriodaphnia dubia." Ecotoxicology and Environmental Safety 74(3): 238-243.
Pahlman, J. E. and S. E. Khalafalla (1988). Use of lignochemicals and humic acids to remove heavy metals from process waste streams. Pgh. [i.e. Pittsburgh] Pa., U.S. Dept. of the Interior, Bureau of Mines.
Pehlivan, E. and G. Arslan (2006). "Uptake of Metal Ions on Humic Acids." Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 28(12): 1099-1112.
Rendal, C., S. Trapp, et al. (2012). "Critical evaluation and further development of methods for testing ecotoxicity at multiple pH using Daphnia magna and Pseudokirchneriella subcapitata." Environmental Toxicology and Chemistry 31(8): 1843-1852.
Sanjay, H. G., K. C. Srivastava, et al. (1995). Development of HUMASORBTM, A lignite derived Humic Acid for removal of metals and organic contaminants from groundwater. Environmental Technology Development through Industry Partnership. Morgantown, West virginia: 1-17.
Steinberg, C. E. W. (2003). Ecology of Humic Substances in Freshwaters, Springer-Verlag Belin Heidelberg, New York.

Suteerapataranon, S., M. Bouby, et al. (2006). "Interaction of trace elements in acid mine drainage solution with humic acid." Water Research 40(10): 2044-2054.

RESPONSE TO REVIEWER #2 COMMENTS

Reviewer #2: The authors address an interesting issue of environmental mitigation. They try combat acidification effects on freshwater shrimps by adding humic substances. The paper appears to be suitable for AECT, but needs some major revisions before.

1)A) The authors only consider the eco-chemical side of the mitigation: binding of metals to humics and adsorption of humics to biological surfaces. What about the organismal side? The authors are obviously not aware that humics have the potential to be internalized by exposed organisms and affect their biochemistry and particulary molecular biology (Menzel, R. et al. 2005. Environ Sci Technol, 39: 8324). In Table 1, the authors mention that the Dee River water contains 11 mg/L. If this DOC is humic substances (most likely), it must be discussed that dissolved humic substances can induce multiple stress resistance (e.g., Suhett, A. L. et al. 2011. Environ Sci Pollut Res, 18: 1004).

RESPONSE: A paragraph discussing the above has been included in the discussion (See page 12 line 7 – page 8 line 2).

HS also have the potential of exerting a mild chemical stress to an organism. Mild chemical stress is exerted by the internalisation of HS which induces the internal biochemical stress defence systems such as molecular chaperones (stress shock proteins), and biotransformation enzymes (Steinberg, Meinelt et al. 2008). It has been suggested that by acting as a mild chemical stressor to aquatic organisms HS may benefit the organism by providing the organism with mechanisms to better cope with exposure to other stressors (Bouchnak and Steinberg 2010). For example, pre-exposure to natural HS was able to alleviate the stress to Moina macrocopa caused by increases in salinity, even across generations (Suhett, Steinberg et al. 2011). Bouchnak and Steinberg (2010) also showed that the addition of huminfeed which is made up of 70-80% HS to daphnids diet, ameliorated the associated food stress on Daphnia magna when fed food of poor quality. HS also increased the recovery of fish to stressful handling (Meinelt, Schreckenbach et al. 2004). Mayflies sourced from humic streams were shown to have a distinctly higher tolerance to AMD than to mayflies sourced from other streams (O'Halloran, Cavanagh et al. 2008). The pre-exposure to high levels of HS in their natal stream may have allowed these organisms to develop defence mechanisms against another stressor, in this case AMD. The shrimp used in this experiment may have developed some stress resistence from exposure to HS in their source stream as this system is most likely to contain HS as indicated by the DOC level of 11mg/L. However, it is unlikely that this stress resistance influenced the survival results provided as all shrimp used in all treatments came from the same site along the same river, therefore, it is likely that all shrimp in all treatments had the same stress resistance.

B) The shrimps were taken from this river and acclimated to laboratory condition for a very short period (48 h). Usually, only acclimated F2 animals are taken.

RESPONSE: Acclimation of shrimp followed the recommended time period for macroinverterbates given in the Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians (ASTM 2007).

2) Characterize the upstream DOC

RESPONSE: The upstream DOC was not characterised as there was no reason to believe that the composition of the DOC would affect our results as all treatments used the same water with the same background DOC. The reporting of the DOC value for treatment water (without its characterisation) is common place in ecotoxicology literature. Wood et al. (2011) states that due to the heterogeneous nature of most natural DOCs, it has not been practical or desirable to characterize the properties of individual molecules.

3) Provide chemical Aldrich humics features

RESPONSE: The chemical features of Aldrich humic acid has been added to the document (See page 6 line 7 -9).

The chemical features of the AHA used in this study are as follows: (residue on ignition 29.9%, carbon content 40.15%, hydrogen content 3.60% and nitrogen content 0.92%).

4) Delete the paragraph of the discussion; it is not in the focus of the paper; instead, discuss the potential underlying mechanism in more detail.

RESPONSE: It is not clear which paragraph requires deletion and what potential underlying mechanisms the reviewer would like expanded. The underlying mechanisms involved in the complexation and precipitation of metals has been expanded (In response to reviewer 1 comment 1.) along with the underlying mechanism of HS effect on organisms (See response to comment 1.).

5) Discuss the binding of metals by humics in detail. How does ion exchange function under acidic conditions?

RESPONSE: The discussion into the binding of metals by humics has been expanded (see response to reviewers 1, comment 1) and a sentence into how ionic exchange functions under acidic conditions has been added (see page 11 line 15 – 17).

The pH of solution can also influence the binding of different metals to humic substances, with decreases in pH below 5 generally leading to decreases in heavy metal binding (Liu and Gonzalez 2000).

6) How did the authors prove that the borderline Aldrich material comprises only humic acids? Water soluble material usually contains a high moiety of fulvic acids. If the authors cannot differentiate between humic and fulvic acids, they should instead use the term humic material consistently.

RESPONSE: Humic acid has been replaced by the term humic substances.

7) Is Fig. 1 really needed? If the authors describe a general mechanism of mitigation of acid conditions, it should not depend on the study site.

RESPONSE: Fig. 1 has been deleted.

8) The authors should discuss how realistic the application of Aldrich humics for the real-world mitigation of the studied AMD is. Is there a real source of humics for this purpose?

RESPONSE: A paragraph discussing the use of humics in real world AMD mitigation has been added (see page 13 line 13 -23).

The present study appears to be the first to show that the addition of HS to AMD-contaminated water results in decreased metal concentrations, and increased survivorship of a freshwater organism. The use of AHA in this experiment allowed this to be demonstrated, however, further investigations into a cost-effective, sustainable source of humics for the mitigation of AMD affected waterways is still needed. Humics obtained from Leonardite and brown coal have been commercialised previously for use in agriculture and water treatment. These may provide a real world source for the treatment of AMD .

Minor issues:

a)Provide toxicity data of MOPS towards the used shrimps

	RESPONSE: A paragraph outlining why MOPS was deemed appropriate for use in trials has been added and a statement outlining the toxicity of MOPS to the freshwater shrimp has been added (see also response to reviewer #1 comment 2). b)Table 1: several dimensions are missing RESPONSE: It is unclear what dimensions are missing in Table 1. A sentence stating that the units recorded in the table are mg/L unless otherwise indicated has been added. c) Delete the reference from the title RESPONSE: Reference has been deleted from the title. d) Correction of typos RESPONSE: Manuscript has been checked again for grammatical errors. e) Provide statistics for Figs. 4 and 5 RESPONSE: Statistics are provided for figures 4 and 5 (now figures 3 and 4 (as figure 1 has been deleted) on page 9 line 8 -11). Non linear regressions were significant for each treatment (0 mg/L HA $R2 = 0.8925$, $p<0.000$; 10mg/L HA $R2 = 0.9273$, $p<0.000$; 20mg/L HA $R2 = 0.9163$, $p<0.000$). References ASTM (2007). Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. West Conshohocken, United States, ASTM International: 22. Bouchnak, R. and C. E. W. Steinberg (2010). "Modulation of longevity in Daphnia magna by food quality and simultaneous exposure to dissolved humic substances." Limnologica - Ecology and Management of Inland Waters 40(2): 86-91. Liu, A. and R. D. Gonzalez (2000). "Modeling adsorption of Copper (II), Cadmium (II) and Lead (II) on purified Humic Acid." Langmuir 16(3902-3909). Meinelt, T., K. Schreckenbach, et al. (2004). "Humic substances affect physiological condition and sex ratio of swordtail (Xiphophorus helleri Heckel)." Aquatic Sciences 66(2): 239-245. O'Halloran, K., J.-A. Cavanagh, et al. (2008). "Response of a new zealand mayfly (Deleatidium spp.) to acid mine drainage: Implications for mine remediation." Environmental Toxicology and Chemistry 27(5): 1135-1140. Steinberg, C. E. W., T. Meinelt, et al. (2008). "Humic substances - Part 2: Interactions with organisms." Environmental Science and Pollution Research 15(2): 128-135. Suhett, A. L., C. E. W. Steinberg, et al. (2011). "Natural dissolved humic substances increase the lifespan and promote transgenerational resistance to salt stress in the cladoceran Moina macrocopa." Environmental Science and Pollution Research: 1-11. Wood, C. M., H. A. Al-Reasi, et al. (2011). "The two faces of DOC." Aquatic Toxicology 105S: 3-8.

26/6/2012

To whom this may concern,

I am submitting my manuscript ‘Humic acid increases survivorship of the freshwater shrimp *Caridina* sp. D (sensu Page, Choy & Hughes, 2005) to acid mine drainage.’ for possible publication in Archives of environmental contamination and toxicology. This manuscript provides a contribution to the understanding of how humic acid can influence survivorship of a freshwater organism to acid mine drainage. This paper is important as freshwater systems all over the world are currently under threat from acid mine drainage or heavy metal pollution. This paper has international significance as it is believe to be the first paper to assess the ability of humic acid to influence survivorship of an aquatic organism to acid mine drainage. It also shows that humic acid can also significantly decrease metal concentrations in solution.

Thankyou for the opportunity to submit my manuscript to your journal.

Aleicia Holland

Title: Humic substances increase survivorship of the freshwater shrimp *Caridina* sp. D to acid mine drainage.

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Abstract

Humic substances (HS) are known to decrease the toxicity of heavy metals to aquatic organisms, and it has been suggested that they can provide buffering protection in low pH conditions. Despite this, little is known about the ability for HS to increase survivorship to acid mine drainage (AMD). In this study, the ability of HS to increase survivorship of the freshwater shrimp *(Caridina sp.* D sensu Page, Choy & Hughes, 2005) to acid mine drainage was investigated, using test waters collected from the Mount Morgan open pit in Central Queensland and the addition of Aldrich humic acid (AHA). The AMD water from Mount Morgan open pit is highly acidic (pH 2.67) as well as being contaminated with heavy metals (1780 mg/L Al; 101 mg/L Cu; 173 mg/L Mn; 51.8 mg/L Zn and 51.8 mg/L Fe). Freshwater shrimp were exposed to dilutions in the range of 0.5 - 5 % AMD water, with and without the addition of 10 or 20 mg/L AHA treatments. In the absence of HS, all shrimp died in the 2.5% AMD treatment. By contrast, addition of HS increased survivorship in the 2.5% AMD by up to 66%, as well as significantly decreasing the concentration of dissolved Cu, Co, Cd and Zn. The decreased toxicity of AMD in the presence of HS is likely to be due to the complexation and the precipitation of heavy metals with the HS; it is also possible that HS caused changes to the physiological condition of the shrimp, thus increasing their survival. These results are valuable in contributing to an improved understanding of potential role of HS in ameliorating the toxicity of AMD environments.

Keywords: Dissolved organic carbon, metals, DOC, toxicity, Mount Morgan, passive treatment, mixture

Introduction

Acidification of freshwaters by anthropogenic means such as acid mine drainage (AMD) or aerial deposition is a global problem. The pH decreases experienced in these systems has a severe effect on the biota, leading to decreased diversity and poor ecosystem functioning. However, many of the countries that feature anthropogenically-acidified waterways also contain naturally acidic environments. Here, the acidity is caused by the high levels of humic substances (HS) leached from the surrounding vegetation. Unlike streams acidified by anthropogenic means, these systems may contain diverse populations of aquatic organisms. It has been suggested that the presence of high amounts of HS in naturally acidic waterways buffers the organisms present against the detrimental effects of low pH (Collier et al. 1990; Dangles et al. 2004). HS have also been shown to decrease the toxicity of heavy metals to aquatic organisms across a number of different trophic levels (Gillis et al. 2010; Kim et al. 1999; Schwartz et al. 2004). Despite this, the ability of HS to increase survivorship to anthropogenically acidified waters such as those affected by acid mine drainage is unknown. This is a critical gap: as both low pH and high metal content are commonly recorded from AMD-affected systems, there may be an important role for HS in reducing the toxicity of waterways polluted by AMD.

The toxicity of AMD varies according to metal and sulphate concentrations as well as the water chemistry of the receiving environment (van Dam et al. 2008). Consequently, the specific impacts of AMD should be directly assessed on a case-by-case basis, to ensure that individual sites are managed correctly (van Dam et al. 2008).

Mount Morgan Mine located at Mount Morgan, Central Queensland is undoubtedly one of the most controversial abandoned mine sites within Australia, with respect to AMD. Mining first began at Mount Morgan in 1882 and continued for over a hundred years until 1991 (Wels et al. 2006). During Mount Morgan Mine’s life time, 262 tonnes of gold, 37 tonnes of silver and 387 000 tonnes of copper have been recovered from underground and open cut operations;

with an accompanying 134 million tonnes of waste rock and tailings also be generated (Wels et al. 2006). In 2007, it was estimated that the oxidation of these tailings has resulted in the production of 90,000 ML of AMD containing high levels of Al, Fe, Cu, and Zn. Conservative models indicate that AMD will continue to be generated at the site for at least the next 400 years if not effectively remediated (Gasparon et al. 2007). Between 1982 and 1990, the open cut pit was backfilled with 28 Mt of retreated tailings (Wels et al. 2006). Since 1990, the open cut pit has been allowed to fill via natural inflows and captured seepage. Over time, the probability of an uncontrolled release has been greatly increasing (Chapman and Simpson 2005). Subsequently, the State Government in charge of the mine has started implementing control releases of 100% AMD water from the open pit during high flow events. These releases are then diluted on contact with the receiving body, the Dee River.

This paper aims to investigate the ability of HS to increase survivorship of the freshwater shrimp *Caridina* sp. D (Page et al. 2005) to AMD-contaminated water collected from Mount Morgan open pit water. As the test design includes the collection and use of organisms from the Dee River upstream of the mine, this project will provide information on the ability of HS to decrease toxicity of the open pit water to organisms commonly found in the area. Investigations into the ability of HS to increase survivorship of biota to AMD at Mount Morgan may therefore help to underpin the development of a new, sustainable treatment option for other waterways affected by AMD, based on rehabilitation using the addition of HS.

Materials and Methods

Mount Morgan Mine is located approximately 40 kilometres south-south west of Rockhampton, Queensland (Australia). The Dee River originates in the Mount Morgan ranges and flows in a south-south westerly direction until it enters the Fitzroy River (Mackey 1988).

The River runs within a few hundred metres of Mount Morgan Mine and sites downstream of this point are highly contaminated by AMD. AMD-contaminated water was collected from the Mount Morgan open cut pit (AMD) during May 2011 for use in the following trials. Dee River water upstream of the influence of the mine was collected for use as diluent and for use in the control treatment.

Shrimp were collected from sites along the Dee River, upstream of the mine, using a 250 µm dip net. On arrival in the laboratory, shrimp were placed in acclimation tanks within a controlled climate room, at 25ºC and 16:8-h light:dark photoperiod, for at least 48 hours before commencing experimental trials. Toxicity tests were conducted over 96 hours in 400mL plastic containers, each containing 200 mL of test solution and four shrimp. Organisms were checked every 24 h for mortality, and dead individuals were removed daily, in order to avoid any adverse influence on the remaining test organisms. The criteria for determining mortality were: no movement and no reaction to gentle prodding. Organisms were not fed during the trial as heavy metals may bind to food items. Test solutions were maintained using the static technique. Air was supplied to the containers continuously from an electric aerator. Lids were placed on top of the test chambers to stop contamination and the escape of test subjects.

Test waters (treatments and controls) were prepared by diluting the AMD with, filtered (0.7µm, GF/F) Dee River water (collected upstream of the mine) containing 0.75g/l MOPS (3-N morpholinopropanesulfonic acid) buffer. Pilot trials showed MOPS to be non-toxic to the freshwater shrimp *Caridina sp. D*. Previous studies have also shown MOPS to be non toxic to other freshwater species (De Schamphelaere et al. 2004; Rendal et al. 2012). MOPS has previously been shown to not affect metal speciation (Kandegedara and Rorabacher 1999) and has been used by a number of authors in experiments involving toxicity of heavy metals to freshwater organisms (Deleebeeck et al. 2007; Mager et al. 2011; Clifford and McGeer 2010).

Treatment groups were prepared with and without the presence of 10mg/L and 20mg/L AHA (Sigma Aldrich Humic Acid). The chemical features of the AHA used in this study are as follows: (residue on ignition 29.9%, carbon content 40.15%, hydrogen content 3.60% and nitrogen content 0.92%). The pH of each AMD dilution for every treatment was recorded, and solutions were then left for 48 hours to stabilise as pilot trials showed increases in pH of treatment waters over the first 24 hours. Test solutions were then acidified back to starting pH using sulphuric acid to mimic acid mine drainage conditions, as pilot trials showed that this methodology ensured that pH remained stable throughout the trial period.

To investigate whether HS increases survivorship to AMD, trials 1 and 2 were performed using the test dilutions: 0% (control), 0.5%, 1%, 1.5%, 2%, and 2.5% AMD. These dilution factors were chosen as range-finding trials indicated that the pit water was highly toxic at concentrations above 2.5%, where it was linked with 100% shrimp mortality. In trial 3, the dilution factors were increased to 0% (control), 2.5%, 3%, 3.5%, 4% and 5% to determine the percentage of AMD at which shrimp are able to survive, due to the presence of HS. Control waters for all trials consisted of 100% Dee River water collected upstream of the mine. The treatments were no AHA, 10 mg/L and 20 mg/L AHA. Each treatment in each trial was replicated three times with 4 shrimp in each chamber, giving a total of 12 shrimp exposed to each treatment and a total n = 54 test vessels. All trials, where possible, followed the recommendations made in the 'Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians' (ASTM 2007).

During the experiment temperature, pH (TPS 80A), conductivity (TPS LC84), oxygen (TPS WP-82Y), and ammonia levels (Aquarium Pharmaceuticals Inc., freshwater total ammonia test kit) were measured every 24 hours. Temperature loggers (TG-4100 Tiny Tag) recorded temperature readings every hour. Disturbance to test organisms was minimal during water quality analyses. For all trials, metal samples were filtered for the determination of dissolved fractions and analysed using inductively coupled plasma mass spectroscopy. During trial 1, a subsample (pooled from 20ml of each replicate) was collected from each dilution/treatment combination for the determination of selected dissolved metals. During trial 2, samples for metal analyses were only collected at 24 and 96 hours from the 1.5 and 2.5% dilutions in the 0 mg/L AHA and 20 mg/L AHA treatments. This allowed an assessment of any differences in metal content experienced over time. Samples were also collected from each replicate in the 1.5% dilution at 96 hours to determine variability amongst replicates. In trial 3, samples were collected from the highest dilution containing surviving shrimp and the lowest dilution causing 100% mortality of shrimp in both the 10 mg/L AHA and the 20 mg/L AHA treatments.

Mortality results from all three trials were pooled together and analysed using non-linear (three-parameter sigmoidal) regressions to determine LC_{50} values and their corresponding ninety-five percent confidence limits (SigmaPlot 11.0). Multiple line and scatter plots were prepared for each trial to show the differences in the proportion of organisms surviving between treatments. Independent sample T-tests on pooled data were used to determine any significant differences in metal concentrations at the 2.5% dilution between the 0mg/L and the 20mg/L treatments.

Results

Water quality

Table 1 provides details of the composition and water quality of the raw treatment water (100% AMD) and the control/dilution water (collected from the Dee River, upstream of the mine site). Table 2 displays key parameters recorded during the 96 hour trial periods. The pH remained stable (±0.2) in all treatments and in all trials (Table 2). Oxygen levels were above 75%; temperatures were 21.5 ± 1°C and ammonia was low, with 1 ppm or less recorded for all treatments during all trials (Table 2). Conductivity of treatments increased with increasing concentration of mine water (Table 2).

Heavy metal concentrations for Al, Cd, Co, Cu, Mn and Zn exceeded the 99% ANZECC (2000) trigger values for all dilutions without the presence of HS (Table 3). Cu exceeded the 99% ANZECC (2000) trigger values in the control water collected from the site upstream of the mine (Table 4). Heavy metals concentrations generally varied in line with the relevant dilutions of AMD. The coefficient variation between metal concentrations within replicates was generally less than 10% (standard deviation/average). Differences in metal concentrations over time and between trials were minimal (Table 5). Precipitates were seen forming in the AHA treatments. A significant difference in Cu, Cd, Co, Zn concentration was found between the 0mg/L and the 20mg/L AHA treatments at 2.5% AMD (T-test Cu (T_3 = 13.195, p =0.001); Cd (T_3 = 7.589, p =0.005); Zn (T_3 = 5.894, p =0.010); Co (T_3 = 5.692, p =0.011)). Metal concentrations in the 100% AMD have increased over time from 2001-2011 (Table 4).

Ecotoxicity

The control treatments had 100% survival of shrimp during all three trials (Fig. 1 & 2). Meanwhile, 100% mortality of shrimp was always recorded for the 2.5% dilutions where HS was not added (Fig. 1a). By contrast, the addition of HS was shown to increase the survival of shrimp exposed to 2.5% AMD by up to 58% and 66%, at the 10 and 20 mg/L treatment levels, respectively (Fig. 1b & c). The addition of HS in treatments containing 4% AMD was not able to prevent 100% mortality of shrimp (Fig. 2).

Non linear regressions were significant for each treatment (0 mg/L AHA $R^2 = 0.8925$, $p<0.000$; 10mg/L AHA $R^2 = 0.9273$, $p<0.000$; 20mg/L AHA $R^2 = 0.9163$, $p<0.000$). The LC_{50} values at 96 hours were established as 1.9% (1.7 - 2) for no AHA; 2.6% (2.5 - 2.7) for 10mg/L AHA and 2.7% (2.6 - 2.8) for 20 mg/L AHA (Fig. 3 and 4).

Discussion

The AMD water collected from the Mount Morgan open pit is highly toxic, with as little as 2.5% needed to cause 100% mortality to the freshwater shrimp *Caridina* sp. D. The LC_{50} of 1.9% recorded during this study is considerably lower than the 2.7% recorded by Chapman and Simpson (2005), which also used the Mount Morgan open pit water in tests with the freshwater shrimp *Caridina indistincta*. This is likely to be due to increases in metal concentrations within the pit water since 2001 (Table 4) (Chapman and Simpson 2005). The increase in metal content within the open pit is likely a by-product of the lime dosing water treatment plant built in 2006. This facility treats the AMD water before releasing it into the Dee River. The addition of hydrolysed lime via the plant raises the pH from around 2.9 to 7.5 and precipitates out the heavy metals (Gasparon et al. 2007). This results in large amounts of heavy metal rich sludge being produced, which is then pumped back into the pit, thus causing the increase in heavy metals within the pit water. It is likely that the cocktail of heavy metals led to the death of the shrimp, with Cu playing a major part, however, all metals exceeded the ANZECC (2000) guidelines. Concentrations of copper in the test concentrations closest to the LC_{50} value (2%) were close to or exceeded the range of LC_{50}'s for copper (3.5 – 110µg/L) previously reported for freshwater shrimp in Australia (Markich et al. 2002).

Shrimp survival to AMD substantially increased in the presence of both 10mg/L and 20 mg/L of AHA. Decreases in toxicity of heavy metals to invertebrates and fish have been shown to occur in the presence of HS (Dobranskyte et al. 2006,Gillis et al. 2010,Schwartz et al. 2004). The decrease in toxicity of heavy metals in the presence of DOC such as HS has been suggested to be due to the complexation of metals by DOC. Heavy metals once in contact with HS, are bound to the carbon skeleton through heteroatom's, with the carboxylic and phenolic groups playing major parts by forming complex compounds with the heavy metals (Pehlivan and Arslan 2006; Sanjay et al. 1995). This complexation with HS causes a shift in the speciation of different metals from a free/bioavailable form such as Al^{3+} to one that is less available thereby decreasing the metals toxicity (Steinberg 2003). Our findings suggest that the decrease in toxicity of the mine water to the shrimp is likely to be due to the complexation and precipitation of heavy metals with HS, with a significant decrease in Cu, Cd, Co, and Zn being recorded in treatments containing HS. At high metal ion to HS ratios, the colloid charge of HS becomes neutralised by complete saturation with metal ion, this causes the agglomeration of HS/metal ions which then precipitate out of solution (Suteerapataranon et al. 2006; El-Eswed and Khalili 2006). This precipitate can then be filtered out or may settle out of solution and become part of the sediments (Suteerapataranon et al. 2006). In the case of this study the precipitate was filtered out when samples were collected for the determination of dissolved metal concentrations, leading to the decrease in the amount of dissolved metals in solution.

The dissolved metals were shown to decrease by different extents in treatments with HS, with Cu displaying a higher sorption affinity for HS than the other metals. In the 10 mg/L treatment the removal of metals through precipitation compared to treatment without AHA follows the following order Cu (67.5%) > Zn (18.7%) > Cd (12%) > Co (6.3%) > Mn (5.7%)

>Ni (5.5%). In the 20 mg/L treatment the precipitation of metals followed a similar pattern Cu (80.4%) > Zn (41.7%) > Cd (19%) > Co (9.5%) > Ni (11.1%) > Mn (5.7%), however, the amount of Mn removed from solution was shown to not change between the 10 and 20 mg/L solutions. Other studies exploring the ability of HS to remove metals from wastewater or AMD have also shown similar absorption affinities (Brown et al. 2000; Pahlman and Khalafalla 1988; Bogush and Voronin 2010). Competition between the metals for binding sites on HS often leads to dissolved metals being decreased by different extents. Metals of high ionic potential (ratio of charge to ionic radius) and higher charge are attracted more strongly to HS and are likely to be bound first, out competing the other metals for binding sites (Brown et al. 2000). The pH of solution can also influence the binding of different metals to HS, with decreases in pH below 5 generally leading to decreases in heavy metal binding (Liu and Gonzalez 2000).

The results in this study, however, do not exclude the possibility that HS itself may have caused changes in the physiology of the freshwater shrimp, hence increasing their ability to cope with AMD. DOC has been shown to bind to the gill cells of fish (Campbell et al. 1997), reduce cellular permeability (Campbell et al. 1997; Wood et al. 2003), stimulate active Na^+ uptake (Glover et al. 2005; Glover and Wood 2005), increase enzymatic activity such as Na^+, K^+ ATPase (McGeer et al. 2002), and alter the transepithelial potential of gill epithelia (Wood et al. 2011; Galvez et al. 2008). Since the primary action of a number of metals such as Cu has been generally accepted to be the inhibition of Na^+ uptake, increased ion leakage and the destabilisation of the paracellular pathway, the positive effects of DOC to the physiology of an organism may counterbalance the negative impacts related to the heavy metals exposure (Wood et al. 2011; Niyogi and Wood 2004).

HS also have the potential of exerting a mild chemical stress to an organism. Mild chemical stress is exerted by the internalisation of HS which induces the internal biochemical stress defence systems such as molecular chaperones (stress shock proteins), and biotransformation enzymes (Steinberg et al. 2008). It has been suggested that by acting as a mild chemical stressor to aquatic organisms HS may benefit the organism by providing the organism with mechanisms to better cope with exposure to other stressors (Bouchnak and Steinberg 2010). For example, pre-exposure to natural HS was able to alleviate the stress to *Moina macrocopa* caused by increases in salinity, even across generations (Suhett et al. 2011). Bouchnak and Steinberg (2010) also showed that the addition of huminfeed (made up of 70-80% humic acid) to daphnid's diet, ameliorated the associated food stress on *Daphnia magna* when fed food of poor quality. HS also increased the recovery of fish to stressful handling (Meinelt et al. 2004). Mayflies sourced from humic streams were shown to have a distinctly higher tolerance to AMD than to mayflies sourced from other streams (O'Halloran et al. 2008). The pre-exposure to high levels of HS in their natal stream may have allowed these organisms to develop defence mechanisms against another stressor, in this case AMD. The shrimp used in this experiment may have developed some stress resistence from exposure to HS in their source stream as this system is most likely to contain HS as indicated by the DOC level of 11mg/L. However, it is unlikely that this stress resistance influenced the survival results provided as all shrimp used in all treatments came from the same site along the same river, therefore, it is likely that all shrimp in all treatments had the same stress resistance.

Information regarding the ability of DOC such as HS in ameliorating the acute toxicity of mixtures of heavy metals is scant. To date, 24 and 48 hr acute toxicity tests of mixture of Cu and Cr on *Daphnia magna* (Jo et al. 2010), and chronic toxicity studies using a mixture of Cu, Cd, and Zn (Kamunde and MacPhail 2011) or Cu and Cd (Richards et al. 1999) have showed DOC decreases the toxicity and physiological effects of heavy metals on aquatic organisms. The limited amount of knowledge on the influence that DOC has on metal mixtures is quite alarming, given that metal contamination of aquatic environments generally involves the presence of more than one metal (Markich et al. 2002).

The use of HS to treat waterways high in heavy metals has been suggested previously by Bollag and Myers (1992). The use of HS to remove heavy metals from AMD contaminated water has also been studied, with peat-derived HS shown to successfully remove up to 80 % of heavy metals such as Cd, Co, Ni, Fe, Cu, Al and Zn (Bogush and Voronin 2010). However, the present study appears to be the first to show that the addition of HS to AMD-contaminated water results in decreased metal concentrations, and increased survivorship of a freshwater organism. The use of AHA in this experiment allowed this to be demonstrated, however, further investigations into a cost-effective, sustainable source of humics for the mitigation of AMD affected waterways is still needed. HS obtained from Leonardite and brown coal have been commercialised previously for use in agriculture and water treatment. These may provide a real world source for the treatment of AMD.

The establishment of similar LC_{50} values for the 10 mg/L and 20 mg/L HS treatments was not expected as other studies have reported that the toxicity of heavy metals decreases with increasing HS content (Kim et al. 1999; Heijerick et al. 2003). In this case, the use of 10 mg/L HS to increase survivorship of aquatic organisms to AMD may be a more cost efficient and viable option than the use of 20 mg/L HS as increases in survivorship with increasing concentration of HS was minimal. However, due to the highly variable nature of HS and AMD, and the different tolerances of aquatic biota, investigations into the optimal concentration of HS for the treatment of a specific sites AMD should be conducted on organisms common to that specific area to determine correct dosage response curves.

Conclusion

The ability of HS to increase survivorship of aquatic organisms to AMD has important consequences for the possible remediation and rehabilitation of AMD impacted waterways. These results are valuable in contributing to an improved understanding of the potential role of HS in ameliorating the toxicity of AMD environments. The ability of HS to decrease toxicity of AMD containing a mixture of heavy metals (including Al, Cd, Co, Cu, Mn, and Zn) that exceed the recommend ANZECC guidelines is encouraging and increases knowledge on the ability of DOC to affect toxicity of heavy metal mixtures.

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References

ANZECC (2000) Australian Guidelines for Water Quality Monitoring and Reporting. Australian and New Zealand Environment and Conservation Council, Canberra,

ASTM (2007) Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. ASTM International, West Conshohocken, United States

Bogush AA, Voronin VG (2010) Application of a Peat-humic Agent for Treatment of Acid Mine Drainage. Mine Water Environ 30 (3):185-190

Bollag J, Myers C (1992) Detoxification of aquatic and terrestrial sites through binding of pollutants to humic substances. Sci Total Environ 117/118:357-366

Bouchnak R, Steinberg CEW (2010) Modulation of longevity in Daphnia magna by food quality and simultaneous exposure to dissolved humic substances. Limnologica - Ecology and Management of Inland Waters 40 (2):86-91

Brown PA, Gill SA, Allen SJ (2000) Metal removal from wastewater using peat. Water Res 34 (16):3907-3916. doi:10.1016/s0043-1354(00)00152-4

Campbell PGC, Twiss MR, Wilkinson KJ (1997) Accumulation of natural organic matter on the surfaces of living cells: Implications for the interaction of toxic solutes with aquatic biota. Can J Fish Aquat Sc 54 (11):2543-2554

Chapman HF, Simpson SL (2005) Toxicity of acid water from Mt Morgan mine site, Central Queensland, to the freshwater shrimp *Caradina Indistincta*. Aust J Ecotoxicol 11:93-99

Clifford M, McGeer JC (2010) Development of a biotic ligand model to predict the acute toxicity of cadmium to Daphnia pulex. Aquat Toxicol 98 (1):1-7. doi:10.1016/j.aquatox.2010.01.001

Collier KJ, Ball OJ, Graesser AK, Main MR, Winterbourn MJ (1990) Do organic and anthropogenic acidity have similar effects on aquatic fauna? Oikos 59 (1):33-38

Dangles O, Malmqvist B, Laudon H (2004) Naturally acid freshwater ecosystems are diverse and functional: Evidence from boreal streams. Oikos 104 (1):149-155

De Schamphelaere KAC, Heijerick DG, Janssen CR (2004) Comparison of the effect of different pH buffering techniques on the toxicity of copper and zinc to Daphnia Magna and Pseudokirchneriella Subcapitata. Ecotoxicology 13 (7):697-705

Deleebeeck NME, De Schamphelaere KAC, Janssen CR (2007) A bioavailability model predicting the toxicity of nickel to rainbow trout (Oncorhynchus mykiss) and fathead minnow (Pimephales promelas) in synthetic and natural waters. Ecotoxicol Environ Saf 67 (1):1-13. doi:10.1016/j.ecoenv.2006.10.001

El-Eswed B, Khalili F (2006) Adsorption of Cu(II) and Ni(II) on solid humic acid from the Azraq area, Jordan. J Colloid Interface Sci 299 (2):497-503. doi:10.1016/j.jcis.2006.02.048

Galvez F, Donini A, Playle RC, Smith DS, O'Donnell MJ, Wood CM (2008) A matter of potential concern: Natural organic matter alters the electrical properties of fish gills. Environ Sci Technol 42 (24):9385-9390

Gasparon M, Smedley A, Jong T, Costagliola P, Benvenuti M (2007) Acid mine drainage at Mount Morgan, Queensland (Australia): Experimental simulation and geochemical modelling of buffering reactions. Paper presented at the IMWA Symposium 2007: Water in Mining Environments, Cagliari, Italy, 27th - 31st May 2007

Gillis PL, McGeer JC, Mackie GL, Wilkie MP, Ackerman JD (2010) The effect of natural dissolved organic carbon on the acute toxicity of copper to larval freshwater mussels (glochidia). Environ Toxicol Chem 29 (11):2519-2528

Glover CN, Pane EF, Wood CM (2005) Humic substances influence sodium metabolism in the freshwater crustacean Daphnia magna. Physiol Biochem Zool 78 (3):405-416

Glover CN, Wood CM (2005) The disruption of Daphnia magna sodium metabolism by humic substances: Mechanism of action and effect of humic substance source. Physiol Biochem Zool 78 (6):1005-1016

Heijerick DG, Janssen CR, De Coen WM (2003) The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to D. magna: Development of a surface response model. Arch Environ Contam Toxicol 44 (2):210-217

Jo H-J, Son J, Cho K, Jung J (2010) Combined effects of water quality parameters on mixture toxicity of copper and chromium toward Daphnia magna. Chemosphere 81 (10):1301-1307

Kamunde C, MacPhail R (2011) Effect of humic acid during concurrent chronic waterborne exposure of rainbow trout (Oncorhynchus mykiss) to copper, cadmium and zinc. Ecotoxicol Environ Saf 74 (3):259-269

Kandegedara A, Rorabacher DB (1999) Noncomplexing Tertiary Amines as "Better" Buffers Covering the Range of pH 3−11. Temperature Dependence of Their Acid Dissociation Constants. Anal Chem 71 (15):3140-3144. doi:10.1021/ac9902594

Kim SD, Ma H, Allen HE, Cha DK (1999) Influence of dissolved organic matter on the toxicity of copper to Ceriodaphnia dubia: Effect of complexation kinetics. Environ Toxicol Chem 18 (11):2433-2437

Liu A, Gonzalez RD (2000) Modeling adsorption of Copper (II), Cadmium (II) and Lead (II) on purified Humic Acid. Langmuir 16 (3902-3909)

Mackey AP (1988) The Biota of the River Dee (Central Queensland, Australia) in relation to the effects of acid mine drainage. Proc R Soc Queensl 99 (9-19)

Mager EM, Brix KV, Gerdes RM, Ryan AC, Grosell M (2011) Effects of water chemistry on the chronic toxicity of lead to the cladoceran, Ceriodaphnia dubia. Ecotoxicol Environ Saf 74 (3):238-243

Markich SJ, Warne MS, Westbury A-M, Roberts CJ (2002) A Compilation of data on the toxicity of chemicals to species in Australasia. Part 3: Metals. Aust J Ecotoxicol 8:1-72

McGeer JC, Szebedinszky C, McDonald DG, Wood CM (2002) The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. Comp Biochem Phys C 133 (1-2):147-160

Meinelt T, Schreckenbach K, Knopf K, Wienke A, Stu□ber A, Steimberg CEW (2004) Humic substances affect physiological condition and sex ratio of swordtail (Xiphophorus helleri Heckel). Aquatic Sciences 66 (2):239-245

Niyogi S, Wood CM (2004) Biotic ligand model, a flexible tool for developing water quality guidelines for metals. Environ Sci Technol 38 (23):6177-6192

O'Halloran K, Cavanagh J-A, Harding JS (2008) Response of a new zealand mayfly (Deleatidium spp.) to acid mine drainage: Implications for mine remediation. Environ Toxicol Chem 27 (5):1135-1140. doi:10.1897/07-199.1

Page TJ, Choy SC, Hughes JM (2005) The taxonomic feedback loop: symbiosis of morphology and molecules. Biol Lett 1:139-142

Pahlman JE, Khalafalla SE (1988) Use of lignochemicals and humic acids to remove heavy metals from process waste streams. Report of investigations / United States. Bureau of Mines ;9200, vol 9 p. U.S. Dept. of the Interior, Bureau of Mines, Pgh. [i.e. Pittsburgh] Pa.

Pehlivan E, Arslan G (2006) Uptake of Metal Ions on Humic Acids. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 28 (12):1099-1112. doi:10.1080/009083190910451

Rendal C, Trapp S, Kusk KO (2012) Critical evaluation and further development of methods for testing ecotoxicity at multiple pH using Daphnia magna and Pseudokirchneriella subcapitata. Environ Toxicol Chem 31 (8):1843-1852

Richards JG, Burnison BK, Playle RC (1999) Natural and commercial dissolved organic matter protects against the physiological effects of a combined cadmium and copper exposure on rainbow trout (Oncorhynchus mykiss). Can J Fish Aquat Sc 56 (3):407-418

Sanjay HG, Srivastava KC, Walia DS (1995) Development of HUMASORB™, A lignite derived Humic Acid for removal of metals and organic contaminants from groundwater. Paper presented at the Environmental Technology Development through Industry Partnership, Morgantown, West virginia, October 3-5

Schwartz ML, Curtis PJ, Playle RC (2004) Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 23 (12):2889-2899

Steinberg CEW (2003) Ecology of Humic Substances in Freshwaters. Springer-Verlag Belin Heidelberg, New York,

Steinberg CEW, Meinelt T, Timofeyev MA, Bittner M, Menzel R (2008) Humic substances - Part 2: Interactions with organisms. Environmental Science and Pollution Research 15 (2):128-135

Suhett AL, Steinberg CEW, Santangelo JM, Bozelli RL, Farjalla VF (2011) Natural dissolved humic substances increase the lifespan and promote transgenerational resistance to salt stress in the cladoceran Moina macrocopa. Environmental Science and Pollution Research:1-11

Suteerapataranon S, Bouby M, Geckeis H, Fanghänel T, Grudpan K (2006) Interaction of trace elements in acid mine drainage solution with humic acid. Water Res 40 (10):2044-2054

van Dam R, Hogan A, Harford A, Markich S (2008) Toxicity and metal speciation characterisation of waste water from an abandoned gold mine in tropical northern Australia. Chemosphere 73 (3):305-313. doi:10.1016/j.chemosphere.2008.06.011

Wels C, Findlater L, McCombe C (2006) Assessment of groundwater impacts at the historic Mount Morgan Mine Site, Queensland, Australia. Paper presented at the In proceedings of the 6th International Conference on Acid Rock Drainage, Missouri, USA, March 26-30

Wood CM, Al-Reasi HA, Smith DS (2011) The two faces of DOC. Aquat Toxicol 105S:3-8

Wood CM, Matsuo AYO, Wilson RW, Gonzalez RJ, Patrick ML, Playle RC, Val AL (2003) Protection by natural blackwater against disturbances in ion fluxes caused by low pH exposure in freshwater stingrays endemic to the Rio Negro. Physiol Biochem Zool 76 (1):12-27

Tables

Table 1. Composition and water quality of Dee River water (control, diluent water) and the 100% Mount Morgan Open pit water (AMD raw water source). Units are mg/L unless otherwise indicated.

	Cond (µS/cm)	pH	DOC	Hardness ($CaCO_3$)	Alkalinity ($CaCO_3$)	Ca	Mg	Na	K	Cl	SO_4
Dee River	1326	7.7	11	329	385	61.7	42.7	161.6	2.9	200	17
100% AMD	11920	2.67	N/A	N/A	N/A	352	1479	266	4.2	130	7100

Table 2. Ranges of physicochemical variables recorded every 24 hours during the 96 hour trial period for all three trials.

% AMD	pH	Conductivity (µS/cm)	Oxygen (% sat)	Ammonia (ppm)
0[a]	7.3 - 7.4	1284 - 1368	79 - 92	0.25 -1
0.5[a]	6.9 - 7	1397 - 1498	77 - 93	0.25 -1
1[a]	6.7-6.9	1501 - 1607	80 - 93	0.25 -1
1.5[a]	6.6-6.7	1557 - 1698	79 - 92	0.25 -1
2[a]	6.4 -6.5	1639 - 1730	81 - 92	0.25 -1
2.5[b]	6.1-6.3	1655 - 1795	82 - 91	0.25 -1
3[c]	5.8-5.9	1819 - 1840	79 - 91	0.25 -1
3.5[c]	4.5-4.7	1865 - 1886	79 - 91	0.25 -1
4[c]	4.3-4.5	1912 - 1927	81 - 89	0.25 -1
5[c]	4-4.2	1953 - 1962	83 - 89	0.25 -1

[a]n = 108; [b]n = 162; [c]n =54

Table 3. Dissolved metal concentrations (mg/L) recorded from each dilution/treatment combination. Values were obtained from a subsample of each replicate, pooled together before analysis. Trigger values for 99% protection from ANZECC (2000) are also supplied for comparative purposes. Values in bold exceed the trigger values.

Treatment	% AMD	Al	Cd	Co	Cu	Mn	Ni	Zn
0 mg/L AHA	0.5	**0.11**	**0.0007**	**0.010**	**0.075**	**0.443**	0.008	**0.116**
	1	**0.14**	**0.0014**	**0.023**	**0.073**	**0.985**	0.008	**0.184**
	1.5[a]	**0.11**	**0.0022**	**0.035**	**0.104**	**1.50**	0.011	**0.262**
	2	**0.12**	**0.0030**	**0.047**	**0.100**	**2.00**	0.014	**0.383**
	2.5[a]	**0.09**	**0.0041**	**0.063**	**0.383**	**2.62**	0.018	**0.613**
10 mg/L AHA	0.5	**0.20**	**0.0006**	0.001	**0.054**	**0.477**	0.005	**0.092**
	1	**0.13**	**0.0012**	**0.021**	**0.052**	**0.960**	0.007	**0.133**
	1.5	**0.13**	**0.0020**	**0.034**	**0.058**	**1.52**	0.010	**0.238**
	2	**0.10**	**0.0027**	**0.047**	**0.068**	**2.05**	0.014	**0.327**
	2.5	**0.12**	**0.0036**	**0.059**	**0.128**	**2.47**	0.017	**0.498**
	3.5	**17.6**	**0.0066**	**0.104**	**1.9**	**3.77**	0.029	**1.27**
	4	**25.3**	**0.0077**	**0.120**	**2.2**	**4.36**	0.033	**1.45**
20 mg/L AHA	0.5	**0.19**	**0.0005**	**0.011**	**0.050**	**0.486**	0.005	**0.071**
	1	**0.12**	**0.0009**	**0.018**	**0.054**	**0.874**	0.007	**0.101**
	1.5[a]	**0.16**	**0.0017**	**0.032**	**0.070**	**1.46**	0.010	**0.169**
	2	**0.12**	**0.0030**	**0.049**	**0.074**	**2.12**	0.015	**0.377**
	2.5[a]	**0.07**	**0.0033**	**0.057**	**0.075**	**2.47**	0.016	**0.357**
	3.5	**19.2**	**0.0074**	**0.111**	**1.98**	**4.04**	0.030	**1.340**
	4	**23.1**	**0.0076**	**0.115**	**2.09**	**4.19**	0.032	**1.400**
ANZECC value		0.02	0.0003[b]	0.005	0.0052[b]	0.2	0.0416[b]	0.0104[b]

[a] average of three readings collected. [b] adjusted for hardness

Table 4. Metal concentrations (mg/L) for control and dilution water (Dee River water upstream of mine site) and the AMD water (100% Mount Morgan Mine pit), and historical metal concentrations recorded from the Mount Morgan Mine pit water, provided in Chapman and Simpson (2005).

waters	Al	Cd	Co	Cu	Pb	Mn	Ni	Zn	Fe
Dee River[a]	0.01	<0.0001	<0.001	0.01	<0.001	0.007	0.003	0.033	<0.05
100% Mine pit[a]	1780	0.291	4.29	101	0.003	173	1.16	51.8	51.8
100% Mine pit (2001)[b]	730	0.15	2.3	35	-	80	0.71	25	-

[a] dissolved metals; [b] total metals recorded Chapman and Simpson (2005). Dashes indicated not tested

Table 5. Dissolved metal concentrations (mg/L) within the 1.5% and 2.5% dilutions, for treatments 0 mg/L AHA and 20 mg/L AHA, during trials 1 collected at 0 hours and trial 2 collected at 24 and 96 hours. Values are made up of a subsample of each replicate pooled together before analysis.

% AMD	Treatment	Trial	hours	Al	Cd	Co	Cu	Mn	Ni	Zn
1.5%	0 mg/L AHA	1	0	0.12	0.0022	0.035	0.071	1.49	0.011	0.264
		2	24	0.10	0.0022	0.034	0.129	1.48	0.011	0.252
			96	0.11	0.0022	0.035	0.112	1.52	0.011	0.271
	20 mg/L HA	1	0	0.13	0.0019	0.033	0.060	1.48	0.011	0.239
		2	24	0.18	0.0016	0.031	0.069	1.44	0.009	0.125
			96	0.17	0.0017	0.032	0.081	1.47	0.010	0.143
2.5%	0 mg/L AHA	1	0	0.09	0.0042	0.064	0.362	2.66	0.018	0.649
		2	24	0.08	0.0040	0.062	0.426	2.54	0.018	0.541
			96	0.09	0.0042	0.064	0.362	2.66	0.018	0.649
	20 mg/L AHA	1	0	0.11	0.0032	0.056	0.069	2.47	0.016	0.360
		2	24	0.05	0.0033	0.057	0.077	2.46	0.016	0.352
			96	0.07	0.0034	0.058	0.079	2.48	0.016	0.359

Figure. 1. % Survival of shrimp in trials 1 and 2 where a) 0 mg/L AHA; b) 10 mg/L AHA; and c) 20 mg/L AHA; and i = trial 1; ii = trial 2. ● = control (0% AMD); ○ = 0.5% AMD; ▼ = 1% AMD; △ = 1.5 % AMD; ■ = 2% AMD; □ = 2.5% AMD. Each point is the mean of three replicates ± standard error (SE).

Figure. 2. % Survival of shrimp in trial 3, where a) 10 mg/L AHA; and b) 20 mg/L AHA; ● = control (0% AMD); ○ = 2.5% AMD; ▼ = 3% AMD; △ = 3.5 % AMD; ■ = 4% AMD; □ = 5% AMD. Each point is the mean of three replicates ± standard error (SE).

Figure. 3. Concentration response plot for Mount Morgan open pit water (AMD) and Dee River water, with 0, 10 or 20 mg/L AHA. Curves represent the non-linear regression (three-parameter sigmoidal) of pooled data from the three trials at 96 hours.

Figure. 4. Concentration response plots showing the upper and lower 95 % confidence limits for a) 0 mg/L AHA; b) 10 mg/L AHA; c) 20 mg/L AHA. Curves represent the non-linear regression (three-parameter sigmoidal) of pooled data from the three trials at 96 hours.

Figure 1
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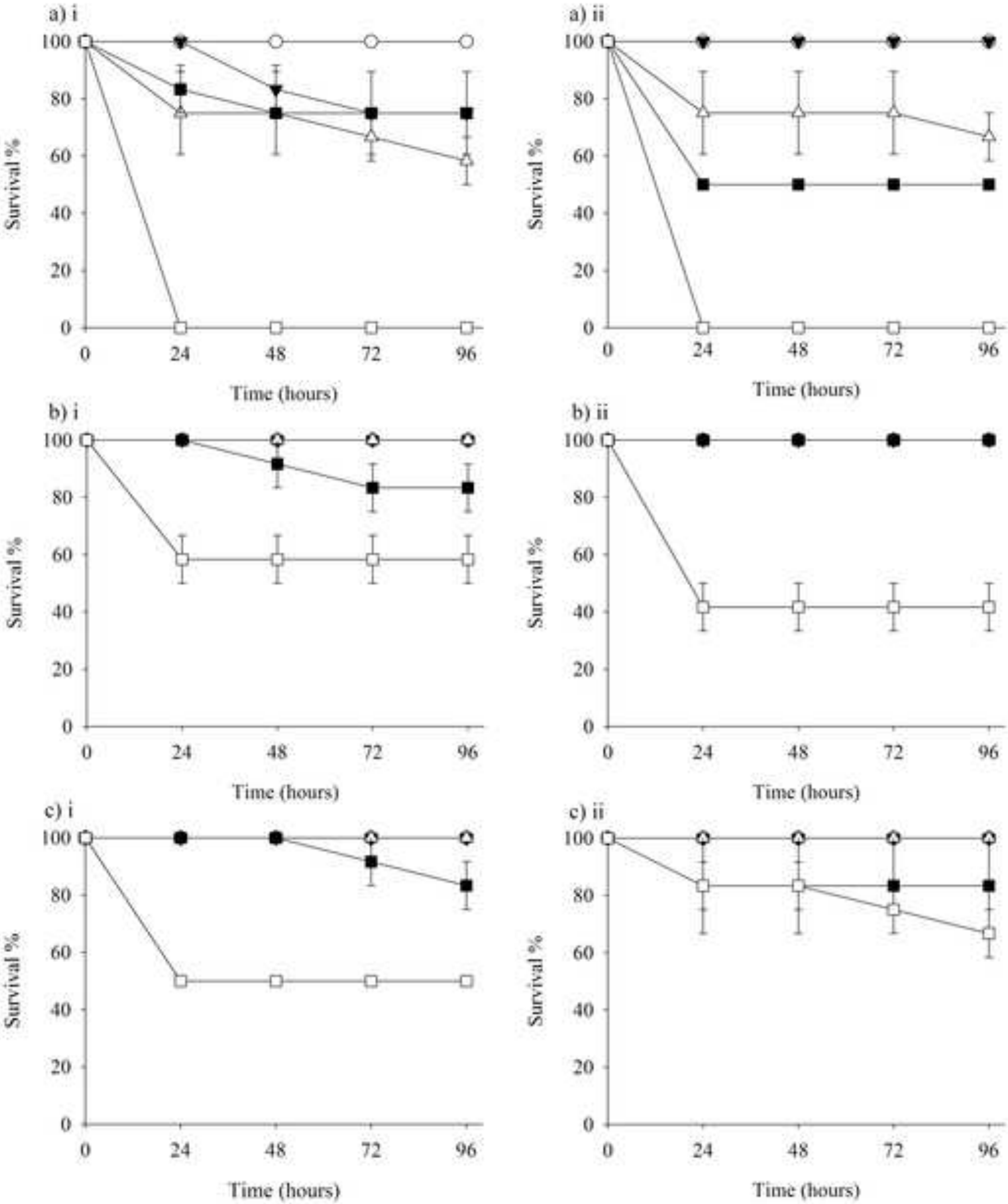

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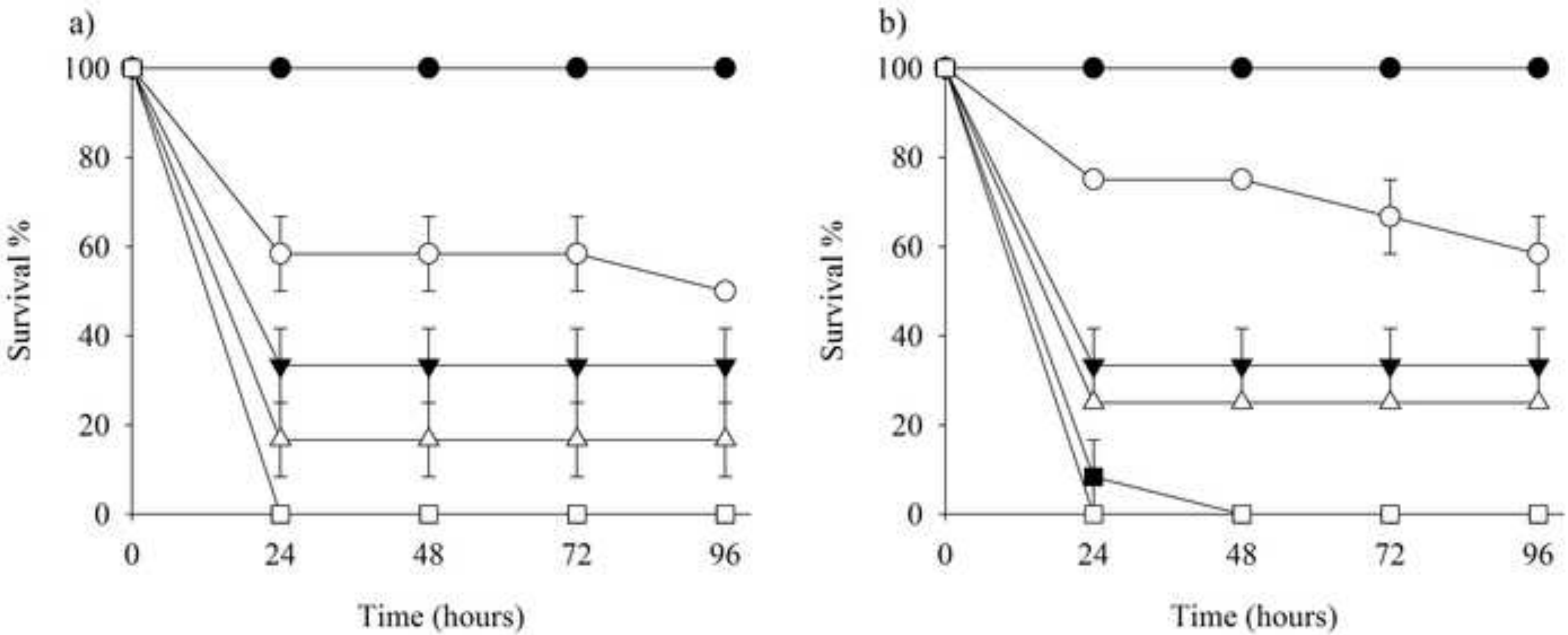

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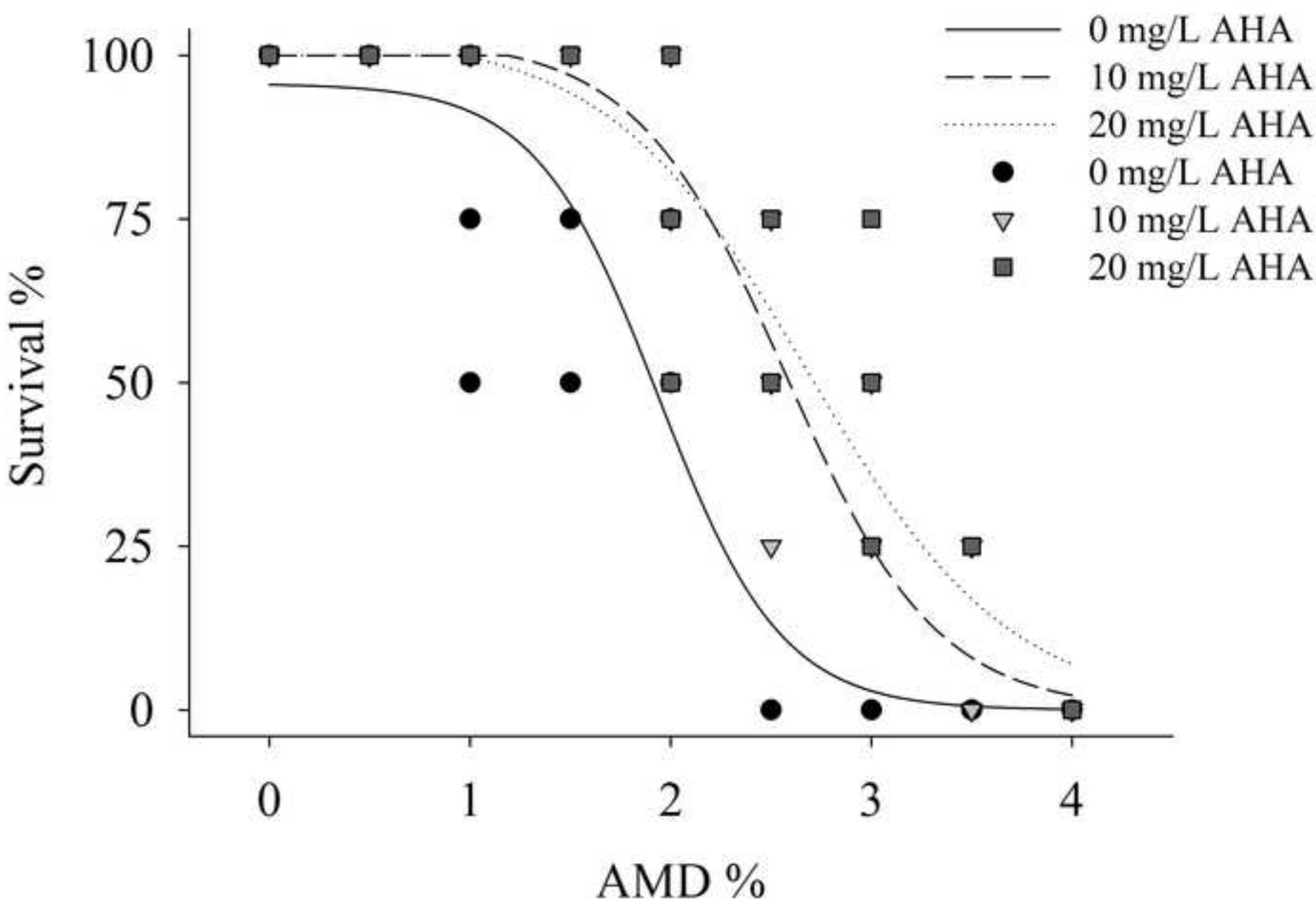

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