A comparison between *Daphnia pulex* and *Hydra vulgaris* as possible test organisms for agricultural run-off and acid mine drainage toxicity assessments

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ABSTRACT

Bioassays, consisting of a diverse selection of organisms, aid in assessing the ecotoxicological status of aquatic ecosystems. *Daphnia pulex* and *Hydra vulgaris* are commonly used test organisms belonging to different trophic levels. The current study focused on comparing the sensitivity of *H. vulgaris* to *D. pulex* when exposed to geometric dilutions of two different water sources, the first (Site 1) from a source containing agricultural run-off and the second (Site 2), acid mine drainage. These sources were selected based on the contribution that the agricultural and mining sectors make to water pollution in South Africa. The bioassay method followed in this study was a modified version of the method described by the USEPA and additional peer-reviewed methods. The mortalities as well as morphological changes (*H. vulgaris*) were analysed using Microsoft Excel. The LC_{50}-values were statistically determined using the EPA Probit Analysis Model and the Spearman-Karber analysis methods. Prior to being used, analysis of the physico-chemical properties, nutrients and metals of both water samples was performed. These results showed a relationship to the results obtained from the *D. pulex* and *H. vulgaris* bioassays, as Site 1 (lower concentration of contaminants) was less hazardous to both test organisms than Site 2 (higher concentration of contaminants). Both organisms can be used for ecotoxicity testing, with *D. pulex* being a more sensitive indicator of toxicity with regards to water sampled from the acid mine drainage site. Due to the sensitivities of sub-lethal endpoints observed over time, *H. vulgaris* may be used for chronic toxicity testing and *D. pulex* for acute toxicity testing.

Keywords: *Hydra*, *Daphnia*, toxicity, ecotoxicity, definitive toxicity test

INTRODUCTION

Man's production and use of chemicals and minerals, and his dependence thereon, has led not only to valuable products and services, but also to the release of numerous hazardous substances into the natural environment (Wharfe, 2005). Over the years, the agricultural and mining sectors have grown in South Africa. Although these sectors provide economic profits, the activities have resulted in increased pollution of South African water sources (Bezuidenhout, 2013). Irrigation and surface run-off water has been found to contain pesticides, fertilisers, harmful chemicals and/or pathogenic microorganisms (Britz and Sigge, 2012). Acid mine drainage (AMD) is characterised by a low pH, high metal concentration, high specific conductivity and a ferric oxyhydroxide precipitate commonly known as 'yellow boy' (Akcil and Koldas, 2006). The aims of ecotoxicology have evolved over the years, from establishing the concentrations at which chemicals exert adverse effects, estimating environmental risk based on measured toxicity endpoints, and predicting environmental concentrations for specific chemicals, to defining toxicant concentrations harmful for specific organism groups and/or for assemblages of species (Blaise and Fèrard, 2005).

The past few decades have produced and utilised a variety of bioassays to assess the toxicity and quality of the surrounding aquatic environment. These bioassays have involved the use of a diverse selection of organisms and can be conducted as acute, sub-chronic or chronic bioassays (Persoone and Janssen, 1993; Cairns, 1995; Slabbert and Venter, 1999; Persoone et al., 2003; Blaise and Fèrard, 2005; Goodfellow, 2005). These bioassays have aided in the establishment and promulgation of water quality criteria (regarding safe release of single chemicals into aquatic ecosystems), providing aquatic safety assessments for chemicals, biomonitoring initiatives, registration of pesticide products, assessing industrial and mine effluent, urban and agricultural run-off and the ranking of chemicals with respect to their hazardous potential (Bitton et al., 1995; Blaise and Fèrard, 2005).

Test organisms that have been used range from plants (*Lepidium sativum*, *Lemna minor*, *Sorghum saccharatum*, *Sinapis alba*) to a multitude of unicellular (*Vibrio fischeri*, *Selenastrum capricornutum*) and small multicellular organisms (*Daphnia pulex*, *Hyalella azteca*, *Chironomus* spp., *Hydra* spp., *Poecilia reticulata*) (Hall and Golding, 1998; Pardos et al., 1999; Gallagher et al., 2005; Goodfellow, 2005; Sanchez et al., 2005; Czerniawska-Kusza et al., 2006; Shuhaimi-Othman et al., 2010). Associated with each bioassay are lethal (mortality) and sub-lethal (e.g. growth inhibition, reproduction teratogenic effects) endpoints, which give an indication of expected toxicity of a contaminant(s) (Suter, 1995; Diaz-Baez and Dutka, 2005; Holdway, 2005; Jonczyk and Gilron, 2005).

Ecotoxicological testing has become a compulsory requirement today as many countries advocate its use to determine the toxicity of effluents, chemicals, metals, wastewaters and solid wastes, and to calculate limits for the discharge of these substances (Persoone et al., 2003). In South Africa, the National Water Act (Act 36 of 1998) (RSA, 1998) governs the protection, use, development, conservation, management and control of its water resources. Stemming from this Act was the National Toxicity Monitoring Programme (NTMP), whose responsibility is to measure, assess

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and regularly report on the state of South African water resources (Murray et al., 2003). The NTMP utilises and promotes the use of bioassays to assess the quality of water resources (Murray et al., 2004).

Globally, *Hydra* species have been used extensively for toxicity testing, i.e., assessing the toxicity of: pharmaceuticals (Pascoe et al., 2003; Quinn et al., 2008a; Quinn et al., 2008b; Quinn et al., 2009), bottled drinking waters (Arkhipchuk et al., 2006), chemicals such as glycol ethers (Bowden et al., 1995), Endosulfan (Pollino and Holdway, 1999), industrial effluents (Blaise and Kusui, 1997) and waste waters (Pardos et al. 1999), and metals (Beach and Pascoe, 1998; Pollino and Holdway, 1999; Karntanut and Pascoe, 2000, Karntanut and Pascoe 2002; Karntanut and Pascoe, 2005). *Hydra* species commonly used are *H. viridissima*, *H. vulgaris*, *H. attenuata H. oligactis* and *H. pseudoligactis* (Bell and Wolfe, 1985; Blaise and Kusui, 1997; Fukuhori et al., 2005; Holdway, 2005).

Holdway (2005) suggests that *Hydra* toxicity testing can be used to determine the teratogenic potential of chemicals in terms of the acute lethality, sub-lethality (morphological changes, behaviour and feeding response), chronic reproductive effects and *Hydra* regeneration effects that are displayed by the test organisms. Hence, these organisms are appropriate for acute and chronic bioassays. Acute toxicity tests are conducted over a maximum of 96 h with the only endpoint being survival/mortality. *Hydra* chronic toxicity tests can be conducted over a period 18–21 days and take into account survival, morphological changes and reproductive capacity (Arkhipchuk et al., 2006). Another test procedure, the *Hydra* reproduction test, occurs over 7 days and evaluates survival and population growth (Holdway, 2005). In a study by Pardos et al. (1999) *H. attenuata* displayed a higher sensitivity to wastewater when compared to the Microtox test (*Vibrio fischeri*).

Daphnia species have been widely used in aquatic ecotoxicology with *D. pulex* being one of the more preferred test species for a number of reasons: these organisms can be easily cultured and maintained; the age of the organisms is always known; biology of *Daphnia* has been thoroughly researched and documented; numerous studies have expressed the sensitivity of *D. pulex* to several chemicals (large toxicity database), their tolerance, ability to outcompete other species and provide the most toxicological information per unit effort (Sprules, 1972; Lynch, 1983; Pennak, 1989; Persoone and Janssen, 1993; Muller and Palmer, 2002; Jonczyk and Gilron, 2005). *Daphnia* species including *D.* pulex have been used internationally in acute and chronic toxicity tests, assessing the toxicity of potentially hazardous chemicals, and bio-monitoring of effluents discharged by industrial companies (Slabbert and Venter, 1999; Jonczyk and Gilron, 2005), municipal wastewater systems (Logue et al., 1989), produced- and receiving waters (Jonczyk and Gilron, 2005), insecticides (Wood and Stark, 2002; Stark and Vargas, 2003, 2005; Zalizniak and Nugegoda, 2006), and metals – zinc in a biotic ligand model (Clifford and McGeer, 2009), copper (Koivisto and Ketola, 1995), nickel (Kozlova et al., 2009; Leonard and Wood, 2013), and lead (Offem and Ayotunde, 2008). *Daphnia* have also been used in various chronic toxicity tests in which they were exposed for a period of 18–21 days. Here, the organisms' survival and total number of young produced were observed (Truter, 1994). *D. pulex* bioassay has also been found to be an alternative to the mouse bioassay due to its advantages and ability to detect cyanobacterial neurotoxins in raw water samples (Ferrao-Filho et al., 2010).

The current study focused on comparing the sensitivity of *H. vulgaris* to *D. pulex* in 96 h bioassays when exposed to geometric dilutions of water samples collected from a source containing agricultural run-off (Site 1) and a source containing acid mine drainage (Site 2).

METHODS

Test organisms and culture maintenance

D. pulex and *H. vulgaris* were obtained from laboratory monocultures in the Aquarium of the Department of Zoology at the University of Johannesburg. The test organisms were cultured in accordance with, and adapted from, the methodologies explained by Truter (1994) and USEPA (2002) for *D. pulex*, and Trottier et al. (1997) and Holdway (2005) for *H. vulgaris*.

The *Daphnia* and *Hydra* cultures were maintained in an environmental room with a constant temperature of 20 ± 1°C and a daily photoperiod of 16 h light and 8 h dark using ambient fluorescent lighting. A *Daphnia* stock solution (also known as *Daphnia* medium) was prepared and used for both cultures (Truter, 1994). Although previous studies utilised a *Hydra* medium for culturing *Hydra* (Blaise and Kusui, 1997; Beach and Pascoe, 1998; Holdway, 2005; Arkhipchuk et al., 2006), better culturing success was achieved for this study using the *Daphnia* medium (Truter 1994). The *Daphnia* cultures were kept in 3 L glass beakers and fed YTC (a suspension of commercial yeast, trout pellets and cerophyll) 3 times a week (Truter, 1994; U.S. EPA, 2002). The *Hydra* cultures were maintained in 1 L circular glass bowls and fed *D. pulex* and freshly-hatched *Artemia salina* nauplii 3 times a week (Sorgeloos and Persoone, 1975; Trotter et al., 1997; Holdway, 2005, Arkhipchuk et al., 2006). Feeding of test organisms were discontinued 48 h prior to and during the bioassays. This practice diminishes the risk of particles in the organism's digestive tract influencing the end result of the toxicity tests.

Water samples

Experiments were conducted using water samples from 2 different sites. Site 1 contained agricultural run-off and Site 2 contained acid mine drainage. Grab water samples were collected, transported on ice, and stored at 0–6°C prior to toxicity testing, as suggested by the relevant standard operating procedures (EC, 1996; USEPA, 2002). Water samples were collected from 2 different projects – one focusing on agricultural pollution and the other on AMD. Names of the sample locations are withheld.

Analytical techniques

Physico-chemical analysis of the water samples is required when conducting bioassays and was performed in 3 parts according to standard operational procedures. Firstly, physical parameters such as pH, dissolved oxygen (DO) (mg/L), percentage oxygen saturation, temperature and conductivity were quantified. These parameters were measured at the beginning of the test with the undiluted samples according to USEPA (2002). Secondly, photometric analysis was used to measure parameters such as ammonium, chloride, nitrate, nitrite, phosphate, sulphate, total hardness and turbidity (EC, 1996; USEPA, 2002). Thirdly, inductively coupled plasma spectrometry (ICP) was conducted to determine concentrations of metals such as aluminium, cobalt, iron, manganese, nickel, uranium and zinc in the water samples (EC, 1996; USEPA, 2002).
The metal concentrations found in the water samples were compared to the Target Water Quality Range (TWQR), Chronic

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TABLE 1
A toxicity classification system for natural and wastewaters (Persoone et al., 2003)

Percentage effect (PE)	Class	Hazard	Symbol
≤ 20%	Class I	No acute hazard	
20% ≤ PE ≤ 50 %	Class II	Slight acute hazard	
50% ≤ PE ≤ 100%	Class III	Acute hazard	
PE 100% in at least 1 test	Class IV	High acute hazard	
PE 100% in all tests	Class V	Very high acute hazard	

Effect Value (CEV) and Acute Effect Value (AEV) guidelines described in DWAF (1996).

Toxicity test procedure and toxicity classification

The acute toxicity test procedure followed in this study was a modified version of the *Daphnia* method described in USEPA (2002) and Truter (1994), and incorporated aspects of *Hydra* toxicity testing (Trottier et al., 1997; Holdway, 2005; Arkhipchuk et al., 2006). The test duration was extended to 96 h, ensuring a better comparison between the two test species. *Daphnia* medium was used as the control- and dilution water in both bioassays. *H. vulgaris* and *D. pulex* were exposed to geometric dilutions (100%, 50%, 25%, 12.5% and 6.25%) of water samples from Sites 1 and 2. To enhance the accuracy of the results, exposures were done in triplicate. The 96th hour LC_{50}-value was calculated for each test using Spearman-Karber analysis and the EPA Probit analysis model (Finney, 1971; Hamilton et al., 1977; Finney, 1978). The primary endpoint (lethal) observed for both bioassays was mortality at 24-h intervals spanning the duration of the bioassays. Morphological change, an additional secondary endpoint (sub-lethal), was observed for *H. vulgaris* at 24-h intervals spanning the duration of the bioassays. Modifications to the test method were aided by Truter (1994); Slabbert and Venter (1999); USEPA (2002); Holdway (2005); Jonczyk and Gilron (2005); and Arkhipchuk et al., 2006. The only modifications were testing both organisms to the same geometric dilution series, and exposing both test organisms for the same test duration, i.e., 96 h. This was done to create a common environment for comparing the organisms and to determine whether the test organism(s) were suitable for toxicity testing, as well as to compare which organism displayed a higher suitability to the ecotoxicity testing. All testing was done in an environmental room with the same controlled conditions described above. *Daphnia* were tested in 50 mL glass beakers with a final dilution volume of 40 mL. *Hydra* were tested in 500 mL glass beakers with a final dilution volume of 300 mL. Five (5) organisms were placed in each beaker, respectively.

Persoone et al. (2003) developed a water toxicity classification ranking system based on mortalities (percentage effect) of test organisms (Table 1). This system was used in this study to rank the water quality of the water samples based on the percentage mortality in the 100% concentration after the 96 h exposures.

Hydras have been shown to display morphological changes in response to contaminants and an unfavourable environment (Wilby, 1988; Holdway, 2005; Quinn et al., 2009). Table 2, designed by Wilby (1988), illustrates the concept where the condition of the *Hydra* is given a score, based on the observed morphology. This score rated the effects of toxicity on the hydroid morphology. The score ranged from 10 (healthy, extended tentacles and body, body reactive) to 0 (body disintegrated). Any score ≤ 5 was concluded to be irreversible and the endpoint for lethality (Blaise and Kusui, 1997; Quinn et al., 2009). Although a subjective observation, previous studies by reputable authors in the ecotoxicity field have utilised this observation and score criterion. As a result, and similar to previous studies, it is only used as a secondary observation with the aim of informing the primary endpoint, i.e., mortality.

TABLE 2
Score criterion based on the effects of toxicity on *Hydra* morphology (Wilby, 1988)

	Score*	Morphology
Reversible	10	Extended tentacles and body, reactive body
	9	Partially contracted, slow reactions
	8	Clubbed tentacles, body slightly contracted
	7	Shortened tentacles, body slightly contracted
	6	Tentacles and body shortened
Irreversible	5	Totally contracted, tentacles visible
	4	Totally contracted, no visible tentacles
	3	Expanded, tentacles visible
	2	Expanded, tentacles not visible
	1	Dead but body and/tentacles intact
	0	Disintegrated

**Scores ≥ 6 until 9 are sub-lethal whilst scores ≤ 5 are considered lethal*

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Statistical analysis

Statistical analysis of the data from the lethality exposure was performed. Spearman-Karber analysis and the EPA Probit analysis model were used to calculate the 96-h LC_{50}-values with 95% confidence intervals (Finney, 1971; Hamilton et al., 1977; Finney, 1978). Graphical representation of the *Hydra* and *Daphnia* sensitivity to toxicity was done using Microsoft Excel. The calculation of the LC_{50} depends on certain factors. The Probit Method, a parametric statistical procedure, requires that the observed proportion mortalities should bracket 0.5, and 2 or more of the observed proportion mortalities must be between 0 and 1. The Spearman-Karber Method is recommended when the data does not fit the Probit model. It is a non-parametric statistical procedure for estimating the LC_{50} requiring that the smoothed adjusted proportion mortality for the lowest effluent concentration should be 0 and for the highest effluent concentration, 1 (USEPA, 2002).

RESULTS

Physical and chemical analysis of water from Site 1 (agricultural run-off) and Site 2 (acid mine drainage) was performed. Table 3 summarises the physico-chemical results that were obtained. For an aquatic ecosystem to optimally support life, a water source should ideally have a pH between 6.0 and 9.0, dissolved oxygen (DO) percentage greater than 40% (preferably 80%–120%) and a DO concentration greater than 4.0 mg/L (DWAF, 1996; U.S. EPA, 2002).

Parameters were measured in duplicate and are expressed as averages. Site 2 presented a lower pH and higher electrical conductivity than Site 1. Additionally, a high concentration of sulphates at Site 2 was noted which may be attributed to being impacted by acid mine drainage (Akcil and Koldas, 2006). Included in Table 3 is the rank of the water samples based on the toxicity (Persoone et al., 2003). Conforming to this hazard classification system, Site 1 presented no acute hazard whilst Site 2 presented a very high acute hazard.

Metal analysis of Sites 1 and 2 (Table 4) revealed elevated levels of aluminium, iron and manganese at Site 2 whilst Site 1 had low metal concentrations when compared to the water quality guidelines for aquatic ecosystems specified in DWAF (1996). Metal concentrations found in the water samples were compared to the TWQR, CEV and AEV guidelines specified in the water quality guidelines for aquatic ecosystems (DWAF, 1996).

The high levels of iron may not only be due to the geology of the sample's location but also as a result of acid mine drainage in the immediate vicinity/upstream (DWAF, 1996; Akcil and Koldas, 2006). From the available information in DWAF (1996), water quality from Site 1 did not exceed the TWQR, CEV and AEV guidelines whilst water from Site 2 had metals that exceeded the acceptable TWQR, CEV and AEV guidelines.

Figure 1 summarises the 96-h bioassay, comparing the responses of *D. pulex* and *H. vulgaris* at each concentration, using water sampled from Site 2. Throughout the exposure duration for Site 1, both test organisms showed zero percentage mortalities in all concentrations. This indicated that water from Site 1 poses no acute hazard to the two species of test organisms (USEPA, 2002; Persoone et al., 2003).

TABLE 3
Physico-chemical water quality analysis of water sampled from Site 1 and Site 2. The toxicity of the sites was ranked according to the hazard classification described by Persoone et al. (2003).

Parameter	Unit	Site 1 (100%)		Site 2 (100%)	
pH		6.3		2.6	
O_2 saturation	%	90.8		97.1	
Dissolved O_2	mg/L	8.5		9.0	
Temperature	°C	17.5		17.4	
Conductivity	mS/m	19.8		181.0	
Ammonium	mg/L	1.5		0.03	
Chloride	mg/L	9.9		20.3	
Nitrate	mg/L	0.6		1.9	
Nitrite	mg/L	0.1		0.08	
Phosphate	mg/L	0.7		> 5.00	
Sulphate	mg/L	20		2 200	
Total hardness	mmol/L	1.02		5.36	
Turbidity	FAU*	87		52	
Rank (From ecotoxicity tests)	Class	***D. pulex***	***H. vulgaris***	***D. pulex***	***H. vulgaris***
	Symbol	Class I 	Class I 	Class V 	Class V 

** Formazin Attenuation Unit*

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TABLE 4
Mean metal concentrations (mg/L) for Site 1 and Site 2, which are compared to the Target Water Quality Range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV) guidelines (DWAF, 1996)

Metal	Site 1	Site 2	TWQR	CEV	AEV
Aluminium	ND*	2.5	≤ 0.005 mg/L (pH < 6.5); ≤ 0.010 mg/L (pH > 6.5)	0.010 mg/L (pH < 6.5); 0.020 mg/L (pH > 6.5)	0.100 mg/L (pH < 6.5); 0.150 mg/L (pH > 6.5)
Cobalt	< 0.015	1.4	**	**	**
Iron	1.4	440.0	Should not be allowed to vary by more than 10% of the background dissolved iron concentration	Insufficient data to derive CEV	Insufficient data to derive AEV
Manganese	0.02	90.0	≤ 0.18 mg/L	0.37 mg/L	1.3 mg/L
Nickel	< 0.015	2.5	**	**	**
Uranium	ND*	0.04	**	**	**
Zinc	< 0.008	0.7	≤ 0.002 mg/L	0.0036 mg/L	0.036 mg/L

Not detected
**No information available*

Percentage mortality of *D. pulex* at Site 2 increased with an increase in sample concentration and exposure time (Fig. 1). After 96 h, the percentage mortality of *D. pulex* from the lowest concentration to the highest concentration was 26.67%, 40%, 46.67%, 60% and 100%. After 96 h, the percentage mortality of *H. vulgaris* from the lowest concentration to the highest concentration was 0%, 0%, 0%, 6.67% and 100%.

After 24 h, all daphnids and hydras in the 100% concentration were dead (Fig. 1). A low percentage mortality was observed with *H. vulgaris* in the 50% sample concentration after 48 h, and remained the same throughout the duration of the test (Fig. 1). Exposure concentrations 6.25% and 12.5% resulted in zero mortality at the 48th hour, with a sudden increase in mortality between the 48th and 96th hour observations for *D. pulex*. *D. pulex* showed more sensitivity to the water from Site 2 as mortalities were observed from the 25% sample concentration at the 24-h reading and increased from this point onwards.

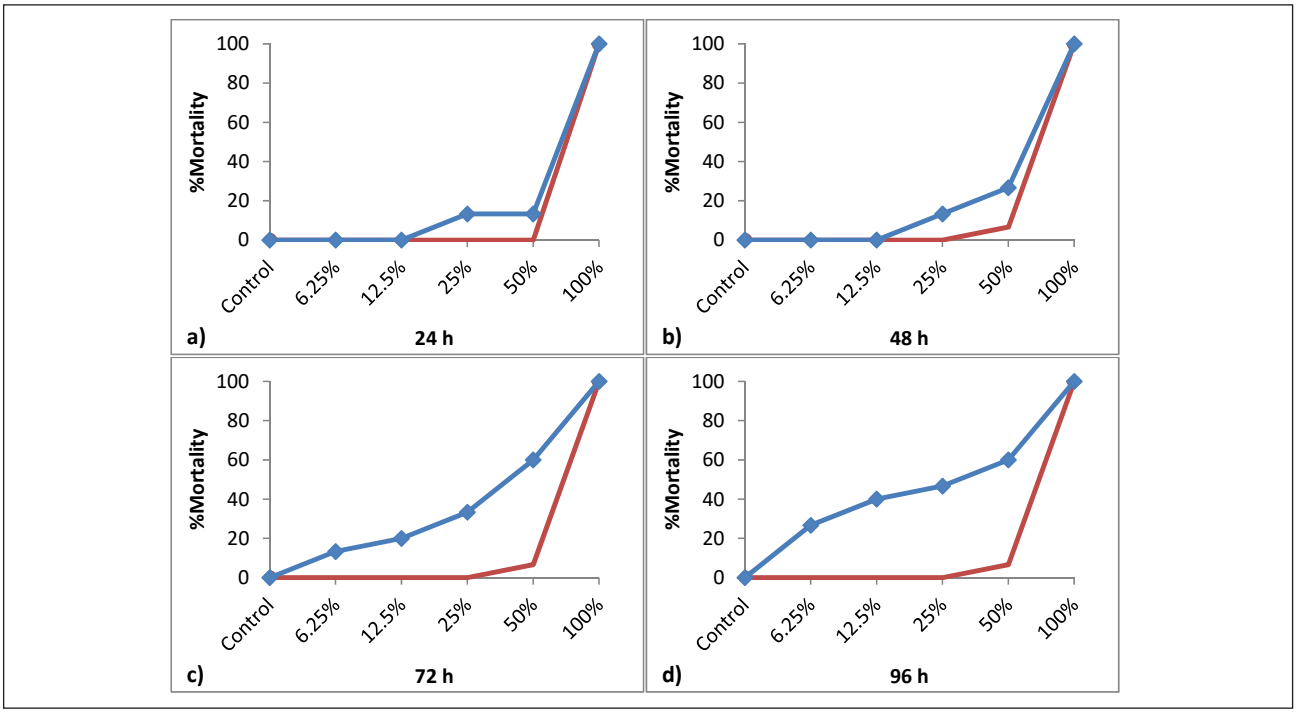

Figure 1
Percentage mortality of Daphnia pulex *and* Hydra vulgaris *observed from the definitive toxicity test with water from Site 2 after (a) 24 h, (b) 48 h, (c) 72 h, and (d) 96 h.* D. pulex — ; H. vulgaris —

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TABLE 5 96 hour LC_{50} values determined using the Spearman-Karber method and EPA Probit analysis				
Site	Spearman-Karber		EPA Probit analysis	
	D. pulex	*H. vulgaris*	*D. pulex*	*H. vulgaris*
1	Minimum required trim is too large; 100.0, therefore SK not calculable	Minimum required trim is too large; 100.0, therefore SK not calculable	Probit model not appropriate for concentration response data	Probit model not appropriate for concentration response data
2	**LC_{50} = 26.49%** 95% lower confidence: 11.13% 95% upper confidence: 63.02%	**LC_{50} = 70.71%** 95% lower confidence limits not reliable	**LC_{50} = 22.54%** 95% lower confidence: 6.552% 95% upper confidence: 63.337%	Probit model not appropriate for concentration response data

The number of hydras increased in the control, 6.25% and 100% concentrations at Site 1. This increase was due to budding (asexual reproduction) which occurs in favourable conditions. There was no change in the number of hydras for concentrations 12.5% to 50% of Site 1. In concentrations 25%, 12.5% and 6.25% (including the control) of Site 2 no mortalities were observed with *H. vulgaris*. There was, however, an increase in *Hydra* numbers through budding (not graphically presented) in both water samples during the test.

Statistical analysis of observations at the end of the bioassays is presented in Table 5. The Spearman-Karber and EPA Probit programs could not calculate the LC_{50} for both organisms exposed to water from Site 1 due to no significant mortalities (conditions as discussed in Methods section: Statistical analysis). Spearman-Karber and EPA Probit determined the LC_{50} for *D. pulex* exposed to water from Site 2 as 26.49% and 22.54%, respectively. Only the Spearman-Karber method could calculate the LC_{50} value for *H. vulgaris* exposed to water from Site 2, i.e., 70.71%, since the data did not fit the Probit model.

Morphological changes of *H. vulgaris* observed during the course of the bioassay were scored using the criteria developed by Wilby (1988) (Table 2) and are presented in Fig. 2. At Site 1 the score began to decrease after 72 h and ranged between 7 and 8 at the end of the test. At Site 2 the score began to decrease after 24 h from the 25% concentration onwards. At the end of the bioassay exposure, the hydra's scores at Site 2 ranged between 0 (in the highest concentration) and 9 (in the lowest concentration). From Fig. 2 it can be seen that *H. vulgaris* showed more sensitivity (morphological changes) at an earlier time at Site 2 than Site 1. This suggested that Site 2 has a higher toxicity than Site 1.

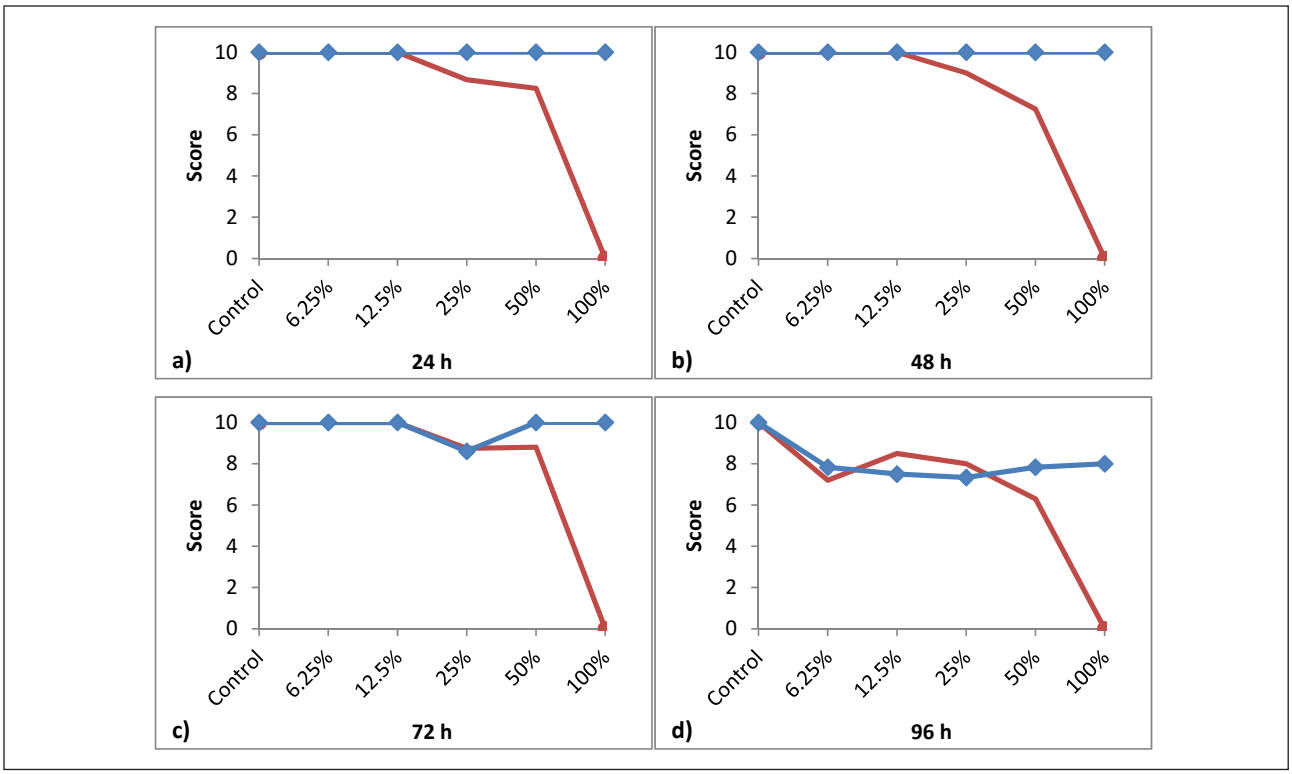

Figure 2

Average score (Wilby, 1988) of Hydra vulgaris *observed from the definitive toxicity test with water from Site 1 and Site 2 after 24 h (a), 48 h (b), 72 h (c) and 96 h (d). (Site 1 — ; Site 2 —)*

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DISCUSSION

According to DWAF (1996) and USEPA (2002), for an aquatic ecosystem to support diverse life forms (and be suitable for biological toxicity testing), a water source should have a pH between 6.0 and 9.0, dissolved oxygen (DO) percentage greater than 40% (preferably 80%–120%) and a DO concentration greater than 4.0 mg/L. Physico-chemical analysis of the two water samples (Table 3) was performed in order to verify results obtained from the bioassays and aid in comparing the sensitivities of *H. vulgaris* and *D. pulex* (USEPA, 2002; Baderna et al., 2011). Site 1 (containing agricultural run-off) presented the constituents and water quality to support aquatic life and to be used for the bioassays, based on the results obtained in this study (Table 3). Site 2 (impacted by acid mine drainage) presented a pH of 2.6, as well as high levels of conductivity and sulphates (Table 3). At such levels the water sample may be rendered unsafe for aquatic life (DWAF, 1996; Lidman, 2005). Elevated levels of these parameters may have been as a result of acid mine drainage and a low pH (DWAF, 1996; Akcil and Koldas, 2006; Liang-qi et al., 2010). The dissolved oxygen measured for this sample was within limits for conducting toxicity tests as required by the standard operational procedure (USEPA, 2002). Both water samples were ranked according to a hazard classification system designed by Persoone et al. (2003) by comparing the response of the test organisms in the 100% concentration after 96 h. A consensus between the physico-chemical results and the results from the bioassay could be seen in Table 3, since water from Site 1 was ranked as Class I, having no acute hazard, whilst water from Site 2 presented a very high acute hazard (Class V).

Metal analysis of the water from Site 1 recorded lower metal concentrations than Site 2 (Table 4). Water from Site 2 presented elevated levels of aluminium, iron and manganese. High concentrations of certain metals such as iron could be attributed to the geology of the surrounding sampling area, acid mine drainage and physical properties of the water (DWAF, 1996; Akcil and Koldas, 2006; Dinelli et al., 2010; Liang-qi et al., 2010). pH values of less than 4.0 and higher than 6.5 increase the solubility of aluminium, which may explain the high concentration at Site 2 (DWAF, 1996). Metals present in the water from Site 1 fell within the limits for Target Water Quality Range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV) guidelines. At Site 2, aluminium, manganese and zinc detected in the water exceeded the TWQR, CEV and AEV resulting in metals being present in concentrations that may be detrimental to aquatic life. This further correlated the physico-chemical results to that of the bioassays. The low pH and presence of other possible toxicants could have contributed to the high mortalities observed during this exposure (U.S. EPA, 2002).

The toxicity test method was a modified bioassay incorporating the methodologies explained in Truter (1994), Trottier et al. (1997), USEPA (2002), Holdway (2005) and Arkhipchuk et al. (2006). Doing so enhanced the comparison between the *H. vulgaris* and *D. pulex* as the only variables were the organisms themselves. Exposures were done in triplicate ensuring a more reliable statistical estimation of the toxicity of the samples and simultaneously minimising the effects of natural deaths to a certain degree. At Site 1, *D. pulex* and *H. vulgaris* showed no mortality in all the exposure concentrations (Fig. 1). From these results, it was concluded that the water from this sample was safe for their survival. According to Blaise and Ferard (2005), *Hydra vulgaris* and *Daphnia pulex* are representative aquatic invertebrates; therefore their sensitivities may be indicative of the toxicity of Site 1 and Site 2 to other aquatic invertebrates. The *Hydra* at Site 1 had population escalations which suggested a favourable environment, as asexual reproduction occurred (Mitchell and Holdway, 2000; Holdway, 2005). The rate at which asexual reproduction occurs when hydras are exposed to a water sample can be used as an indication of its toxicity (Mitchell and Holdway, 2000).

One hundred percent (100%) mortality was observed for both test organisms in the highest exposure concentration at Site 2 after 24 h (Fig. 1a). This observation could be attributed to the very low pH (2.6) and high level of conductivity, phosphates, metals and sulphates (Table 3 and 4) (Blaise and Kusui, 1997; Mitchell and Holdway, 2000; USEPA, 2002; Holdway, 2005). Blaise and Kusui (1997) found that a correlation exists between conductivity (contributed by the presence of metal ions) and the response of *Hydra* – an increase in conductivity spurs an increase in toxicity to *Hydra*. According to Mitchell and Holdway (2000), *Hydra* have been found to display sensitivity to metal and organic contaminants. It was found that further dilutions of water from Site 2 led to organisms being able to survive (Fig. 1). *D. pulex* mortality decreased with the decrease in concentration of the sample (Fig. 1). *H. vulgaris* showed a high sensitivity to the 100% concentration and a lower sensitivity to the 50% concentration of the sample. Further dilutions of water from Site 2 resulted in an increase in the *Hydra* populations (by budding). The dilution of acid mine water created a more favourable environment for the *Hydra* (Bell and Wolfe, 1985; Holdway, 2005). In a study by Loehr et al. (2006), dilution of wastewater discharges resulted in the effluent being less toxic to aquatic organisms when conducting WET (whole effluent toxicity) testing. The decrease in sensitivity could also have been as a result of the *Hydra* metabolising toxicants in the water sample (Quinn et al., 2009) and thereafter adapting themselves to the conditions. It was further observed in this experiment that *D. pulex* had a higher sensitivity to toxicants in water with poor water quality than the hydras.

The LC_{50}-values were determined using the Spearman-Karber (SK) method and the EPA Probit analysis model (Finney, 1971; Hamilton et al., 1977; Finney, 1978). From Table 5 it is evident that the LC_{50} for both test organisms at Site 1 was incalculable due to no significant mortality rate (Refer to Methods section for conditions to the statistical programme). The LC_{50} for the daphnids at Site 2 was calculated as 26.49% (SK method) and 22.54% (Probit Model) (Table 5). The average of these two LC_{50} values suggested that a lethal concentration of 24.52% would kill off half the population of daphnids after 96 h. Using the SK method, the LC_{50} for the hydras at Site 2 was calculated as 70.71% but the 95% confidence limits were not reliable (Table 5). This was due to insignificant mortality response (variability in mortality) in the different exposure concentrations. The Probit model could not determine the lethal concentration for the hydras at Site 2, since there was no considerable mortality response to the different concentrations (Table 5).

The condition of test organisms is a reasonable sub-lethal endpoint as organisms should not only be able to survive but also to thrive in an aquatic environment. Such an endpoint was achieved with the *Hydra* bioassay. Scores presented in Fig. 2 that were less than and including 5 were considered a lethal endpoint (Wilby, 1988; Arkhipchuk et al., 2006). *H. vulgaris* showed an increased sensitivity to water from Site 1 after the 96th hour based on their morphological changes (Fig. 2). This may infer that if *H. vulgaris* had been exposed for a longer duration

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(chronic testing), higher sensitivity and even a population decrease may be observed as was experienced by Arkhipchuk et al. (2006). It also indicated that water from Site 1 presented a low hazard for aquatic life.

The *Hydra*'s net population growth in the acid mine water (Site 2) (not graphically presented) did not necessarily indicate that they had no sensitivity to toxicity, since their morphologies presented scores that suggested their sensitivities over time (Fig. 2). After the 96th hour there was a greater variety of scores. Hence, even with water from Site 2, had the exposure time been extended, significant mortalities at the lower concentrations may eventually have been observed. This experiment provided a platform for future biological toxicology studies in South Africa as both *H. vulgaris* and *D. pulex* displayed sensitivity to water quality and proved to be suitable organisms for the acute toxicity testing method.

CONCLUSION

Advances in ecotoxicology have led to the development of various bioassays utilising a diverse selection of organisms belonging to different trophic levels. *D. pulex* and *H. vulgaris* have been and are currently used as test organisms. The focus of the study was to compare the sensitivities of both organisms and to suggest which organism(s) may be more applicable for acute toxicity testing of aquatic resources inundated/contaminated with either agricultural run-off or acid mine drainage. The study further focused on determining whether *H. vulgaris* may be used concurrently with *D. pulex* when observing possible effects of water samples on organisms belonging to two different trophic levels. This comparison was achieved by simultaneously exposing the organisms to 2 water samples following a modified bioassay method. The physico-chemical and metal analyses of the water samples showed a relationship to the results obtained from the bioassays: in the 100% sample concentration of Site 1 both organisms presented no mortality, whilst in that of Site 2, both organisms presented 100% mortality after 24 h. *H. vulgaris* showed a lesser degree of sensitivity for the endpoint mortality when compared to *D. pulex* but displayed morphological changes in response to toxicity, signifying sensitivity as a sub-lethal endpoint which can be useful for further studies. *Hydra* reproduces asexually in a favourable environment and this was observed in the water from Site 1 and dilutions of water from Site 2 where the number of *Hydra* increased. This may be a useful observation when assessing the acute hazard of a water sample and the effects of dilution on biota. In conclusion, both organisms can be used for ecotoxicity testing with *D. pulex* being a more sensitive indicator of toxicity. Due to the sensitivities observed over time, *H. vulgaris* may be used for chronic toxicity testing and *D. pulex* for acute toxicity testing.

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