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The toxicity of zinc to a selected macroinvertebrate, *Adenophlebia auriculata* (Ephemeroptera, Leptophlebiidae): method development

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The South African water quality guidelines for the protection of the aquatic environment are constantly being updated. The Centre for Aquatic Toxicology of the Institute for Water Research, Rhodes University, uses artificial streams and toxicological methods to contribute to the development and refinement of these guidelines. This study involved the use of 96-hour acute toxicity tests, using zinc sulphate as the toxicant, to determine the suitability of *Adenophlebia auriculata* as a potential indicator species of zinc pollution. As it is known that experimental environments (e.g. still versus flowing water systems) can influence the tolerance of a test species, the experimental system most suited to this species' abiotic requirements was determined. Static and recirculating systems were compared, with LC50 values calculated for the static systems being 91% lower than those calculated for the recirculating systems. The increased sensitivity to the toxicant under static conditions may be due to the animals being additionally stressed by the less favourable static environment. This suggests that recirculating systems are more suited for toxicity tests using this mayfly. *Adenophlebia auriculata* appears to be tolerant to zinc when compared to zinc LC50 values for other test species in the literature, and may therefore not be a suitable indicator of zinc pollution in an aquatic environment.

Key words: artificial streams, *Adenophlebia auriculata*, acute toxicity tests, zinc sulphate

Introduction

Current legislation in South Africa requires that all effluents, subject to certain standards, be returned to the water body of origin (DWAF 1996). While this augments the quantity of water, it also affects the quality of the water as pollutants contained in the effluents are returned to the source waters. In the last decade, the Department of Water Affairs and Forestry (DWAF) implemented the Receiving Water Quality Objectives (RWQO) approach (renamed Resource Quality Objectives), to existing South African water quality management strategies, in an attempt to prevent further pollution and degradation of South Africa's rivers. This approach is based on maintaining a river's fitness for use for specified users (which are categorised into domestic, industrial, agricultural and recreational users), and enables an agreed limit to be placed on the levels of pollution which can be tolerated by the designated user of each water body (National Water Act No. 36 of 1998). Guideline levels or ranges have been published depicting the qualitative assessments of known effects for incremental changes in concentration of selected variables for domestic, agricultural, recreational and industrial users (DWAF 1996). The policy regarding the management of the natural aquatic environment is that the environment is not a 'user' of water, in competition with other users, but is the resource base, without which no development or water use can be sustained (DWAF 1996). To sustain the natural aquatic environment, the ability of the aquatic system to accommodate waste discharge and the effects of the discharges on the aquatic biota must be defined.

In order to evaluate the health of an aquatic environment, managers need to be able to compare current conditions with acceptable levels or guideline values for key variables (Roux *et al.* 1996). Chemical and physical conditions alone are not sufficient to assess the effects of pollutants on an aquatic ecosystem, because the integrated effects of pollutants on the biota are not taken into consideration. Ecotoxicology, which can be defined as understanding the exposure and toxicity of a pollutant on any component of the ecosystem (Gerhardt 1993), utilises toxicity tests, where the responses of aquatic organisms are used to detect or measure the presence or effect of pollutants, in order to set a guideline value. The lack of a generally accepted set of local guidelines has forced scientists and managers to make use of various substitutes, including international guidelines such as the Canadian (CCREM 1987) and Australian guidelines (ANZECC 1992), and the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000). Thus existing local guidelines are currently being expanded and developed for additional water quality variables, and include criteria which are specified to protect against both acute and chronic effects (Roux *et al.* 1996).

The Centre for Aquatic Toxicology of the Institute for Water Research (CAT–IWR), Rhodes University, Grahamstown, uses the experimental methods of ecotoxicology to contribute to water quality management, including providing data for the development and refinement of environmental water quality guidelines (Palmer *et al.* 1996). This includes establishing procedures for the selection and maintenance of indigenous stream macroinvertebrates, and to design suitable experimental procedures (see DWAF 2000 for detailed information). This study was the first metal toxicity research undertaken at the IWR. Ninety six hour (4 days) acute toxicity tests were conducted using *Adenophlebia auriculata*, a leptophlebiid mayfly, as the test species and zinc sulphate ($ZnSO_4$) as the toxicant. Experiments were conducted in three experimental chambers and results compared.

Zinc sulphate ($ZnSO_4$) was chosen as the test toxicant for this study as its chemical speciation is fairly well known and its toxic effects on other aquatic insects is fairly well researched. During chemical speciation, the zinc ion becomes dominant, with sulphate forming a less toxic complex or a precipitate (Wade, Environmentek, CSIR, pers. comm.). Zinc exists mainly as the divalent cation in natural waters, which is the potentially toxic form (CCREM 1987, DWAF 1996), and has been shown to form precipitates on the body, and with mucus onto the gills of *Gammarus pulex* (Crustacean) (Brooks *et al.* 1995). These insoluble precipitates damage the gill epithelium of fish (McKee and Wolff 1963, Mallatt 1985).

It is known that experimental conditions markedly effect the sensitivity of a test species to the toxicant (Buikema *et al.* 1982). Gerhardt (1992) showed that the sensitivity of cadmium to two mayfly species was increased in a flow-through system compared to a static system. In addition to determining the toxicity of zinc to the selected test organism, the objective of this study was therefore to determine which experimental system, static or recirculating, was more appropriate for further metal toxicity studies using *Adenophlebia auriculata* as test organism.

Materials and methods

Test organisms: Collection and handling — Pontasch and Cairns (1991) have suggested that mayflies are amongst the macroinvertebrates most sensitive to pollution, but a potential biological indicator should also reflect the tolerances of other aquatic species in its structural and functional group. Its geographic distribution, availability and abundance throughout the year, along with its abiotic requirements and biology, should also be well researched (APHA 1992). *Adenophlebia auriculata* is locally abundant in the Palmiet River, a relatively unimpacted river (Davison 1993, cited in Haigh and Davies-Coleman 1997) in the Eastern Cape. Collection records from the national collection at Albany Museum, Grahamstown, show that this species' known distribution appears to follow the Drakensberg mountains, occurring from the Eastern Cape coast, up through KwaZulu-Natal and into Gauteng and Mpumalanga. A study by Hunt (1996) did not find *A. auriculata* in the nearby polluted Bloukrans River, despite the availability of physically suitable biotopes, suggesting that this mayfly species is a clean water species and therefore a potentially good indicator of pollution. The biology of this species has also been researched by Haigh and Davies-Coleman (1997).

Adenophlebia auriculata nymphs were collected from the Palmiet River (33°22'S 26°28'E), Eastern Cape, using hand nets. The river has a clear turbulent flow, typical of a mountain stream, and as it is not affected by point source pollution above the collection site, has a good water quality (Table 1).

Nymphs were transported from the collection site to the laboratory in cooler boxes filled with river water. There they were sorted into size classes and those with head widths between 0.9mm and 1.7mm were used in toxicity tests. All injured or late instar mayflies were rejected.

Experimental systems

Static experimental systems: Bubble pots — Plastic 500ml honey jars were used as bubble pots, which were aerated through the lid from a common air source. A square piece of gauze at the bottom of each pot acted as a substrate. Nine to eleven nymphs were placed in each pot and left to acclimate for 36 hours, after which all dead animals were removed and the correct volume of zinc sulphate stock solution was added to make up the relevant concentration for each selected treatment. Each treatment was duplicated. The stock solution of 100.0mg l^{-1} zinc was prepared by adding 0.44g zinc sulphate to 1l dechlorinated tap water (see Table 1 for chemical profile). Dechlorinated tap water served as experimental medium. The zinc solution was stable to precipitation (Wade, Environmentek, CSIR, pers. comm.) and was used throughout the tests. Test concentrations ranged between 0.2mg l^{-1} and 50.9mg l^{-1} zinc. Selected concentrations were based on information available from the literature.

Table 1: Chemical profiles for the Palmiet River and dechlorinated Grahamstown municipal water (TDS: Total Dissolved Solids, TAL: Total Alkalinity, EC: Electrical Conductivity)

Variable	Palmiet River	Dechlorinated tap water
pH	6.7	7.6
TDS	102mg l^{-1}	260mg l^{-1}
TAL as $CaCo_3$	7mg l^{-1}	44mg l^{-1}
EC	16.3mS m^{-1}	47.9mS m^{-1}
NH_4-N	0.03mg l^{-1}	<0.04mg l^{-1}
NO_3+NO_2-N	0.01mg l^{-1}	0.04mg l^{-1}
F	0.1mg l^{-1}	0.1mg l^{-1}
Na	23mg l^{-1}	54mg l^{-1}
Mg	3mg l^{-1}	10mg l^{-1}
Si	3.5mg l^{-1}	3.3mg l^{-1}
PO_4-P	0.008mg l^{-1}	0.008mg l^{-1}
SO_4	37mg l^{-1}	28mg l^{-1}
Cl	16.1mg l^{-1}	95.0mg l^{-1}
K	0.9mg l^{-1}	3.9mg l^{-1}
Ca	1.0mg l^{-1}	14.0mg l^{-1}
Zn	0.000mg l^{-1}	0.066mg l^{-1}
Cu	0.014mg l^{-1}	0.008mg l^{-1}
Al	0.00mg l^{-1}	<0.02mg l^{-1}
Mn	0.000mg l^{-1}	0.002mg l^{-1}

Recirculating experimental systems: Recirculating channels and modified bubble pots — The channels were made of 1m polyvinylchloride (PVC) guttering lengths, with a screen of fine netting at one end, through which the water flows into a 20 litre bucket and is recirculated via a submersible aquarium pump. The current in each channel is approximately 3cm s^{-1} (Binder 1999). The constant recirculation of water aids oxygenation. Two river stones and a strip of plastic gauze are used as a substrate for the animals. See DWAF (2000) for a diagrammatic representation of the channels.

Two modified bubble pots, which are 500ml plastic jars with windows cut on either side and covered with fine netting, were suspended in a bucket at the end of each channel. This allowed for a direct, paired comparison between the bubble pot and channel methods, as there may have been space constraints in the bubble pots. Twenty to 25 nymphs were placed in each unreplicated channel and 9–11 in each replicated modified bubble pot. The organisms were left to acclimate for 36 hours, after which the dead were removed and zinc added to the treatments to a range between 0.2 and 58.7mg l^{-1} zinc.

Experimental procedure — Ninety six hour acute toxicity tests were conducted, and mortality, determined by the lack of response to mechanical stimulation, was defined as the end point. Animals were unfed during testing as 96 hours has been recorded as the no feeding limit for many invertebrate species (APHA 1992). Water was not changed or replaced during the test period (i.e. non-renewal testing) in either the static or the recirculating experimental systems.

As the toxicity of any pollutant is influenced by physical conditions (APHA 1992), physico-chemical conditions (pH, dissolved oxygen, EC and temperature) were measured daily within each treatment for the duration of the test period. A Hanna HI 193 meter and a Hanna pH probe were used to measure dissolved oxygen and pH respectively, and an Amel 160 digital conductivity meter (model 160, graphite electrode model 193) was used to record EC. Nutrient concentrations (ammonium, nitrate, nitrite and phosphate) were recorded spectrophotometrically at the beginning and end of the experimental period using a Merck Spectroquant 118 photometer. Ammonium was analysed by the indophenol blue method, and nitrate by reacting samples with nitrospectral in concentrated H_2SO_4 to produce a dark red-coloured nitro compound. Samples were reacted with sulphanilic acid and N-1-naphthylethylenediamine dihydrochloride to produce a magenta azo dye (Griess' Reaction) in order to analyse for nitrite, and orthophosphate was measured by the phosphomolybdenum blue (PMB) method (Merck Manual Photometer SQ118). These measurements are essential in ecotoxicological studies, as metabolites may increase to unacceptably high levels, resulting in the stress and death of the test organisms (APHA 1992).

Air temperature in the artificial streams laboratory was regulated to approximately 18°C by two Sanyo SA 2465E air conditioners, and light was provided by OSRAM 'biolux' tubes, which provide a full spectrum of wavelengths similar to sunlight (Palmer *et al.* 1994). The lights were on an adjustable daylength timer and set to a photoperiod of 12 hours light, 12 hours dark. Mortality and the frequency of moult was recorded twice daily, and as no water was added to compensate for possible evaporative losses, duplicate water samples were taken throughout the test period from each treatment and analysed using an Atomic Absorption Spectrophotometer (AAS). In order to obtain accurate readings, the concentrations of the samples were diluted to within the optimum concentration range of the spectrophotometer (0.4–1.5mg l^{-1}) (Athanasopoulos 1993).

Experimental design and statistical methods — Experiments were designed to take advantage of the linear regression approach, as opposed to the more traditional Analysis of Variance (ANOVA) approach (APHA 1992). A regression design is used to describe a concentration-response relationship. Linear regression also treats each treatment independently and therefore replicates are not required (Stephan and Rogers 1985). This is advantageous in toxicity testing as the fluctuation in zinc concentration is difficult to control.

The parametric probit method (US EPA version 1.4) was used to calculate the (lethal) concentration at which fifty percent of the population was adversely affected (LC50). It is the most widely used method in ecotoxicology and uses the probit transformation of mortality data in combination with a standard curve-fitting technique (APHA 1992). This method however has some disadvantages. Hamilton *et al.* (1977) suggest that the probit model should not be used for routine analysis of toxicity data. The non-parametric Spearman-Karber method for analysing mortality data is a widely accepted alternative to the probit method. The data in this study was analysed using probit and Spearman-Karber methods for calculating LC50 values, and the two methods were compared.

A chi-square test was used to determine the adequacy of the probit analysis model to the mortality data (Fowler and Cohen 1993). Multivariate ANOVA was used to determine if the physico-chemical conditions and nutrient concentrations changed significantly within a treatment over the 96 hours and to determine if there were any differences between treatments. A paired t-test was used to determine differences between the channels and the modified bubble pots. Statistical tests were conducted at the 95% level of confidence.

Results

No organisms died during acclimation in the experimental system. Control mortalities were 0% in the recirculating systems and up to 9% in the static systems, which are well within the required 10% mortality range (APHA 1992), indicating that the dechlorinated tap water did not influence mortality and was therefore a suitable dilution water.

Physico-chemical and nutrient concentration during the test period — Temperatures in the recirculating systems ranged between 16°C and 17.5°C, with a mean of 16.7°C, and did not differ significantly between the treatments ($p = 0.17$). Temperatures in the bubble pots showed a similar trend, where conditions were similar in all treatments ($p = 0.21$), and temperatures ranged from 18.4°C to 19.9°C. These fluctuations reflected those of ambient temperature changes and therefore were not considered biologically significant.

Conductivity and pH changes showed the same trend as temperature. Conditions were similar in all treatments ($P < 0.1$), and ranged between 7.79 and 6.42 in the recirculating systems, with a mean of 6.95. Values were between 6.51 and 8.26 in the static bubble pots, with a mean of 7.29. Conductivities showed a mean of 35.4mSm^{-1} and tended to increase with an increase in temperature. Dissolved oxygen levels in the channels and modified bubble pots were not significantly different ($p = 0.2$), with a mean of 90.57% in the channels and 88.49% in the modified bubble pots. Dissolved oxygen remained fairly constant both over the test period and within treatments ($p = 0.493$ and $p = 0.890$ respectively). Oxygen levels were not measured in the static bubble pots due to equipment failure, but it is unlikely that oxygen stress alone resulted in increased mortality as the control mortality was only 9%.

Ammonium and phosphate did not increase significantly over the test period ($p = 0.3069$ and $p = 0.548$ respectively) and were lower than the Special South African Effluent Standard in both experimental designs (Table 2). Nitrate and nitrite increased significantly over the test period in all three experimental designs ($p = 0.0163$ and $p = 0.047$ respectively), but these increases were below the Special South African Effluent Standards (Table 2).

Zinc concentration — Zinc concentrations in the recirculating systems showed less fluctuation (average coefficient of variation = 6.2%) than in the static bubble pot system (average coefficient of variation = 17.88%). Fluctuation in the zinc concentration differed significantly over the test period, tending to increase in both systems and ranged between 2.6 and 12.0% ($p = 0.045$) in the recirculating systems and 2.6 and 55.0% ($p < 0.001$) in the static system (Figure 1).

Comparison between statistical methods — The LC50 is the median response of a given test population and is an estimate of the 'true' median lethal concentration of that test material (APHA 1992). The percentage mortality is plotted against zinc concentration for all three systems in Figures 2 to 4 and the LC50 values indicated. The LC50 values and confidence intervals analysed by both methods and for all three experimental systems are shown in Table 3. The values given by the two methods are significantly different in the bubble pots, with a concentration of 4.32mg l^{-1} zinc (42%) separating the two LC50 concentrations.

Comparison between experimental systems — In both the recirculating and the static systems the physico-chemical conditions and nutrient concentrations were within the water quality standards used for comparative purposes (see Table

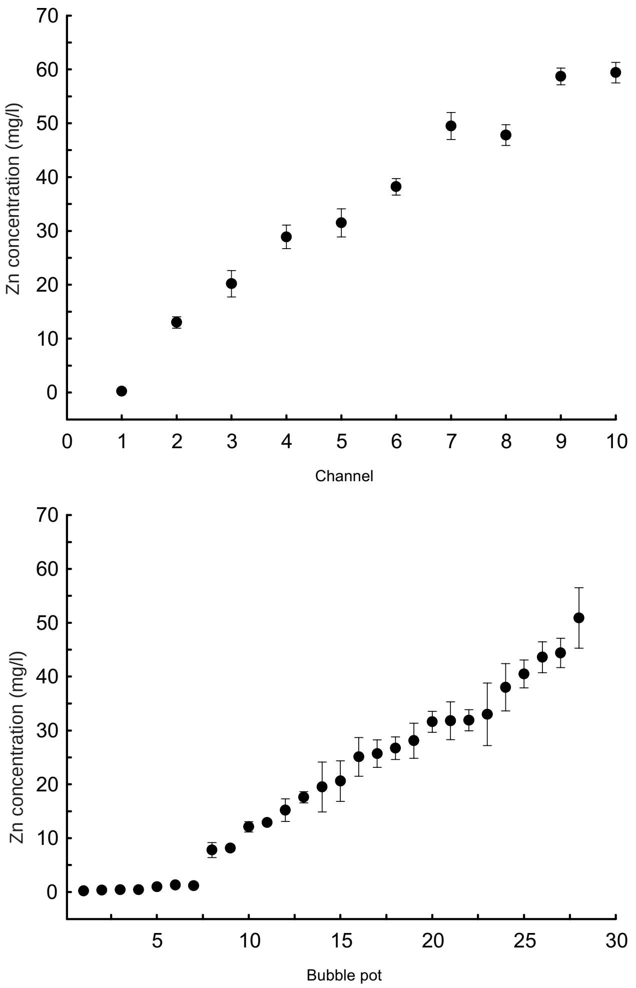

Figure 1: The daily fluctuation in zinc concentration is indicated by the standard error bars for both the recirculating and static experimental systems

Table 2: Physico-chemical and nutrient concentration ranges for each experimental system, and the recommended guidelines (Dallas and Day 1993)

Variable	Channels	Modified bubble pots	Bubble pots	Guidelines
T°	16.0–17.5°C	16.0–17.5°C	17.0–20.5°C	Current SA standard: 35°C
pH	6.42–7.97	6.42–7.97	6.51–8.26	Recommended that the pH should not change by more than one pH unit
DO	71.2–93.5%	84.0–97.1%		Recommended SA minimum: 65% @ 16°C 68% @ 21°C
NH_4	0.01–0.38mg l^{-1}	0.01–0.38mg l^{-1}	0.01–0.41mg l^{-1}	Special effluent standard: 1.0mg l^{-1}
NO_2	0.01–0.06mg l^{-1}	0.01–0.06mg l^{-1}	0.01–0.05mg l^{-1}	UK and USA standard: 0.03–0.06mg l^{-1}
NO_3	0.13–2.68mg l^{-1}	0.13–2.68mg l^{-1}	0.08–2.77mg l^{-1}	Special effluent standard: 6.6mg l^{-1}
P	0.01–0.05mg l^{-1}	0.01–0.05mg l^{-1}	0.02–0.07mg l^{-1}	Special effluent standard: 1.0mg l^{-1}

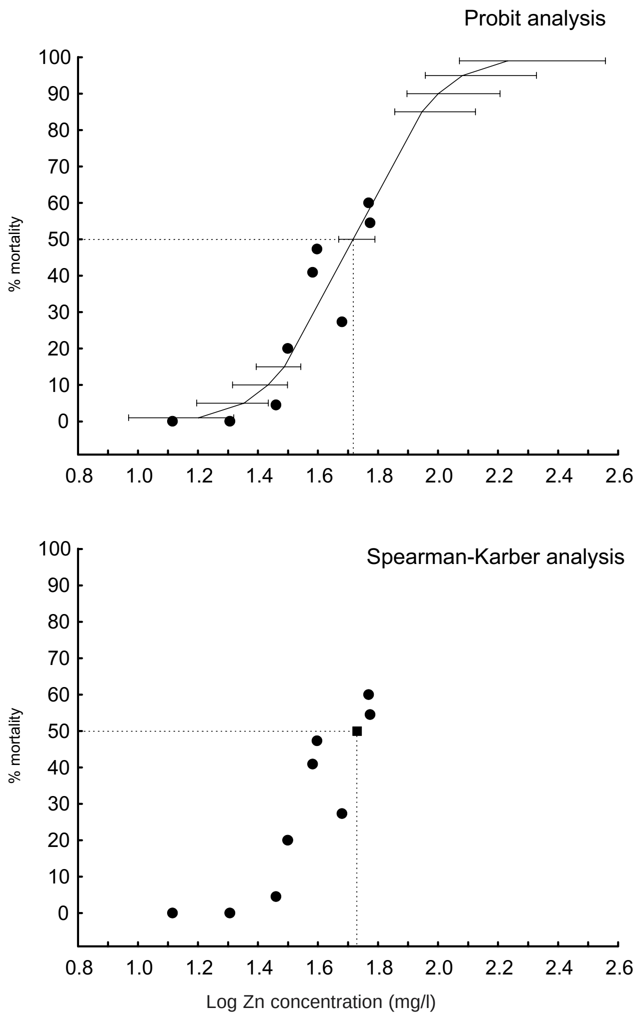

Figure 2: Probit and Spearman-Karber LC50 estimations for zinc in the recirculating channels

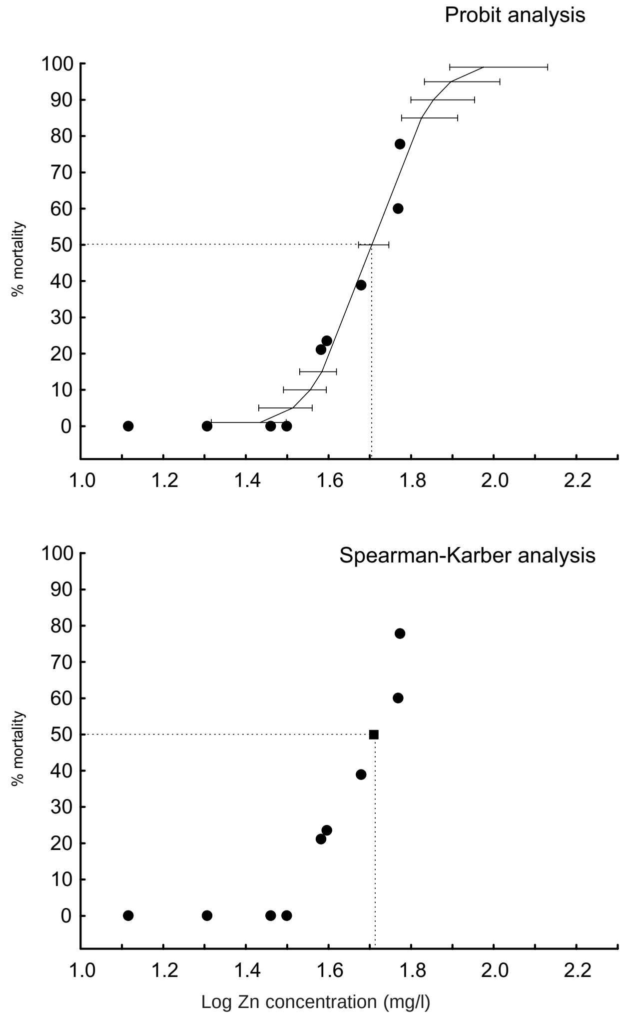

Figure 3: Probit and Spearman-Karber LC50 estimations for zinc in the modified bubble pots

2). The exception is pH, where the fluctuation was greater than 1pH unit in all the experimental systems.

Recirculating experimental systems: Recirculating channels and modified bubble pots — Mortality in the control was 0% for both the channels and the modified bubble pots, and a paired t-test showed that there was no significant difference in mortality between the channels and the modified bubble pots ($P = 0.49$). The result therefore suggests that the stress experienced by the nymphs in the modified bubble pots was similar to that experienced in the channels, and the competition for space in the modified bubble pots did not appear to influence mortality.

Static experimental systems: Bubble pots — The LC50 value calculated for the bubble pots was 5.95mg l^{-1} zinc by probit analysis, and 10.27mg l^{-1} zinc by Spearman-Karber, which is 88.6% and 80.1% lower than the LC50 values calculated for the modified bubble pots (Table 3). The control mortality during the test period was 9%. Based on the measured physico-chemical and nutrient results, and the results of the channels versus modified bubble pots, it is unlikely that the increased sensitivity evident in these results is due to space constraints or fluctuations in measured water quality variables. However, increased sensitivity to zinc may have been aided by reduced oxygen levels. Due to the dependent relationship between zinc and pH, a pH fluctuation from 6.51–8.26 may also have contributed to increased sensitivity to zinc.

Discussion

Artificial streams are used as standard tools for the testing of aquatic organisms, and may include static, recirculating and flow-through systems. Static toxicity tests (in this case, bubble pots) are simple and inexpensive, generate minimum volumes of waste, making waste disposal easier and require minimum laboratory space (Buikema *et al.* 1982). The disadvantages of static systems is that the test duration is potentially shortened due to oxygen depletion and metabolic waste accumulation (Buikema *et al.* 1982, APHA 1992, Brooks *et al.* 1995). This study found that the concentration

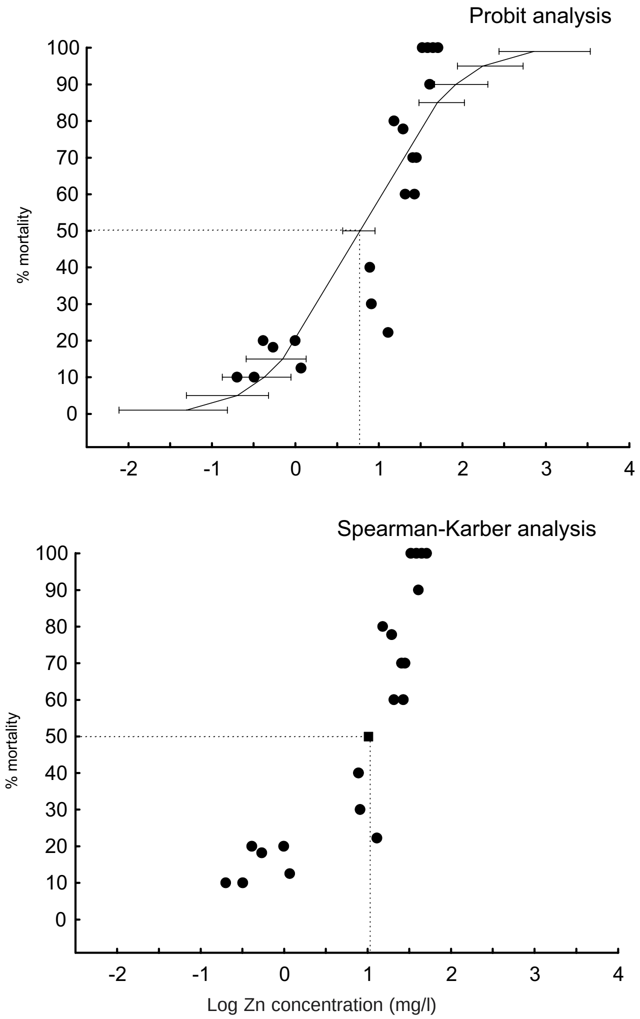

Figure 4: Probit and Spearman-Karber LC50 estimations for zinc in the bubble pots

of zinc fluctuated by as much as 55% over the test period in the static system, and tended to increase over the 96 hours. This is undesirable in a toxicity test, where conditions should be kept constant in order to be able to quantify the effects of the toxicant accurately. Recirculating test systems therefore proved to be more reliable at maintaining high oxygen levels and reducing fluctuations in the toxicant concentration (Figure 1). Organisms from lotic systems can also be used successfully in recirculating systems (Buikema *et al.* 1982). Disadvantages include the expense associated with constructing flowing water systems, high space requirement and electricity demand. They also generate larger volumes of waste, creating a waste disposal problem. Flow-through systems are most efficient at maintaining a constant test concentration throughout an experiment, but systems are more complex and expensive to construct, and generate larger volumes of waste.

However, in order to simulate conditions in the natural environment, the experimental systems must take the test species abiotic requirements into consideration. *Adenophlebia auriculata* occupies a wide range of biotopes in the Palmiet River, occurring in all biotopes with a solid substratum (Haigh and Davies-Coleman 1997). *Adenophlebia auriculata* is essentially a lotic species found in riffles with current speeds up to 20cm s^{-1} (Binder 1999), although it has been shown that current is not a prerequisite for *A. auriculata's* distribution (Hunt 1996), and that as nymphs mature they appear to actively seek out regions of quieter water (Hall *et al.* 1980).

The absence of a current in the static system may have resulted in the slow delivery of dissolved oxygen to the gills, which may have additionally stressed the organisms, leading to increased sensitivity to the toxicant compared to the sensitivity observed in the recirculating systems. In a study by Lowell *et al.* (1995), *Baetis tricaudatus* showed signs of stress at velocities of 0cm s^{-1}, resulting in a lower LC50 value compared to the LC50 values calculated at higher current speeds. However due to the design of the aeration system in the bubble pots, the water was fairly turbulent. The exposure to this turbulence may also have stressed the animals, as in the field the nymphs are found in biotopes which are protected from the direct influence of turbulence. Thus the interaction of a number of variables, such as the possible lack of adequate oxygenation, the small water volume, and the turbulence in the bubble pots may have increased the stress experienced by the nymphs during the test period, making the nymphs more vulnerable to the toxic effects of zinc. The effects of changes in physico-chemical conditions, particularly pH and nutrients, on the sensitivity of *A. auriculata* to zinc contamination, warrants further investigation. It is important to note that the water quality guidelines used for comparative purposes were the Special Effluent Standards. The use of aquatic ecosystem guidelines (DWAF 1996), therefore looking at levels in the receiving environment, are more relevant. However, for inorganic phosphorus and nitrogen these guidelines (DWAF 1996) set the Target Water Quality Range (TWQR) in terms of deviation from background levels normally found in a water body, making

Table 3: Comparison between calculated LC50 concentrations and the confidence interval for all three experimental systems, using two different statistical methods

System		Probit analysis	Spearman-Karber analysis	Difference in LC50 values
Channels	LC50	52.13mg l^{-1}	54.24mg l^{-1}	2.11mg l^{-1}
	95% confidence interval	46.63–61.62mg l^{-1}	46.48–63.29mg l^{-1}	
Modified bubble pots	LC50	50.69mg l^{-1}	51.54mg l^{-1}	0.85 mg l^{-1}
	95% confidence interval	47.09–55.79mg l^{-1}	46.46–57.19mg l^{-1}	
Bubble pots	LC50	5.95mg l^{-1}	10.27mg l^{-1}	4.32mg l^{-1}
	95% confidence interval	3.68–9.01mg l^{-1}	6.17–17.1mg l^{-1}	

the comparison with laboratory-based experimental data difficult. The levels of nitrate reported during this experiment compare favourably with nitrate levels toxic to freshwater insects, reported in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000).

Although *A. auriculata* appears to be suitable for toxicity studies based on its abundance and availability throughout the year, many authors caution against the use of a randomly selected species, as it may not give accurate information on the toxicity of a compound to other species and life stages or to an entire biota (APHA 1992). Bacher and O'Brien (1990, cited in ANZECC 1992) report acute toxicities for Australian species which range from 0.14–6.90mg l^{-1} zinc, and the Australian guidelines for the aquatic environment report that zinc concentrations in fresh water should not exceed 0.005–0.050mg l^{-1} zinc, depending on the hardness of the water (ANZECC 1992). The South African recommended maximum for the aquatic environment is 0.05mg l^{-1} zinc and the reported LC50 for *Daphnia magna* is 0.1mg l^{-1} zinc (Dallas and Day 1993). Comparing the LC50 values in this study (mean LC50 in the recirculating system is 53.2mg l^{-1} zinc) with those in the literature, *A. auriculata* appears to be highly tolerant to zinc. Ideally a test species' sensitivity should only be compared to that of other members in its structural or functional group, because of the vast differences in tolerance between different taxa (Hoekstra *et al.* 1993). Unfortunately information on other leptophlebiid mayfly tolerances to zinc in South Africa is very limited, and equally limited for mayfly species and detritivores in general. Hickey and Vickers (1992) compared the LC50 values between a mayfly species, *Delatidium*, and a *Daphnia* species for a number of heavy metals. They found that all levels of metals tested were comparable with *Daphnia* levels except zinc, which had a significantly lower (96-fold) toxicity for the mayfly based on the comparison of the respective LC50 concentrations. They also found that the mayfly was markedly (200-fold) less sensitive to zinc than it was to the other metals. Other studies on leptophlebiid mayflies suggest that this family is fairly tolerant. *Leptophlebia marginata* was shown to have a 120 hour LC50 of 65–106mg l^{-1} Fe, compared to the 16 hour LC_{50} for *Daphnia magna* which was calculated at 152mg l^{-1} Fe (Gerhardt 1994). This species was also found to be more tolerant with respect to cadmium and lead than the baetid *Baetis rhodani* (Gerhardt 1992). In order to confirm the assumption that *A. auriculata* is not a good indicator of metal pollution, more indigenous leptophlebiid studies must be undertaken.

Conclusions

LC50 values calculated for the static systems were 5–10 times lower than those calculated for the recirculating systems. The increased sensitivity to the toxicant under static conditions may be due to the animals being additionally stressed by the less favourable static environment, and suggests that recirculating systems are more suited for toxicity tests using this mayfly. *Adenophlebia auriculata* appears to be tolerant to zinc when compared to zinc LC50 values for other test species in the literature, and may therefore not be a suitable indicator of zinc pollution in an aquatic environment.

Confidence in a water quality criterion increases with the amount of available data. The more comprehensive and representative the information gathered, the less the chance of deriving standards that are either over-protective or under-protective. Locally derived data are, however, limited for many indigenous species, and international data bases are heavily relied on. This research project therefore demonstrates a method which can be used to determine the tolerances of riffle-dwelling indigenous macroinvertebrates, and in so doing, contributes to the development and testing of water quality guidelines currently being generated for the protection of South Africa's aquatic ecosystems.

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