African Journal of Biotechnology Vol. 7 (23), pp. 4298-4300, 3 December, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Short Communication

The acute toxicity of lead nitrate on *Daphnia magna* Straus

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Accepted 17 September, 2008

In this study the acute toxicity of lead nitrate ($Pb(NO_3)_2$) to *Daphnia magna* Straus was investigated in a static bioassay. After 24 h the mobility of daphnids were examined and immobile ones were counted. The 24 h EC_{50} of lead nitrate to *D. magna* was found as 0.44 mg/L. According to Behrens-Karber method the 24 h EC_{50} of lead was found as 0.51 mg/L.

Key words: Lead, daphnid, toxicity, EC_{50}, probit.

INTRODUCTION

Aquatic ecosystems are the final sink for all potentially toxic metals in the environment via transfer from natural and/or anthropogenic sources. The increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brandao et al., 1992). One of the freshwater species most recommended and used as a standard bioindicator organisms for both water and sediment toxicity bioassays is *Daphnia magna* Straus (Persoone and Janssen, 1993; Lilius et al., 1994; Martins et al., 2007). The extremely fast growth rates, high reproductive rates and short life cycles associated with *Daphnia* were all perceived as positive features for an ideal test organism.

Lead is one of the non-essential and non-biodegradable heavy metals and it is highly toxic to many organisms even at low concentrations (Biesinger et al., 1972; LeBlanc, 1982) and it can accumulate in aquatic ecosystems. This study was aimed to investigate the acute toxicity (24 h) of lead nitrate to laboratory cultured *Daphnia magna* Straus.

MATERIALS AND METHODS

All reagents were of analytical grade and all laboratory glassware were soaked in 10% HNO_3 for at least 48 h and rinsed with distilled water at least 3 times prior to use. Deionised water from a Millipore Milli-Q ultra pure (Milli- Di, France) water system was used through out the study except for daphnid culture.

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The test organism *D. magna* was obtained from the Kepez Aquaculture Research Institute (Antalya, Turkey) and introduced into 30 L aquariums with de-chlorinated tap water, which serves as holding tanks, and maintained with a 12 h light and 12 h dark photocycle at 20.2 ± 1.3°C. The dissolved oxygen leves and electrical conductivity in holding tanks were 6 mg/L and 250 µS/cm, respectively. *D. magna* was cultured and handled according to the procedures outlined in the ISO-6341 (ISO, 1996). Acute 24 h toxicity tests for lead nitrate ($Pb(NO_3)_2$) (Merck) was examined under static non-renewal conditions in 100 mL reconstituted water in 250 ml erlenmayer flasks. Reconstituted water was used as dilution water ($CaCl_2.H_2O$ 290 mg/L, $MgSO_4.7H_2O$ 120 mg/L, $NaHCO_3$ 65 mg/L, KCl 6 mg/L with a pH of 7.1 ± 0.7). Toxicity tests were performed according to the ISO 6341 standart protocol (ISO, 1996). The toxicity is expressed by the initial concentration that inhibits the mobility of 50% of the daphnids during a 24 h period of exposure (EC_{50} 24 h). After 24 h, the mobile ones were counted and after gently shaking the glass containers, the ones that could not move were regarded as immobile.

Twenty neonates (age < 24 h) obtained from the original culture, were introduced into erlenmayer flasks having different concentrations of lead (0.2, 0.4, 0.6, 0.8, 1.0 mg/L). There was no feeding during the test and the containers were slightly aerated (not to disturb daphnids with air bubles). Immobilization was determined visually per 8 hour and dead ones were removed. Exposure to the different concentrations was carried out in triplicate while the control was carried out in duplicate. EC_{50} (24 h) (median effective concentration) values were calculated using a regression line obtained by plotting the concentration (on a logarithmic scale) against the immobilization percentage on a probit scale and the results were evaluated with probit analysis. The data were also evalutaed according to Behrens-Karber method (Klassen, 1991).

RESULTS AND DISCUSSION

Acute toxicity tests indicated that lead at higher concentrations had a detrimental effect on the survival of

Table 1. Acute 24 h toxicity of lead nitrate to *D. magna*.

Point	Concentration (mg/L)	95% Confidence limits	Slope (95% C.L)
EC 1.00	0.128	0.062 - 0.187	4.34 (2.89 - 5.79)
EC 5.00	0.184	0.106 - 0.247	
EC 10.00	0.224	0.141 - 0.288	
EC 15.00	0.225	0.170 - 0.319	
EC 50.00	**0.441**	**0.361 - 0.517**	
EC 85.00	0.764	0.641 - 1.002	
EC 90.00	0.870	0.717 - 1.201	
EC 95.00	1.055	0.841 - 1.581	
EC 99.00	1.514	1.119 - 2.667	

D. magna. The calculated 24 h EC_{50} value of lead nitrate, using a static bioassay system for *D. magna* was 0.44 mg/L. Control mortality was zero and controls did not show any behavioural abnormalities. EC_{50} values calculated with Behrens-Karber method were 0.51 mg/L. The results of acute toxicity test for lead nitrate are presented in Table 1 and expressed as median effectice concentration (EC_{50}). The immobilization observed in the controls was allways 0%.

In the exposure groups it was observed that daphnids showed erractic swimming. The carapax of the dead daphnids were abraded, and in higher concentrations partial ruptures were observed especially in the carapax.

Understanding the problems associated with the degradation of water quality requires detailed knowledge of the state of an aquatic system and the way in which it changes with time. The development of new methods that can be used to identify the presence of toxic susbtances that effect water quality is extremely important to gurantee a continious supply of high-quality water suitable for human consumption. Several of these methods are based on the use of test organisms including fish and invertebrates. Among these, the cladoceran *D. magna* is widely used as a test organism in a variety of ecological studies. *D. magna* is relatively easy to maintain in the laboratory, has a short life cycle, and can be maintained at high population densities in relatively small volumes. Furthermore, it has been studied extensively in a wide range of ecotoxicological investigations (Guilhermino et al., 2000). It is also known to be sensitive to many chemicals that are commonly found in the aquatic environment, and can respond to these susbtances with a variety of physiological and behavioural characteristics (Michels et al., 2000).

Theegala et al. (2007) found that *Daphnia pulex* exposed to lead nitrate at a concentration of 1.0 mg/L completely died. They found the 48 h LC_{50} value of lead nitrate to *D. pulex* as 4.0 mg/L which is very high when compared to our results. This may simply rise from the difference of the chosen daphnid species. LeBlanc (1982) found the 48 h LC_{50} for lead nitrate as 0.15 mg/L for *D. magna*. Biesinger et al. (1972) found 48 h LC_{50} for lead nitrate as 0.45 mg/L. These results are similar to our findings. However, Bodar et al. (1989) stated that high concentrations of lead nitrate (1, 10 and 25 mg/L) had no significant effect on death rates of early life stages and they concluded that early life stages of *D. magna* is more tolerant to heavy metals than adult stage. Gordillo et al. (1998) found the 24 h LC_{50} for lead nitrate as 4.92 mg/L for *D. magna.* As it can be seen form the results mentioned above, there is a great discrepancy.

Khangarot and Reay (1989) found the 24 and 48 h EC_{50} values for lead acetate to *D. magna* as 4.89 and 3.61 mg/L, respectively. Fargasova (1994) found the 24 h EC_{50} value for lead acetate to *D. magna* as 8.32 mg/L. The LC_{50} values for lead acetate are very high when compared to lead nitrate, which may indicate that lead acetate is more toxic to *D. magna*. Also, the bioavailability of lead as lead acetate may be higher than lead nitrate. However, Baudouin and Scoppa (1974) found the 48 h LC_{50} value of lead acetate as 0.6 mg/L for *D. hyalina*. The discrepancy between those studies makes it difficult to interpret. However, it should be kept in mind that acute toxicity of metal ions vary with changes in the water quality (Khangarot and Ray, 1989).

Although, *D. magna* is the most common test organism in aquatic toxicology studies and there are a few standard methods developed to study the acute toxicity of toxicants, there are still discrepancies between different laboratories which may rise from the local tap water quality and differences in the resistance of different *D. magna* stocks, which should be investigated in future studies.

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