



The temperature dependence of the acute toxicity of heavy metals (cadmium, copper and mercury) to a freshwater pond snail, *Lymnaea luteola* L.

Sangita Das✉, A. K. Sharma and Tabrez Ahmad

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Abstract

Responses of a freshwater pulmonate snail *Lymnaea luteola* L. to copper, cadmium and mercury were observed at four temperatures in the range of 15.0 °C to 30.0 °C. Acute static bioassays were carried out at 15.0 °C, 20.0 °C, 25.0 °C and 30.0 °C to determine the percent mortality and median lethal concentrations (LC₅₀) and their 95 percent confidence limits. The acute toxicity of mercury, copper and mercury increased with the increase of temperature from 15°C to 30°C. The 96 h LC₅₀ values and percent mortality indicate that metals at 15°C was least toxic while at 30°C it was highly toxic.

Keywords: Temperature dependence, heavy metals, LC₅₀, snails

Introduction

Temperature is a single important environmental factor having marked influence on the physiology of aquatic organisms (Pechenik *et al.*, 2003, Perschbacher, 2005; Matias-Peralta *et al.*, 2005). In tropical countries freshwater bodies exhibit great variations in temperature. In order to understand the direct effect of temperature prevailing at the breeding habitats on the biology of snail population, it is necessary to undertake studies in controlled laboratory conditions. Such studies will help to understand how snail populations may fluctuate at different seasons of the year. Lymnaeids have been subject of this type of study, due to their varying responses to thermal stresses (Parsharet *et al.*, 1983). Seasonal temperature changes have profound effects on the physiology of ectotherms, resulting in altered toxicity of chemicals (Garnacho *et al.*, 2000). All most ecotoxicological data are produced at a single temperature supposed to reflect the optimal living conditions of an organism, and thus may not easily be extrapolated to other temperatures. The snail *Lymnaea luteola* is used commonly in toxicological

studies its physiology is both temperature and season-dependent (Gupta *et al.*, 1981). However very few data are shown the lethal toxicity of these snails at different temperatures have been carried out (Mathur, 1995). Here, we report the effect of different temperature (15.0°C, 20.0°C, 25.0°C, 30.0°C) to a acute toxicity of Cu, Cd and Hg.

Material and Methods

Adult *L. luteola* were collected from unpolluted ponds from the Gheru campus, Lucknow, and acclimatized in the laboratory conditions for 15 days before the experiment. Almost same size are chosen for toxicity test. They had an average wet weight of 450 mg (range, 350-550 mg) and shell length 21 mm (range 19-25 mm). In this experiment criteria for death were the failure of snails to respond to prodding of their 'foot' with a needle.

Physico-chemical properties of test water: At the beginning of the experiments physico-chemical properties of test water such as pH, total dissolved solids (TDS), dissolved oxygen, and chloride were determined using the routine standard methods (APHA *et al.*, 2002). The temperature of the experimental water was 21±1°C. The mean and range of test water physico-chemical characteristics were as follows: pH 7.5 (7.35-7.65), dissolved oxygen 6.5 (5.8-6.9) mg L⁻¹; total dissolved solids

Author's Address

Protozoology Laboratory, Department of Zoology, University of Lucknow, Lucknow (U.P)
Email: sangita_rea@yahoo.co.in



940 (910-1023) mg L⁻¹; chloride 13 (10-17) mg L⁻¹; total hardness 320 (218-240) mg L⁻¹ as CaCO₃ and alkalinity 180 (170-210) CaCO₃. Dissolved oxygen was determined by Azide modification of Winkler's methods (APHA *et al.*, 2002). Hardness and alkalinity were measured by titrating with 0.01 EDTA solutions and 0.02N H₂SO₄, respectively. Mean and ranges of selected heavy metals (µg L⁻¹) in control test water were: Zn, 5.4 (4.1-6.5); Cu, 4.3 (3.1-5.6), Ni, 4 (3.2-6.8); Fe, 40 (25-59); Cd, 3 (1-6); and Cr, 4 (1-6.1). Heavy metals were determined using a simultaneous multielement atomic absorption spectrophotometer (AAS). Stock solution of Cu, Cd and Hg were prepared by distilled water. We maintained the different temperature in our laboratory at 96 h of experiment. Following this holding period, ten snails were exposed to 500 ml glass beaker for each metal (Cu, Cd and Cd) concentrations. Three replicates were carried out with each experiment. At 24, 48, 72 and 96 h the mortality of snails and the behaviors of the snails were observed. The static bioassay technique was employed in the study. The toxicity solutions and control water were changed after every 24 h. The experimental results were processed for the determination of LC₅₀ values and their 95 percent confidence limits are determined by Harris method, (1959).

Results and Discussion

Control and metal-exposed individuals were temperature-dependent at 15, 20, 25, and 30°C and both metal exposed snails demonstrated significantly increased mortality at 15 and 30°C compared with controls which are presented in Table 1. In our study the percent mortality of snails at temperature 20°C of 96 h of exposure was lower, but at 30°C with the same period of exposure and concentrations, the percent death was higher. The observations indicate that copper, cadmium and mercury toxicity increases with temperature increase. Similar trend were also observed in other experiments tested at different temperature. The toxicity of metals at different environmental temperatures thus varied considerably. In higher copper, cadmium and mercury concentrations, snails spent most of their time at the bottom closing their opercula, and secreting copious mucus and discharge eggs and embryos in the test solution. This secretion of mucus and delivery of eggs and embryos decreased in low copper and cadmium

concentrations and snails remained attached to the wall of the container. In control jars, most of the animals remained attached to the container surface without secreting mucous and discharging eggs and embryos. Temperature is one of the most important environmental factors controlling the distribution of benthic organisms. (Masilamoniet *al.*, 2002). According to Urban, (1994) the temperature tolerance of mollusks complies with their area of distribution. Heat death of organisms occurs due to many reasons such as denaturation and thermal coagulation of proteins, thermal inactivation of enzyme systems, inadequate oxygen supply and /or effects on membrane structure (Nielsen, 1994; Masilamoniet *al.*, 2002). The temperature at 15, 20 and 25° C the snail mortality was close. Our results were similar to other experiments. Gupta *et al.* (1981) observed an increased mortality to copper as temperature increased. It was noticed that raising the temperature from 20.3°C to 32.5°C reduced the 96 h LC₅₀ value of snail from 0.39 to 0.06 ppm of Cu thereby increasing the toxicity by a factor of 6.52. However, a converse situation was obtained when the 96 h LC₅₀ values from other two temperatures were considered. A rise of temperature from 24°C to 27.3°C increased the 96 h LC₅₀ from 0.06 to 0.09 ppm of Cu. Thus the toxicity was decreased by a factor of 1.34. These results are in excellent agreement with the findings of (Wurtz, 1962; Gupta *et al.*, 1981) who also reported that rise in temperature increased the zinc toxicity in hard water to ramshorn snail (*Helisomacapannulatum*). However, the picture becomes somewhat less clear, when the information from other studies of Wurtz, (1962) is considered whereas he claimed that in the case of another species of freshwater pond snail (*Physaheterostropha*), rise in the temperature decreased the zinc toxicity in hard water but had no effect in soft water. Alternatively, crabs maintained at 5 and 25°C experienced their thermal tolerance limits perhaps rendering them more susceptible to copper exposure as suggested by Heugenset *al.* (2001). Crabs exposed to waterborne copper may sustain gill damage resulting in internal hypoxia (Nonnotteet *al.*, 1993). Cairns *et al.* (1975) reported that increase in temperatures may potentiate the toxicants that act on cellular enzymes. The delayed mortality at low temperatures as compared to higher temperatures may be due to changes in several physiological processes especially



respiratory and circulatory rate as also suggested by Cairns et al., (1975). The physiological activities in *L. luteola* increased as temperature are increased up to 35°C. Similar results were recorded for the species *Mytilusedulis* (Bayne et al., 1976); *Pernaperna* (Bayne, 1967); *Dreissenapolymarpha* (Quigley et al., 1993); *L. luteola* (Gupta et al., 1981; Mathur, 1995;); *Pernaindica* (Rajagopalet al., 1995a) and *Pernaviridis* (Rajagopalet al., 1995b). Increased physiological activities in relation to elevated temperature may be due to higher enzyme activity. Brock et al. (1986) studied 13 bivalve species and reported that the activity of digestive enzymes was maximum at temperatures between 24-32°C. However, the maximum activity temperature is species specific. The biochemical activity in the cellular micro environment depends on the molecular mobility within and across the cell membrane which varies directly with temperature (Tayler, 1987). As a result, reduced temperature slowed down biochemical activity by reducing the molecular movement.

The physiological activities were found to be decreasing in relatively low and high temperatures. Mussels (*Dreissenapolymorpha*) were reported to be more susceptible to lower level temperatures than higher level temperatures (Claudi and Mackie, 1993).

Table-1: Effects of temperature on *L. luteola* L. LC₅₀ values and their 95% C.L.

Temperature (°C)	LC ₅₀ values and 95% confidence limits (mg L ⁻¹)			
	24h	48h	72h	96h
Mercury				
15	0.055 (0.049-0.069)	0.055 (0.044-0.067)	0.048 (0.040-0.059)	0.033 (0.026-0.041)
20	0.050 (0.043-0.060)	0.05 (0.043-0.060)	0.0514 (0.043-0.060)	0.049 (0.040-0.059)
25	0.042 (0.034-0.050)	0.039 (0.034-0.047)	0.0297 (0.020-0.049)	0.027 (0.019-0.040)
30	0.092 (0.064-0.139)	0.092 (0.064-0.139)	0.013 (0.008-0.022)	0.013 (0.008-0.022)
Copper				
15	6.988 (6.406-10.761)	0.650 (0.544-0.762)	0.527 (0.433-0.676)	0.344 (0.259-0.420)
20	1.198 (1.132-1.1555)	0.156 (0.105-0.259)	0.107 (0.080-0.130)	0.0927 (0.075-0.122)
25	0.306 (0.254-0.379)	0.094 (0.075-0.122)	0.068 (0.055-0.080)	0.053 (0.046-0.064)
30	0.320 (0.220-0.415)	0.050 (0.042-0.063)	0.032 (0.030-0.046)	0.0310 (0.020-0.046)
Cadmium				
15	96.88 (84.07-123.020)	96.88 (84.07-123.020)	76.76 (62.54-86.14)	54 ----*
20	88.61 (72.81-117.02)	71.03 (57.40-81.98)	59.60 (49.27-68.80)	5.93 (45.88-68.84)
25	92.90 (75.33-113.08)	79.15 (64.44-97.03)	67.39 (55.77-80.09)	62.47 (49.99-72.83)
30	73.66 (62.56-87.14)	29.56 (22.260-37.33)	29.56 (22.263-37.35)	29.57 (22.27-37.36)

LC₅₀, median lethal concentration; Number of replicates = 3



The present study clearly indicates that heavy metals toxicity greatly varies with the temperature. Furthermore, results also indicate the danger of setting water quality standards on the basis of bioassay tests alone without considering the effects of environmental factors on chemical toxicity. The effect of temperature on pollutant toxicity is an important factor to consider when setting individual pollutant standards. Additional experiments with other heavy metals, test organisms and effects of temperatures changes on acute and chronic toxicity are urgently needed to gain more knowledge of temperature effects on chemical toxicity and predict “safe” concentration to save the aquatic organisms.

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