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TOXICOKINETICS AND BIOACCUMULATION OF COPPER AND LEAD IN *CHIRONOMUS* SP. (DIPTERA: CHIRONOMIDAE) AT DIFFERENT TEMPERATURE UNDER LABORATORY CONDITION

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ABSTRACT

The effect of temperature on metal uptake and toxicity in aquatic organisms has been the subject of numerous studies. In the present paper the kinetics of bioaccumulation, variation of LC_{50} over various temperature ranges and uptake factor in *Chironomus* sp, exposed to the sub- lethal concentration of copper and lead after 72 hr and 96 hr of exposure, has been investigated. The experimental results showed that the LC_{50} value of Pb and Cu decreased with increase in temperature for both durations, while the bioaccumulation of lead was more than copper and it increased with increasing temperature and duration. Thus, temperature also influences the toxicity of the heavy metal in the organism along with its dose and duration of exposure, which should also be taken into account while assessing the toxicity of heavy metals.

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INTRODUCTION

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades (Canli *et al.*, 1998; Voegborlo *et al.,* 1999; Dirilgen, 2001; Vutukuru, 2005; Vinodhini and Narayanan, 2008). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi *et al.,* 2007). Due to rapid industrialization heavy metals are excessively released into the environment and this has created a major global concern. Cadmium, zinc, copper, nickel, lead, mercury and chromium are often detected in industrial wastewater, which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, paint manufacture, pigment manufacture and printing (Kadirvelu *et al.*, 2001; El-Shenawy *et al.*, 2010). Pb is not essential for plant growth and considered as toxic at the concentration of 30-300μg g^{-1} in plant tissues. It has received much attention as a hazardous pollutant to human and animals (Roos, 1994; El-Shenawy *et al.*, 2010).

The invertebrates are largely used as a tool for monitoring of chemicals in the environment. Biological, ecological and toxicological characteristics render invertebrates useful in detecting pollution in species habitats, mainly through their bioaccumulation potential, and in evaluation of individual effects of exposure (Lagadic and Caquet, 1998; Michailova, 2010). Among freshwater macroinvertebrates, benthic organisms such as chironomid larvae are considered a promising indicator of water quality because of their ubiquity and abundance in aquatic ecosystems (Pinder, 1986; El-Shenawy *et al.*, 2010). The extraordinary number of species and ecological ranges within this family group adds a high degree of resolution and sensitivity to environmental interpretations as biomonitors (Mousavi *et al.*, 2003). There are several accounts on chironomid tolerance to pollution, responses to contaminants and their use as indicators of water quality (Seire and Pall, 2000). The family Chironomidae has been used to investigate heavy metal pollution (Bervoets *et al.*, 1997) and changes in lake salinity (Heinrichs *et al.*, 2001). Chironomidae larvae have been used as bioindicators of an acid mine drainage in Portugal (Bervoets *et al.*, 2003 ; El-Shenawy *et al.*, 2010).

The main objective of the present toxicological investigation is to study the kinetics of bioaccumulation of heavy metals which occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Bioaccumulation results from a dynamic equilibrium between exposure from the outside environment and uptake, excretion, storage, and degradation within an organism (Zhou *et al.*, 2008). Additionally, heavy metals (particularly lead and copper) have been chosen as reference contaminants in order to illustrate the main ecotoxicological concepts and the complementarity between field and laboratory studies in aquatic ecotoxicology.

MATERIALS AND METHODS

Chironomus sp. was collected from small ponds as well as water-logged regions of Ranchi in polythene bags. Apparently healthy insects were used in the experiment. The insects were kept in four different aquarium in the labarotary in soft artificial water. The first group was kept under normal condition. The second aquaria contained water mixed with Pb (lead), the third one had water mixed with Cu (copper), whereas the last one contained water mixed with equal amount of Pb and Cu each. They were not fed for one day prior to experiment to reduce the faecal matter output and metal sorption onto faecal material during exposure. Each aquarium contained a batch of ten insects in ten liters of water. They were acclimated to laboratory conditions for a short duration prior to experimentation. A pH of 6.85 was maintained.

Lead nitrate and copper sulphate (Sigma) were purchased as a source of heavy metal. Appropriate quantity of these salts was weighed for the treatment. Concentrated nitric acid (Sigma) was required for the digestion of tissue.

The LC_{50} value for Pb, Cu, and Pb-Cu mixture was calculated by probit analysis (according to Finey, 1964) and it was expressed in terms of mg/L. One fourth dose of LC_{50} value was assumed to be its sub- lethal concentration. The effect was observed at different temperature of 10°C, 15°C, 20°Cand 25°C respectively.

The bioaccumulation of metals in test insect was studied after 72 hr and 96 hr of exposure. One gram of insect tissue was weighed and digested in minimum quantity of concentrated HNO_3 and made upto 25mL by adding deionized water. The kinetics of the heavy metals in the whole insect was calculated as per the method of APHA (1993) by inductively coupled plasma spectrophotometer (Perkin Elmer, USA, model DVD 2100). The values was fit into the first order kinetic equation of Widianarko and Van Straalen(1996) with slight modifications.

$$k = \frac{2.303}{t} \text{ Log } \frac{C_o}{C_t}$$

Where C_o is the initial concentration of heavy metal in the water, C_t is the concentration of heavy metal in water at time t, k is the first order rate constant and t is the time in days. The uptake factor of the different heavy metals in the insect was calculated as per the method of Sample *et al*., (1998).

$$UF = \frac{\text{Concentration of metal in the animal (C)}}{\text{Concentration of metal in the animal } (C_o)}$$

RESULTS

The values of LC_{50} calculated from the probit analysis revealed that metals in combination were more toxic than when given individually. Also it decreased with increase in temperature a shown in the Table 1. There is a decreasing trend in LC_{50} of Cu and Pb with rise in temperature and duration of exposure (Fig. 1). The LC_{50} range of Cu after 72 hr was 3.95 ± 0.28 to 3.35 ± 0.24, Pb was 3.69 ± 0.30 to 2.82 ± 0.25 and Cu-Pb mixture was 2.19 ± 0.10 to 1.86 ± 0.09 mg/L respectively at 10°C to 25°C of exposure. The LC_{50} range of Cu after 96 hr was 3.79 ± 0.24 to 2.89 ± 0.28, Pb was 3.02 ± 0.23 to 2.28 ±0.27 and Cu-Pb mixture was 2.04 ± 0.09 to 1.73 ± 0.12 mg/L respectively at 10°C to 25°C of exposure. The results indicate that lead was more toxic to *Chironomus* sp. than copper.

An examination of the 96 hr LC_{50} values for *Chironomus* sp. indicates a rank order of metal toxicity of Pb > Cu. Lead was toxic at all temperatures. However, when insects were exposed to copper and lead simultaneously, it showed synergistic effect as is evident from LC_{50} values (Fig. 1; Table 1).Lead as a pollutant has assumed particular importance due to its relative toxicity and increased environmental

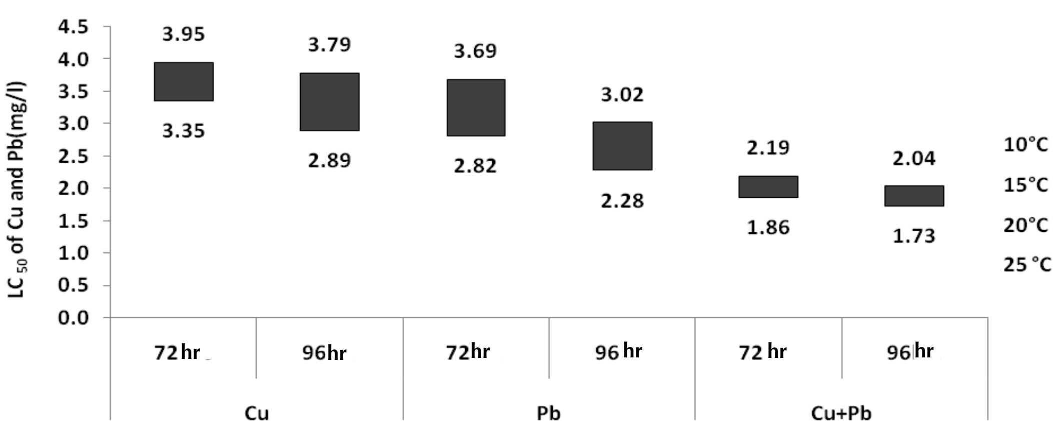

Figure 1: LC_{50} of Cu and Pb at different temperature and duration of exposure. The above plot clearly shows that LC_{50} (mg/L) of Cu and Pb has a decreasing trend both with the increase in temperature and duration. Also when insects were exposed to mixture of salts (copper sulphate and lead nitrate) they acted synergistically and enhanced the toxicity further. Each box represents the value of LC_{50} ranging from 10ºC (upper value) to 25ºC (lower value)

Table 1: LC_{50} of Cu and Pb at different temperature and duration of exposure

	Cu		Pb		Cu+Pb	
Temp.	72 hr	96hr	72 hr	96hr	72 hr	96hr
10°C	3.95±0.28	3.79±0.24	3.69±0.30	3.02±0.23	2.19±0.10	2.04±0.09
15°C	3.8±0.26	3.61±0.24	3.39±0.25	2.85±0.24	2.13±0.10	1.95±0.10
20°C	3.53±0.23	3.61±0.24	3.09±0.26	2.53±0.24	2.02±0.10	1.81±0.10
25 °C	3.35±0.24	2.89±0.28	2.82±0.25	2.28±0.27	1.86±0.09	1.73±0.12

Table 2: Toxicokinetic study of bioaccumulation of different metals in *Chironomus* sp. when exposed to sub lethal concentration at different temperature for 72 hr and 96 hr

	Temp.	C (μg/g)		C_o (mg/L)		C_t (mg/L)		k		$\log(C_o/C_t)$	
		72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr
Cu	10°C	0.228	0.292	9.884	9.473	9.655	9.181	0.008	0.008	0.010	0.014
	15°C	0.290	0.311	9.507	9.038	9.217	8.727	0.010	0.009	0.013	0.015
	20°C	0.312	0.374	8.835	9.038	8.523	8.664	0.012	0.011	0.016	0.018
	25°C	0.340	0.637	8.371	7.245	8.031	6.608	0.014	0.023	0.018	0.040
Pb	10°C	0.377	0.415	9.223	7.540	8.846	7.125	0.014	0.014	0.018	0.025
	15°C	0.411	0.469	8.471	7.121	8.060	6.652	0.017	0.017	0.022	0.030
	20°C	0.529	0.579	7.743	6.320	7.215	5.741	0.024	0.024	0.031	0.042
	25°C	0.619	0.733	7.047	5.694	6.428	4.960	0.031	0.034	0.040	0.060
Cu	10°C	0.313	0.321	7.229	5.093	6.916	4.772	0.015	0.016	0.019	0.028
(Cu	15°C	0.467	0.376	5.334	4.885	4.867	4.509	0.031	0.020	0.040	0.035
+ Pb)	20°C	0.355	0.502	5.047	4.525	4.692	4.023	0.024	0.029	0.032	0.051
	25°C	0.590	0.649	4.642	4.338	4.051	3.689	0.045	0.041	0.059	0.070
Pb	10°C	0.312	0.349	7.229	5.093	6.917	4.744	0.015	0.018	0.019	0.031
(Cu	15°C	0.383	0.433	5.334	4.885	4.951	4.453	0.025	0.023	0.032	0.040
+ Pb)	20°C	0.592	0.666	5.047	4.525	4.455	3.859	0.042	0.040	0.054	0.069
	25°C	0.773	0.861	4.642	4.338	3.869	3.477	0.061	0.055	0.079	0.096

C: Metal uptake at different temperatures in insect tissue after 72 and 96 hr of exposure; C_o: Initial concentration of metal in water; C_t: Concentration of metal in water after time t; k: Rate constant

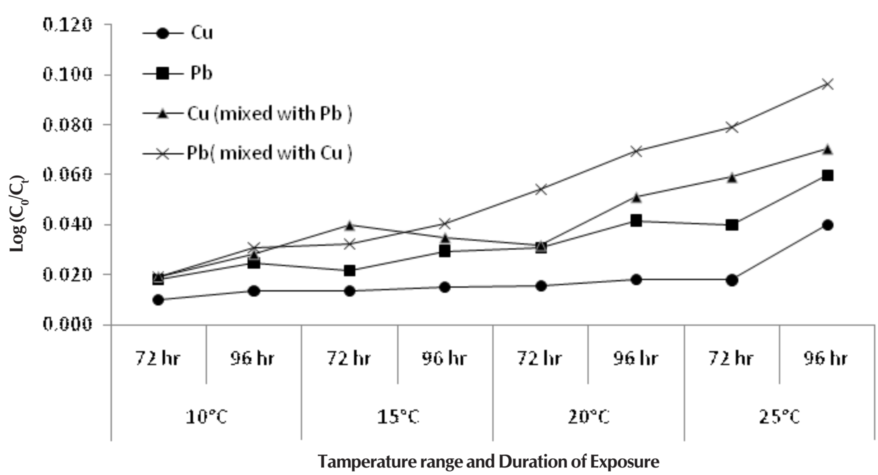

Figure 2 Toxicokinetic study of bioaccumulation of Cu and Pb at different temperature for 72 and 96 hr. Log (C_o/C_t) values calculated indicate that bioaccumulation steadily increased with increasing temperature and duration of exposure. For Cu it increased from 0.010 to 0.018 after 72 hr of exposure. After 96 hr it varied from 0.014 to 0.040. For Pb the values were 0.018 to 0.040 and 0.025 to 0.060 after 72 and 96 hr respectively. The increase was much greater when combination of salts were given.

contamination via car exhaust and highway run-off. Effects of lead in the aquatic environment with the effect of fluctuating temperature, however, have not been studied and relevant literature is scarce.

The bioaccumulation of copper and lead (singly or in combination) in the selected amount of insect tissue, at different temperatures and exposure period was recorded and is presented in Table 2; Fig. 2. The

Table 3: Uptake factor at different temperature and duration of exposure

Heavy metal	10°C		15°C		20°C		25°C	
	72 hr	96hr	72 hr	96hr	72 hr	96hr	72 hr	96hr
Cu	0.07	0.10	0.10	0.11	0.12	0.13	0.13	0.29
Pb	0.13	0.18	0.16	0.21	0.23	0.30	0.29	0.42
Cu (in Cu+Pb)	0.14	0.21	0.29	0.25	0.23	0.37	0.42	0.49
Pb (in Cu+Pb)	0.14	0.22	0.24	0.29	0.39	0.49	0.55	0.66

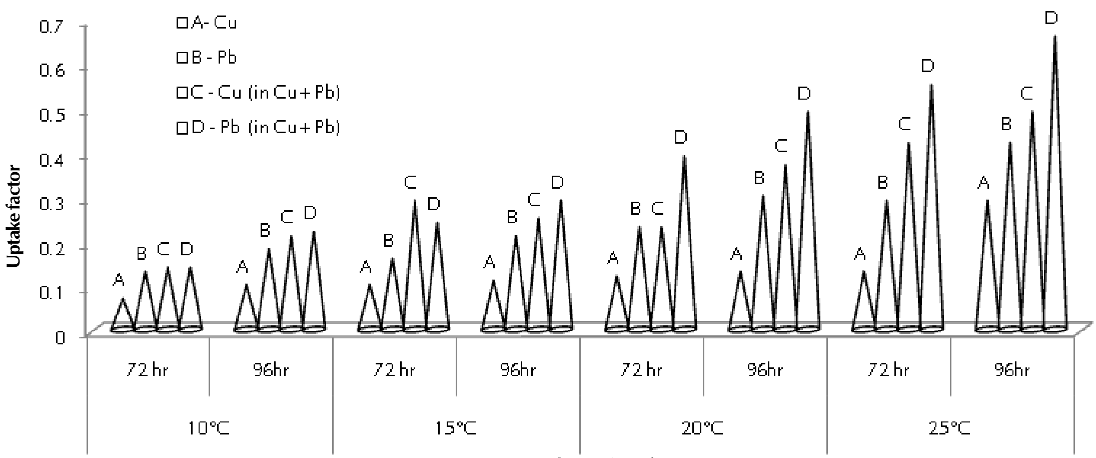

Figure 3: Uptake factor of Pb and Cu at different temperature and duration of exposure. There is a gradual increase of accumulation from 72 hr to 96 hr, with increase in temperature from 10°C to 25°C. The uptake was significantly higher when insects were exposed to mixed metal salts. The Pb uptake, on an average, was significantly higher than Cu at all temperature ranges and duration of exposure.

accumulation of copper varied from 0.228 μg/g (at 10°C) to 0.340 μg/g (at 25°C) and 0.292 μg/g (at 10°C) to 0.637 μg/g (at 25°C) after 72 and 96 hr of exposure respectively. Lead accumulation also reveals a similar pattern of accumulation. After 72 hr of exposure it ranged from 0.377μg/g at 10°C to 0.619 μg/g at 25°C and 0.415 μg/g to 0.733 μg/g at the same temperature ranges for 96 hr. (Table 2; Fig. 2). From the result it is evident that the rate of bioaccumulation, on an average, is maximum for lead. Both copper and lead showed higher accumulation after 96 hr of exposure and at 25°C.

DISCUSSION

Virtually all facets of life history and distribution of aquatic insects are influenced by temperature. It is an important factor determining the distribution, diversity, and abundance patterns over elevation gradients in lentic and lotic water, together with flow regimes that are also very important (Merritt and Cummins, 2005). Temperature, especially, is an important factor affecting metal toxicity, since most aquatic organisms are poikilothermic (Hilmy *et al.*, 1987).

In the present investigation two potent heavy metals, Cu and Pb, that is predominantly present in almost all the aquatic ecosystems, have been chosen, that have profound influence not only on the water quality but also in the life of aquatic insects, causing physiological, behavioral and histopathological alterations. The toxicity of lead is dependent not only on its concentration but also on several other physiochemical factors like pH, salinity, hardness, chemical speciation, temperature etc. Although copper is important, it is toxic when concentrations exceed that of natural concentration ($<0.05\mu$ mol/L) (Stouthart *et al.*, 1996). The concentration of a pollutant in an organism is the result of many variables such as its concentration in the water, the membrane permeability of the organisms, the type and quantity of food and its degree of contamination, the physiological state of the organism and the characteristics of the physical environment, influencing the organism as well as pollutant (Ravera, 2001). Research and literature concerned with assessing toxicity with insects has expanded significantly in the past few years. A review of literature indicates a substantial database existing on aquatic insect responses to nutrients, pesticides and other physiochemical perturbations (Ristola *et al.*, 1996; Stuijfzand *et al.*, 1998; Mafalda *et al.*, 2007). Aquatic invertebrates can serve as indicators of water quality. They accumulate metals in their bodies in high enough concentrations that are detectable whereas the concentration of these metals in the water is too low to be noticed.

The mortality of *Chironomus* sp. increased with increasing copper and lead concentrations and temperature regimes during the calculation of LC_{50} value. However, the toxicity of copper and lead to *Chironomus sp.* decreased with decreasing temperature. These observations are confirmed by toxicokinetic studies of bioaccumulation of copper and lead at different duration, which showed that copper and lead together acts synergistically and accentuated the rate of absorption. The possible reason for the above observation is that ionized heavy metals interact with the charged ligands present on the external surface of cell membranes (glycoprotein, proteins, and polar heads of lipids) and neutral species move across the lipid bilayer by passive diffusion (Blust *et al.*, 1987). Pb and Cu might also interact with $Na^+/K^+/Ca^+$ ATPase channels and increase their reabsorption (Solioz and Vulpe, 1996). Literature surveyed indicated that one of the possible route of lead and copper uptake is through calcium channel, while there is partial inhibition of sodium and potassium pumps (Rogers *et al.*, 2005), resulting in homeostasis disturbances of ionic contents affecting the overall metabolism leading to stress condition.

Using the results of the internal concentration of metal in animals and the concentration in the water, it is possible to calculate the uptake factor (UF). The uptake factor(UF) of the heavy metals at a steady state (Landrum *et al.*, 1992), given in Table 3, reveal that lead showed the maximum uptake factor and that too when applied in combination with copper salt (Fig. 3). Even copper showed significant increase in uptake when applied in combination with lead salt. This clearly indicates that environmental hazard of a toxicant does not depend solely on its concentration in the environment, but on several other factors including temperature, duration and concentration of toxic substance. The study also showed that not only the uptake of lead was more than copper; its bioaccumulation was also higher. Thus lead proved to be more toxic than copper because of its ability to accumulate in the tissue.

In the present study it was also observed that the activity of the insects deteriorated after 96 hr of exposure. The decline in the body functions can be associated with the lowering of the metabolic activity that has inhibitory effect on growth of an organism.

Increase in temperature leads to an increase in toxicity. Rao and Khan (2000) observed that respiration rate increased with increase in water temperature at 15, 20, and 25°C, in Zebra mussels, along with the increase in the toxicity of copper. Similar increase in heavy metal toxicity may also accompany global warming which is expected to raise surface water temperature by 2°C to 3°C.This is a major factor of trouble to the aquatic flora and fauna. Temperature can influence the process of absorption, storage, metabolism and excretion of metals and can thus increase or decrease the toxicity of a given metal to an organism. Thus, contamination of aquatic ecosystem with heavy metals should be monitored and controlled in correlation with fluctuation in temperature (Dallas and Day, 1993). Insects have a largely unexploited potential as biomonitors of metal contamination in nature.

A better understanding of the physicochemical and biological mechanisms mediating trace metal bioavailability and exchange will facilitate the development of general predictive models relating trace metal concentrations in insects to those in their environment. Such models will facilitate the use of insects as contaminant biomonitors (Hare, 1992).

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