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Temperature – Dependent Toxicokinetics of Copper and Lead in *Notonecta glauca* L. (Hemiptera: Notonectidae) Under Laboratory Condition

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Abstract: Temperature is a critical factor due to its effect on both physiology of the organism and the environmental chemistry of the metals. The present study focuses on the toxicity and the bioaccumulation kinetics of Cu and Pb in *Notonecta glauca* exposed to heavy metal contaminated water system at different temperatures. The result showed that the accumulation of heavy metal gradually increases with increase in temperature and duration of exposure while there was a decrease in LC_{50} value of Pb and Cu. The calculated uptake factor of lead at different temperatures showed that it has a higher uptake potential than copper.

Keywords: Temperature, Uptake factor, Bioaccumulation, Toxicokinetics.

1. Introduction

The aquatic environment with its water quality is the principal factor controlling the state of health and disease of all organisms. With the rise of industrialization, there is a steady increase in toxic outputs. The efflux of the waste chemical and agricultural drainage system represents the most dangerous chemical pollution. From the point of view of water pollution, the most important heavy metals are Cu, Pb, Zn, Cd, Hg, Ni and Cr. While some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals to living organisms, majority is toxic at higher concentrations. Others, such as Pb and Cd have no known biological function but are toxic elements[1].

The aquatic invertebrates including aquatic insects are largely used as a tool for monitoring of chemicals in the environment in terms of biological, ecological and toxicological characteristics in detecting pollution in species habitats, mainly through their bioaccumulation potential, and in the evaluation of the individual effects of exposure[2-3].

The logical protocol for evaluating the bioavailability of a heavy metal is to assess its concentration in the test organism. Although, it is not a simple process but depends upon several interlinked physiological processes, like biokinetics of uptake, storage and excretion[4]. The rate of all these processes will govern the accumulation of a particular heavy metal and its toxicity[5]. The vulnerability of a chemical does not depend only on its concentration in the environment. Therefore it is essential not only to understand the pathways and the mechanisms through which a chemical enters the organism but also of its bioaccumulation potential[6]. In this regard, kinetic models are proving to be very useful tool[7].

2. Materials and Methods

Notonecta glauca was collected from freshwater streams of Ranchi in polythene bags. Healthy and mature insects were used in the experiment. The insects were brought into the laboratory and were kept in four glass aquaria maintained with soft artificial water. In the first one, the insect was kept under normal conditions. The second aquaria contained water mixed with Pb [$(PbNO_3)_2$ ≥99%, Sigma], the third one had water mixed with Cu [$(CuSO_4.5H_2O)$ ≥98%, Sigma], whereas the last one contained water mixed with equal amount of Pb and Cu salts. Each experiment was with a batch of ten insects kept in ten liters of water. Insects were acclimated to laboratory conditions for a short

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duration prior to experimentation. A pH of 6.85 was maintained.

An appropriate quantity of salts was weighed for the treatment. Concentrated nitric acid (Sigma) was required for the digestion of tissue. In the present investigation, Probit analysis was performed[8] to calculate LC_{50} value for Pb, Cu and Pb-Cu mixture in mg/l. One-fourth dose of LC_{50} value was given to the test insects to see the accumulation of heavy metals in the insects. The effect was observed at different temperature of 10^0C, 15^0C, 20^0C and 25^0C respectively.

Metal uptake in test insects was studied after 72 hours and 96 hours of exposure. One gram of insect tissue was weighed and digested in minimum quantity of concentrated HNO_3 and made up to 25ml by adding deionized water.

The bioaccumulation of the heavy metals in the whole insect was analyzed[9] by inductively coupled plasma spectrophotometer (Perkin Elmer, USA, model DVD 2100). The data fitted into the first order kinetic equation with slight modifications[7].

$$k = \frac{2.303}{t} \text{Log} \frac{(C_o)}{(C_t)} \qquad (1)$$

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[10] as given below:

$$UF = \frac{\text{Concentration of metal in the animal (C)}}{\text{Concentration of metal in the water } (C_0)} \qquad (2)$$

3. Results

LC_{50} derived from the probit analysis revealed that metals in combination were more toxic than when given individually. Also, it decreased with increase in temperature as shown in Fig. 1. There is a decreasing trend in LC_{50} of Cu and Pb with rise in temperature and duration of exposure.

The LC_{50} range of Cu after 72 hours was 1.1 ± 0.101 to 0.762 ± 0.099, Pb was 1.123 ± 0.125 to 0.737 ± 0.101 and Cu-Pb mixture was 0.609 ± 0.059 to 0.404 ± 0.052mg/l respectively at 10^0C to 25^0C of exposure. The LC_{50} range of Cu after 96 hours was 1.012 ± 0.095 to 0.730 ± 0.112, Pb was 0.922 ± 0.103 to 0.640 ± 0.110 and Cu-Pb mixture was 0.507± 0.051 to 0.355 ± 0.057mg/l respectively at 10^0C to 25^0C of exposure. The results indicate that lead was more toxic to *Notonecta glauca* than copper.

An examination of the 96 hour LC_{50} values for *Notonecta glauca* indicates a rank order of metal toxicity of Pb > Cu. Lead was toxic at all temperatures. Lead as a pollutant has assumed particular importance due to its relative toxicity and increased environmental contamination via car exhaust and highway runoff. Effects of lead in the aquatic environment with the effect of fluctuating temperature, however, have not been studied and relevant literature is scarce.

Mortality was not observed during the experimental period. 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 1; Fig. 2.

The accumulation of copper varied from 0.290µg/g (at 10^0C) to 0.427µg/g (at 25^0C) and 0.368µg/g (at 10^0C) to 0.820µg/g (at 25^0C) after 72 and 96 hours of exposure respectively. Lead accumulation also reveals a similar pattern of accumulation. After 72 hours of exposure, it ranged from 0.396µg/g at 10^0C to 0.794µg/g at 25^0C and 0.422µg/g to 0.888µg/g at the same temperature ranges for 96 hrs. An increase in the value of log (C_o/C_t) was also observed with the increasing temperature and duration. This showed that the bioaccumulation of these metals was enhanced at higher temperature and also their toxicity increased with the duration of exposure due to higher accumulation. Insects, when treated with these heavy metals in combination, showed elevated accumulation for both copper and lead (Table 1; Fig. 2).

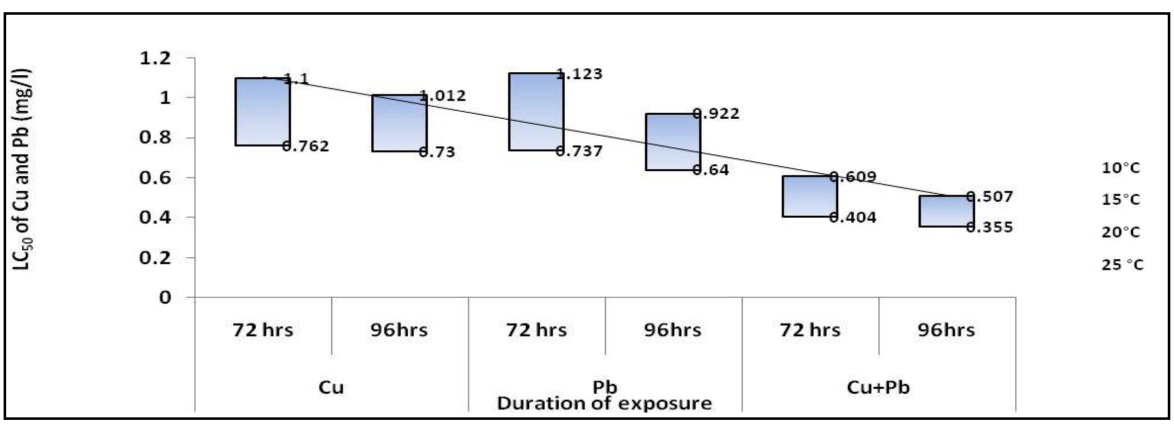

Fig. 1. The above plot clearly shows that LC_{50} (mg/l) of Cu and Pb has a decreasing trend both with the increase in temperature and duration. Also when insects were exposed to a mixture of salts (copper sulphate and lead nitrate) they acted synergistically and enhanced the toxicity. Each box represents the value of LC_{50} under the temperature range of 10^0C (upper value) to 25^0C (lower value).

Table 1. Toxicokinetic study of bioaccumulation of different metals in Notonecta when exposed to its sublethal concentration at different temperature for 72 hrs and 96 hrs.

	Temp.	C (μg/g)		C_o (mg/l)		C_t (mg/l)		k		Log (C_o/C_t)	
		72 hrs	96 hrs	72 hrs	96 hrs	72 hrs	96 hrs	72 hrs	96 hrs	72 hrs	96 hrs
Cu	10°C	0.290	0.368	2.750	2.530	2.460	2.163	0.037	0.039	0.048	0.068
	15°C	0.372	0.454	2.615	2.283	2.244	1.829	0.051	0.055	0.067	0.096
	20°C	0.452	0.554	2.310	2.072	1.858	1.519	0.073	0.078	0.095	0.135
	25°C	0.427	0.820	1.905	1.826	1.478	1.006	0.085	0.149	0.110	0.259
Pb	10°C	0.396	0.422	2.807	2.306	2.411	1.885	0.051	0.050	0.066	0.088
	15°C	0.415	0.488	2.599	2.080	2.184	1.592	0.058	0.067	0.076	0.116
	20°C	0.676	0.770	2.180	1.848	1.503	1.078	0.124	0.135	0.161	0.234
	25°C	0.794	0.888	1.843	1.601	1.049	0.714	0.188	0.202	0.245	0.351
Cu (In Cu-Pb mixture)	10°C	0.411	0.677	1.524	1.268	1.113	0.591	0.105	0.191	0.136	0.332
	15°C	0.524	0.701	1.418	1.156	0.894	0.455	0.154	0.233	0.201	0.405
	20°C	0.587	0.749	1.134	1.103	0.546	0.354	0.243	0.284	0.317	0.493
	25°C	0.751	0.839	1.011	0.887	0.260	0.048	0.453	0.729	0.590	1.265
Pb (In Cu-Pb mixture)	10°C	0.429	0.669	1.524	1.268	1.095	0.599	0.110	0.188	0.144	0.326
	15°C	0.453	0.714	1.418	1.156	0.965	0.441	0.128	0.241	0.167	0.418
	20°C	0.676	0.808	1.134	1.103	0.458	0.295	0.302	0.329	0.394	0.572
	25°C	0.930	0.840	1.011	0.887	0.080	0.047	0.845	0.733	1.101	1.273

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

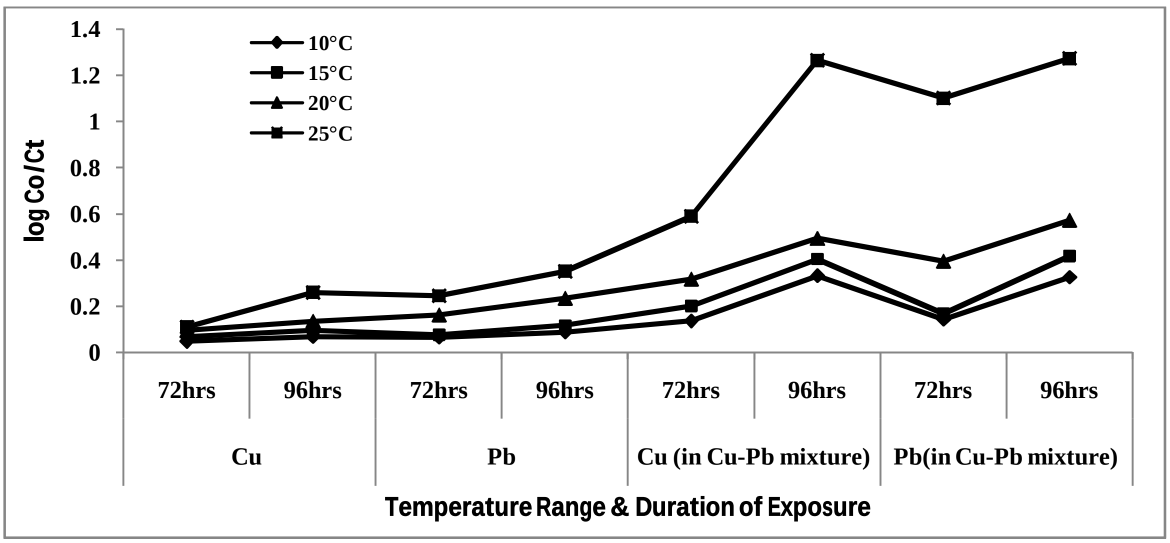

Fig. 2. Toxicokinetic study of bioaccumulation of Cu and Pb at different temperature for 72 and 96 hrs. Log (C_o/C_t) values calculated indicate that bio-accumulation steadily increased with increasing temperature and duration of exposure. For Cu, it increased from 0.048 to 0.110 after 72 hrs of exposure. After 96 hrs it varied from 0.068 to 0.259. For Pb, the values were 0.066 to 0.245 and 0.088 to 0.351 after 72 and 96hrs respectively. The increase was much greater when the combination of salts was given.

Table 2. Uptake Factor at different temperature and duration of exposure.

	10^0C		15^0C		20^0C		25^0C	
	72 hr	**96 hr**	**72 hr**	**96 hr**	**72 hr**	**96 hr**	**72 hr**	**96 hr**
Cu (A)	0.26	0.36	0.36	0.5	0.26	0.69	0.56	1.12
Pb (B)	0.56	0.46	0.34	0.59	0.35	1.04	1.08	1.39
Cu (in Cu+Pb) (C)	1.08	1.33	0.93	1.52	0.67	1.7	1.86	2.36
Pb (in Cu+Pb) (D)	1.86	1.32	0.8	1.54	0.7	1.83	2.3	2.37

Using the results of the internal concentration in animals and the concentration in the water, it is possible to calculate the Uptake Factor (UF). The UF of the heavy metals at a steady state[11], shown in Table 2, reveal that lead showed the maximum UF and that too when applied in combination with copper salt.

4. Discussion

Copper and lead were chosen in the present study because they represent a broad spectrum of potential pollutants in freshwater[12-14]. The present investigation observed a gradual increase in heavy metal uptake with the rising temperature. Similar effects of temperature were shown[15, 16] on the toxicity of chromium, arsenic, nickel and zinc to a variety of marine and estuary invertebrates. The accumulation of lead by aquatic organisms from water and sediments are influenced by various environmental factors, such as temperature, salinity, pH and concentration of a particular heavy metal[17].

Copper is found in natural waters as a trace metal usually at concentrations of <5μg/l but can also be present at much higher concentrations as a result of industrial processes[18, 12, 19-20], although the criteria were given would not protect the more sensitive macroinvertebrate species. There is, therefore, a need for further research on the toxicity of heavy metals to freshwater invertebrates. This should concentrate on the effects of sublethal chronic exposures. Toxicity tests should also be carried out on species from a range of taxa.

From the present study of toxicokinetics of bioaccumulation of copper and lead at different duration, it became evident that copper and lead together act synergistically and accentuate the rate of absorption. Because of the lack of available data on the effects of bioaccumulation of heavy metals in combination at different temperature, the results of the present study have not been compared with those of other studies and discussed accordingly. However, some justifications have been provided following various studies. The accumulation and uptake factor for copper is lower than lead as it forms complexes with other ion[21] and thus causing hindrance in its entry inside the cell. Data suggests that lead increases the cell membrane permeability and so its concentration inside the tissue. One of the possible reasons 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[22]. Pb and Cu might interact with $Na^+/K^+/Ca^+$ ATPase channels and increase their reabsorption[23]. Another possible route of lead and copper uptake is through calcium channel, while there is partial inhibition of sodium and potassium pumps[24], resulting in homeostasis disturbances of ionic contents affecting the overall metabolism leading to stress conditions. It was found throughout the experiment that the pH of the water decreased with increase in temperature. This lowering of pH could also be associated with the bioaccumulation property of these heavy metals[21]. As the pH decreased, there was an increase in the activity of metal transport across the cell membrane of the insect.

5. Conclusion

Thus, lead proved to be more toxic than copper because of its superior ability to accumulate in the tissue[25]. Aquatic organisms are very sensitive to lead because of their readily permeable membranes and high surface-volume ratio, and unlike copper, its excretion rate is much slower resulting in higher accumulation[25]. This principle can also be used for the extrapolation of toxicity data in aquatic insects. In the present study, it was also observed that the activity of the insects deteriorated after 96 hours of exposure. The decline in the body functions can be associated with the lowering of the metabolic activity that has an inhibitory effect on the growth of an organism.

The present authors finally conclude that it is essential to consider the effects of temperature when assessing the toxic effects of heavy metals on the survival of aquatic organisms. Relationships between metal tissue levels and mortality can provide a realistic indication of how the organism interacts with variable metal concentrations over varying time periods and temperatures[26].

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