Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2012, 2 (1):83-87

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Food chain bioaccumulation of lead in *Chrissia halyi* (Ferguson 1969) using cladophora as feed

Prasuna Solomon G[1] Pavani V[2] and Nageswara Rao Amanchi[*2]

[1]*Department of Zoology, Sardar Patel College, Hyderabad, Andhra Pradesh, India*
[2]*Protozoology and Environmental Toxicology Unit, Department of Zoology, Nizam College, Hyderabad, Andhra Pradesh, India*

ABSTRACT

Ostracods form an important part of benthic biota. They form a feed to many fish, insects, insect larvae and even corals. In the vast aquatic biome both freshwater and sea they occupy an important place in the food chain. Experiments were conducted on a freshwater ostracod species, Chrissia halyi, culturing them in the laboratory along with chlodophora the feed. The feed was first exposed to lead at different concentrations and fed to ostracods. The results showed significant amount of accumulation even for 4 days. There was a good increase in accumulation in 1, 2 and 3 ppm from 24 hrs to 96 hrs but in 4 ppm the accumulation was constant. The results are discussed.

Key words: *Chrissia halyi*, lead toxicity, food chain, bioaccumulation, biomagnification.

INTRODUCTION

The ostracods are abundant in both freshwater and marine environments and are usually found scuttling around among the submerged plants and debris at the shallow edges. Their most salient characteristic is the presence of a bivalve carapace that can completely enclose a laterally-compressed and weakly-segmented body [1]. Ecologically, freshwater ostracods can be part of the zooplankton or most commonly they are part of the benthos. They mainly feed on both living and detrital particles and ostracods form an important component in the food chain of some fish. These plankton at the bottom of the food chain absorb the long lasting toxicants like pesticides from water. The poison becomes more and more concentrated in each link and the consumers at the top of the food chain get a heavy dose [2]. The zooplankton play a significant role in transferring the nutrients and poison from water to the higher food chain organisms including man [3]. Ruiz *et al*., [4] suggested that ostracods are highly sensitive to heavy metal pollution, oil-discharges, and anoxic conditions, and a study by Khangarot and Das [5] demonstrated the need to include crustacean ostracods in a battery of biotest to detect the presence of hazardous chemicals in soils, sewage sludge, sediments, and aquatic systems. The relationship between water quality index and population density, species richness and diversity of various macro and micro invertebrates and ostracods was reported by several authors earlier [6], [7], [8], [9], [10]. Bioaccumulation potential of certain heavy metals such as copper, zinc,

cadmium, lead and chromium was determined in different organs of 5 freshwater fish species by Ambedkar and Muniyan [11] but sufficient data is not available using freshwater ostracod, *Chrissia halyi* in labaratory as well as field studies. In this context the present study was aimed to determine the toxicity of lead to freshwater ostracod, *Chrissia halyi* and to examine bioconcentration of lead in the body after 96 hours of exposure.

Food Chain: Heavy metal uptake and concentration in food chains especially those terminating in human beings are topics of interest largely due to several instances of unexpected human intoxication as have occurred with Hg, Cd, and Pb [12]. There are very few data to support a simplistic assumption of step wise heavy metal bioaccumulation through food. There is not much in situ data for accumulation in the food chain. Food can contribute as much as 90% of total body uptake of metals [13]. Heavy metals such as Lead, Zinc and Hg and Cd occupy the least stable sediment fractions and may thus be more easily remobilized and introduced into food chain than others which are inertly bound in the residual sediment particles [14].

MATERIALS AND METHODS

Experimental organism: It is an ostracod belonging to the order stenocypris, class crustacea of arthropoda. The species *Chrissia halyi* is extensively found in almost all aquatic habitats of peninsular India [15].

Identification of the species: The algal clumps on which the ostracod species was found feeding in the field were first washed with water in the tray to separate ostracods from the algae. Adults of the same species were picked up with a pasteur pipette into a cavity block and 90% alcohol was added drop wise until all the ostracods were dead. This is a method adapted specially for ostracods to kill them with their shells open making it easy for dissection and identification [16]. The preliminary identification was done up to generic level and another set of sample was sent to Dr. Maya Deb, Z.S.I Calcutta, India for further identification up to species level (Figure 1).

Medium preparation: A modified method of Shirgur's [17] studies on culture of zooplankton with cow dung was adapted. 6 grams of dried buffalo dung was boiled thoroughly under low heat in a litre of distilled water. It was cooled and filtered with Whatmann No 42. Filter paper. The filtrate was sterilized in an autoclave, cooled and stored. This extract was added to the culture along with chlodophora algae, maintained in a cement tank of the size 4x2x1.5 feet (L x B x H). This tank was filled with 1/2 or ¾ with water depending on the season (1/2 in winter ¾ in summer) (Figure 2).

Sample preparation for Atomic absorption spectrophotometric analysis: The organisms after a period of exposure were picked with a pasteur pipette on to a tin foil. Excess of water was sucked with a filter paper. The tin foil was weighed with and without ostracods on a sensitive balance thus estimating the weight of ostracods. The ostracods were then carefully placed in a crucible, and ashed in a muffle furnace at 450^{o}c for 3 hrs. The ash was dissolved in 5 ml of 2NHNO_3 [18]. The digested samples were analyzed for traces of lead using a single beam varian tetron atomic absorption spectrophotometer at Birla institute for scientific research.

RESULTS

Toxicity evaluation: (LC_{50} evaluation) the result of the toxicity of lead nitrate to *Chrissia halyi* showed increased mortality rate with increase in lead nitrate concentration and exposure time. From the data of the % survival of the ostracods, the median lethal concentration (LC_{50}) was calculated both by the graphical interpolation and probit analysis [19]. A graph plotted between % mortality and the log concentrates gives a characteristic curve, where as plotting a graph between probit mortality versus log concentration; a straight line was obtained. LC_{50} value was determined for the 96 hours by both the methods and was found to be 5 ppm. 50 % and 100% mortality for Lead at 24, 48, 72 and 96 hrs was estimated.

Table 1: 50% and 100% mortality of *Chrissia halyi* for 24, 48, 72 and 96 hrs exposure against Lead

Lead in ppm	24 hrs	48hrs	72hrs	96 hrs
LC_{50} in ppm	36	26	15	5
LC_{100} in ppm	40	35	20	7

Food chain accumulation of lead: 300 adult ostracods of the same size were picked up with a dropper having a wide opening (so that the ostracods will not touch the dropper edges) and introduced 50 organisms into each petri plates filled with 100 ml of filtered culture medium. 100 mg of cladophora was first exposed to 1, 2, 3 and 4 ppm of Pb $(NO_3)_2$ separately for 10 days. After 10 days of exposure, Lead accumulated in caldophora was estimated by the atomic absorption spectrophotometric method. The lead accumulated Cladophora was fed to ostracods for 96 hrs. After 4 days % accumulation of lead was estimated in the ostracods sample of 1, 2, 3 and 4ppm lead. During the experiment algae and ostracods were transferred into fresh concentrations after every 24 hrs. Biomagnifications from water to cladophora and then in ostarcods was estimated by the (AAS) same method.

In the present set of experiment, Cladophora exposed to Pb $(NO_3)_2$ to 1, 2, 3 and 4 ppm for 10 days and fed to *Chrissia halyi* for 96 hrs. The exposure time was the same but the concentrations were different. There was a gradual decrease in uptake with increase in concentration. At 1 ppm, cladophora accumulated 240 µg/ gm. At 2 ppm – 180 µg/ gm, at 3 ppm 120 µg/ gm and 4 ppm 80 µg/gm of Lead (Table 2). In our unpublished data under 36 ppm Lead exposure for 24 hrs, cladophora accumulated 207 µg/ gm and similarly at 0.5 ppm for exposure of 15 days, the accumulation was 238 µg/ gm, it may be noted that cladophora has a tendency to accumulate high quantities of lead when exposed to low concentration for a long period and high concentration for a short period. The capacity of cladophora to eliminate the toxicant is not clear, but this may be one of the reasons why the accumulation declined from 1 to 4 ppm when all condition including the exposure time is same.

Table 2: Comparative analysis of % accumulation of Lead in cladophora and *Chrissia halyi*

Conc of $Pb(NO_3)_2$	Cladophora Species			*Chrissia halyi*		
In water in ppm	Accumulation of Lead in µg/ gms.	% accumulation	Bio Conc. Factor.	Accumulation of Lead in µg/ gms.	% Accumulation	Bio Conc. Factor.
1	240±0.04	38	0.24	340±0.018	141	1.48
2	180±0.01	14	0.06	300±0.013	166	1.69
3	120±0.01	6.3	0.06	220±0.016	183	1.83
4	80±0.01	3.2	3.2	130±0.08	162	1.62

DISCUSSION

Jan Vymazal [20] reported that cladophora accumulated 75% of the total lead during the first hour of exposure and then only slight uptake took place. In the present experiment, cladophora accumulated 240 µg/gm in 10 days exposure, when estimated after 96 hours from 1 ppm concentration. It may be that the uptake increased in first hour and then decreased to 240 µg/ gm, in 10 days. After 10 days exposure, algae were left in control water for 96 hours as a feed to ostarcods. During this period depuration could have occurred which left 38% of the Lead in 1 ppm, 14% in 2 ppm, 6.3% in 3 ppm and 3.2% in 4 ppm i.e., there is decrease in the uptake with the increase in concentration. The Water Hyacinths Weevils, *Neochetina eichorniae* were fed with heavy metals (Zn, Cd, Pb and Hg). Contaminated water, Hyacinth leaves exposed to 25, 50 and 100 ppm. It was found that the uptake of Lead was 77% just after one day of exposure of the plants to lower concentration of Lead i.e., 25 ppm. However at higher concentration, 50 and 100 ppm, the percentage accumulation slowed down over a period of one week, as compared to the uptake at low concentrations [21]. The biological activity or the metabolic rate of an organism often changes due to natural seasonal variations causing the rate of incorporation and release of the heavy metals to change.

Jan Vymajal [20] reported that the presence of humic substances in water a decrease of a specific heavy metal uptake. In the present experiment the water was filtered with whattman No. 42, therefore, the presence of humic substances is minimized. The bioconcentration of metals in *Neochetina eichorniae* feeding on contaminated eichnorneae plants was found to be in the order of Pb > Cd > Zn. The % of lead accumulated in insects through the food chain was 6.7, 6.15 and 6% after one day of feeding. After 2 days of exposure insects accumulated about 7.7%, 7.5% and 6.2% of lead from plant leaves at 25, 50 and 100 ppm. However after 7 days no further significant bioaccumulation of lead occurred at all concentrations tested [21].

The bioaccumulation of lead in ostracod *Chrissia halyi* by feeding on contaminated cladophora was 340 µg/ gm in 1 ppm, 300 µg/ gm at 2 ppm, 220 µg/ gm in 3 ppm and 130 µg/ gm in 4 ppm. There is a magnification seen in all the exposed concentrations from algae to ostracod. But the percentage of magnification increased from 1 ppm to 3 ppm

and then decreased in 4 ppm. The % magnification increase in 1 ppm was 141, in 2 ppm it was 166, in 3 ppm it was 183 and in 4 ppm it was 162. In contrary to this, percentage of accumulation from water to algae decreased gradually. It was 38% in 1 ppm. 14% in 2 ppm, 6.3% in 3 ppm to 3.2% in 4 ppm. The gradation in increase or decrease could not be seen in the case of ostracods. This may be because of the individual differences in the feeding behavior of ostarcods [22]. Ostarcods are voracious feeders. Therefore as habit they may have fed on algae increasingly until in 4 ppm and then they began to feel the effect of toxicity. However toxicity through the food chain is very less compare to toxicity through water. Ortal and Vogal [23] reported that insects of Pimplaturionellae (a pupal parasitoid) fed with lead via food lived longer than those contaminated by water. Therefore contamination via water was more effective than by food. The studies on dietary lead on fish in which guppies *Poecilla reticulate* fed on *Daphnia magna* containing as much as 68 mg/kg of lead for 4 weeks showed accumulation of body lead concentration as much as 24 mg/kg without suffering an over toxic effects. Lead or any other heavy metal therefore gets accumulated through the food chain but does not have any acute toxic effect on the organism. Even in *Chrissaia halyi* no mortality was observed in those feeding on contaminated algae. Though the exposure was for a limited time period of 96 hours the lack of any significant effect on mortality suggests that metal present in the body may be below the toxicity threshold to cause mortality or possibly were bound to metallothionien like protein so that metals may not exert the effect on biological system.

Figure 1: *Chrissia halyi* feeding on chlodophora algae (34 days old organism) (400X).

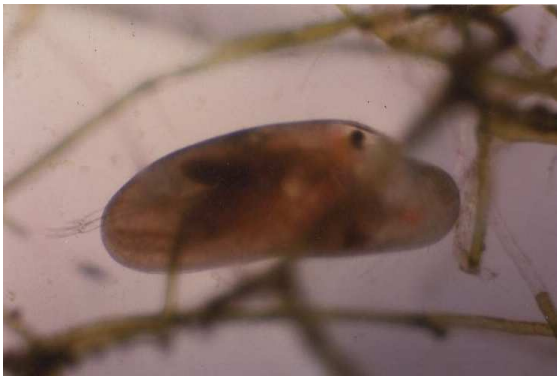

Figure 2: Eggs of an individual *Chrissia halyi* along with chlodophora (400X).

CONCLUSION

This indicates that the bioaccumulation does not affect the experimental organism but allows magnification in the food chain. Ostracods in general form a feed to many fish but it is not known as to how many of our common food fish feed on ostracods. However, it may be deduced that ostracods do occupy an important place in

biomagnifications of heavy metals in the food chain and *Chrissia halyi* here plays an important role in peninsular India – a species which is found abundantly. This study indicates that *Chrissia halyi* is a potential bioindicator organism of lead pollution and in toxicity testing.

Acknowledgments
The authors are thankful to Dr. Maya Deb, Z.S.I Calcutta for identification of organism up to species level and also thankful Prof. S. Sabita Raja, Department of Zoology, Nizam College, Hyderabad, for her suggestions.

REFERENCES

[1] Rossetti G, Koen M, Claude M, Stefano T, Valentina P, *J Limnol*, **2006,** 65: (2). 121-131.
[2] Prasuna SG, Amanchi N, Hussain MM**,** *Bull Environ Sci*, **2007,** 15: (2), 157-162.
[3] Zeba M, Khan MA, Narasimha Rao B, *Bull Environ Conta and Toxicol*, **1996,** 56: (3). 483-485.
[4] Ruiz F, Gonz´alez-Regalado ML, Borrego J, Abad M, Pend JG, *Environ Pollu*, **2004,** 129: (1), 49–61.
[5] Khangarot BS, Das S, *J Hazard Materi*, **2009,** 172: (2-3), 641–649.
[6] Ferda Perçin P, *J Black Sea/Mediterranean Environ*, **2011,** 17: (2), 127-144.
[7] Narasimha Ramulu K, Benerjee G, Srikanth K, Ravindar B, Gowri P, *Inter J Advan Biotechnol and Res*, **2011,** 2: (2), 286-290.
[8] Okan K, Dugel M, Balci M, Aziz D, Derya A, Mustafa K, *Turk J Zool*, **2010,** 34, 429-442.
[9] Amanchi NR, *Adv. Appl. Sci. Res*, **2010**, 1 (2): 124-131.
[10] Anant JD, *Adv. Appl. Sci. Res*, **2011**, 2 (6): 223-228.
[11] Ambedkar G, Muniyan M, *Adv. Appl. Sci. Res*, **2011**, 2 (5): 221-225.
[12] Friberg L, Piscator M, Nordberg GF, Cadmium in the Environment, C.R.C. Press: Cleveland, Ohio, USA, **1971.**
[13] Ewing BB, Pearson JE, *Advan Environ Sci & Technol*, **1974,** 3: 1-126.
[14] Prasuna G, Zeba M, Khan MA, *Bull Environ conta and toxicol*, **1996,** 57: (5), 849 -852.
[15] Deb M, *J Zool Socie India*, **1969,** 1: 91-92.
[16] Pennak RW, Freshwater Invertebrates of the United States, John Wiley & Sons, Inc. New York, NY, **1978,** p 803.
[17] Shirgur GA, *Crustaceana*, **1989,** 56: (2), 113-126.
[18] American Public Health Association, (APHA), Standard methods for the examination of water and wastewater. 18th ed., Washington, D.C. Port city Press. Baltimore, Maryland, **1995.**
[19] Finney DJ, Probit Analysis. 3rd ed. Cambridge Univ. Press, Cambridge, **1971.**
[20] Jan Vymazal**,** *Hydrobiol*, **1987**, 148: 97-101.
[21] Saber Hussain M, PhD Thesis, Osmania University, (OU, INDIA, **1990).**
[22] Armes NJ, King ABS, Carlaw PM, Gadsden H, *Entomologia Experimentalis et Applicata*, **1989**, 51: 5-10.
[23] Ortel J, Vogel WR, *Entomologia Experimentalis et Applicata*, **1989**, 52: 83-89.