

## Excretion of Lead as a Mechanism for Survival in *Chrissia halyi* (Ferguson, 1969)

G. Prasuna, M. Zeba, M. A. Khan

Department of Zoology, Osmania University, Hyderabad 500 007 (A.P.), India

Received: 3 July 1995/Accepted: 2 May 1995

In general organisms develop or use a number of mechanisms to overcome the adverse influence of such toxicants as heavy metals. These mechanisms may be avoidance, immobilization, excretion and mechanisms involving enzymatic changes. Tolerant organisms may possess certain storage organs which are particularly involved in heavy metal immobilization as suggested by Hopkin and Martin (1985).

*Chrissia halyi* (Crustacea, Ostracoda) were observed to be surviving in waters which are highly polluted with lead (Prasuna, 1994). Besides they were found feeding voraciously on contaminated blue green algae, and excreting large pellets. Therefore the present work has been taken up to see the lead in the excreted pellets.

### MATERIALS AND METHODS

The culture of *Chrissia halyi* and cladophora species were maintained in a cement tank of the size 4 x 2 x 1 1/2 ft (lxbxh) approximately the volume of 85,000 cu.cm filled 1/2 or 3/4 full with tap water depending on the season (1/2 in winter & 3/4 in summer). The physico chemical properties of water were as follows - pH - 7.5 to 7.8 mg/L; dissolved oxygen - 7.8 mg/L; Temperature - 27±2°, total hardness - 134 mg/L as CaCO<sub>3</sub>, alkalinity - 1 or 2 mg/L. The medium was prepared by boiling 6 g (dry weight) of dried buffalo excreta in a glass flask under low heat in a liter of distilled water. It was cooled and filtered with Whatmann No.42 filter paper. The filtrate was sterilized in an autoclave, cooled and added to the culture. Identification of the species was done by Dr. Maya Deb (Z.S.I. Calcutta).

Stock solutions of technical grade lead nitrate (99% pure) obtained from Regional Research Laboratories, Hyderabad was prepared in double distilled water. From this four different concentrations (1,2,3 and 4 mg/L) were prepared in filtered pond water with required dilutions. 100 mL of each concentration was added into a clean washed and sterilized petriplate. Fully grown adult ostracods in batches of 25 each were taken and exposed to the selected concentrations for 1,2,3 and 4 d respectively. The ostracods were fed with cladophora throughout the experimental period.

Correspondence to: M. A. Khan

In the lead exposed petriplates, the excreta was very distinct as black pellets which were excreted simultaneously as they fed compared with control. The excreta was picked up with a fine Pasteur pipette under a microscope into a clean washed and dried cavity block. Excreta from all the exposed concentrations were removed and separately washed in ordinary water, and then in distilled water to remove any lead particles adhering from the remaining water to the pellets and then dried in an oven. The dried excreta was ground with a round edged glass rod, before dissolving in 2 mL of 2  $\text{NHNO}_3$ . Then the samples were sent for estimating the quantity of lead in excreta with Atomic Absorption Spectrophotometer (Perkin Elmer model, 2380). The results are expressed as  $\mu\text{g/g}$  wet weight of Ostracods.

## RESULTS AND DISCUSSION

The results obtained in this experiment are given in the Table 1. In the first day of exposure to 1 mg/L of lead the excretion was observed in large quantities. This trend increased as the time period and concentration increased.

In Chrissia halyi excretion was observed to be one of the tolerance mechanisms towards lead. They excreted lead through feces when exposed to different concentrations for different durations.

The hepatopancreas is the main site of metal accumulation in land snails. It seems to play a crucial role in metabolism and detoxification of cadmium, zinc and other metals. Reinhard Dallinger et al. (1986) reported metal accumulating granules in the hepatopancreas of the garden snail Helix aspera and suggested a possible role of these vesicles in detoxification of certain metals. Metal accumulating granules have also been found in the digestive gland of the marine mussel Mytilus galloprovincialis. These granules have been identified as lysosomes, containing copper bound to polymerised thioneins. Association with metal binding proteins has been proposed as another mechanism responsible for detoxification of heavy metals in molluscs (Reinhard Dallinger et al., 1986). Some arachnids like spiders, which feed on isopods rich in heavy metals, store a surplus of metals in intracellular granules formed in the midgut which are excreted by lysis of the cell (Hopkin and Martin 1985). Feces is the main route of elimination. Methyl mercury is secreted in bile, in part absorbed in the intestinal tract and the remainder degraded to inorganic mercury by intestinal microflora. Inorganic mercury is poorly absorbed and therefore excreted in the feces (Clarkson 1989). Nuorteva et al. (1978) observed that mercury as an element is excreted more efficiently than any other form of mercury by blow flies. The blow fly Phormia terraenovae was reared on liver of a seal (Phoca hispida) found dead in the Finnish lake. Pihlajavesi on April 20, 1975, and having 36 mg/L Hg in the liver. In this rearing experiment, freshly emerged adult flies contained 18.2 mg/L dry weight of mercury and they excreted 69% of their mercury in 2 d.

The ratio of excretion for Chrissia halyi varied from 20 - 64% of exposed lead, depending on the duration and concentration of exposure. The efficiency of excretion

Table 1. Lead excreted by *C.halyi* in µg/g.

Duration of exposure, hr.	Nominal Conc. of lead in water,mg/L	Lead in fecal pellets		Ratio of excretion
		µg/g wet wt. of ostracods	S.E.	
24	1	0.4	(±0.01)	64
	2	0.49	(±0.01)	39
	3	0.6	(--)	32
	4	0.8	(±0.09)	28
48	1	0.5	(±0.09)	40
	2	0.79	(±0.01)	32
	3	1.18	(±0.10)	31
	4	1.46	(±0.03)	29
72	1	0.81	(±0.03)	43
	2	1.14	(±0.05)	30
	3	1.38	(±0.02)	25
	4	1.52	(±0.06)	20
96	1	1.15	(±0.04)	46
	2	1.41	(±0.02)	21
	3	1.57	(±0.03)	21
	4	1.70	(±0.05)	17

Values are mean ± SE of six observations.

decreased from 1 - 4 mg/L in all the hours of exposure.

Hutagalung (1989) reported that metal elimination from the whole animal appeared to proceed in three well discernible stages: within a week, a first fraction is lost. This fraction must represent liable metal that could in part be extracellular or otherwise not yet bound to cellular ligands. Borchardt (1983) also confirmed the three compartments of cadmium storage. The first compartment was a liable fraction that was not truly incorporated into the tissues and therefore was released quickly. The second fraction was reversibly bound cadmium, elimination of which may be energy dependent. The last one was the firmly bound metal, the greater part of which was bound to strong ligands, such as metalothionien and other sulphydryl group containing proteins.

In the present study, excretion of lead was observed occurring simultaneously while taking in of the toxicant with the food and water. It might be inferred that this lead like cadmium might belong to the first fraction of elimination. Since as reported by Borchardt (1983) mercury, cadmium and lead all have similar mode of action in the biological system, it may be inferred that excretion of lead also might occur in three well discernible stages.

Lakshmann and Nambisan (1989) suggested that the quantity of lead loss was dependent on the internal lead concentration. This was found to be true in Chrissia halyi as the quantity of lead excreted increased with higher concentration.

Khan et al. (1989) reported that pike Esox lucius collected from highly contaminated to slightly contaminated lakes accumulated comparable levels of mercury. Observations on the mercury excretion in freshly emerged adult Lucilia illustris flies showed that they had excreted during the first 2 d of their adult life about 66% of the mercury.

Therefore we may conclude that excretion is an efficient 'protective mechanism' in Chrissia halyi enabling this species to survive even in highly polluted water bodies, like Hussain Sagar Lake, Hyderabad.

Acknowledgment. The authors are thankful for financial assistance provided by Ministry of Environment (Govt. of India) Project No. 19/126/90-RE.

## REFERENCES

- Borchardt T (1983). Influence of food quantity on the kinetic of cadmium uptake and loss via food and sea water in Mytilus edulis. Mar Biol 76:67-76.
- Clarkson, TW (1989). Mercury. Journal of the American College of Toxicology 8:7 Mary Ann Liebert. Inc. Publishers.
- Herwig HJ, Brands F, Kruitwagen, Zandee DI (1989). Bioaccumulation and histochemical localization of cadmium in Dreissena polymorpha exposed to cadmium chloride. Aquatic Toxicology 15:269-286.
- Hopkin SP, Martin M (1985). Assimilation of Zn, Cd, Pb, Cu and Fe by the spider Dysdera crocata on predator of wood lice. Bull Environ Contam Toxicol 34: 183-187
- Hutaglung HP (1989). Mercury and cadmium content in green mussel, Mytilus viridis L, from onrust waters, Jakarta Bay. Bull Environ Contam Toxicol 42:814-820.
- Khan AT, Judith SW, Lissane D' Andrea (1989). Bioaccumulation of four heavy metals in two populations of grass shrimp Palaemonetes pugio. Bull Environ Contam Toxicol 42:339-343.
- Lakshmann PT, Nambisan PNK (1989). Accumulation and bioconcentration factor of lead in soft tissue by mussel P. Viridis exposed to metal in sea water. Bull Environ Contam Toxicol 43:131-138.
- Nuorteva P, Nourteva SL, Suckcharoen S (1980). Bioaccumulation of Mercury in blow flies collected near the mercury mine of Idrija, Yugoslavia. Bull Environ Contam Toxicol 24:515-521.
- Prasuna, G (1994) Ecological adaptations of Chrissia halyi and their role in bioaccumulation and biomagnification of lead. Ph.D. Thesis, Osmania University, Hyderabad, India.
- Reinhard Dallinger, Janssen HH, Hilty AB, Berger B (1986). Characterization of an inducible cadmium binding protein from hepatopancreas of metal exposed slugs (Arionidae, Mollusca). Comp Biochem Physiol 92C:2 : 355 - 360.