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RESEARCH ARTICLE

Bioaccumulation of Mercuric chloride in the Reproductive Organs of Freshwater Female Crab, *Barytelphusa cunicularis* (Westwood)

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Abstract

Mercury concentrations were recorded in water and tissue of *Barytelphusa cunicularis* from Pimpalwadi site (Jaikwadi Dam) near Aurangabad. The level of heavy metals in the ovary and spermatheca of crabs was investigated using Atomic Absorption Spectrophotometer (AAS). The mean concentration of mercury in the crab was 0.9±0.001 µg/g. In the experimental crab, the percentage of mercury bioconcentration factor was in the range of 2002.9-22252.9 in ovary and 1632-10172.7 in spermatheca. The concentration of mercury was highest in spermatheca and lowest in ovary tissue after exposed to sublethal concentration (24^{th} of LC_{50}:$1/5^{th}$ 0.208 ppm) of mercuric chloride.

Keywords: Mercuric chloride, *Barytelphusa cunicularis*, Jaikwadi dam, sublethal concentration.

Introduction

Increasing concentrations of the metals cause significant increases in the mortality in crabs and prawns. In general, considerations of metal bioavailability and bioaccumulation in aquatic media can be split into direct and indirect exposure and impacts. Direct exposure occurs via the water column where biotic and abiotic factors can influence metal bioavailability and bioaccumulation may lead to toxic impacts on crustacean development (Dennis *et al.,* 2005; Soundarapandian *et al.,* 2010). Bioaccumulation is the general term describing a process by which chemicals are taken up by an organism either directly from exposure to a contaminated medium or by consumption of food containing the chemical (USEPA, 2010). To understand in a better way, not only physiological effects but also toxicological effects affect the hygienic organisms and a great attention is paid to hazardous elements such as mercury, lead, cadmium and arsenic and their ability to accumulate in organs (Spurny *et al.,* 2002; Yilmaz, 2006). In the freshwater crabs, it is observed that the external organs are affected due to the toxic chemicals causing loss of equilibrium, abnormal changes (jumping to avoid toxin, paralysis) and finally lead to death (Paul *et al.,* 2005; Joshi, 2006). This may be attributed to the significant damage to the internal organs. Heavy metal pesticides pollute aquatic ecosystem and find their way in the body of aquatic animals by means of ovary and spermatheca (Paul *et al.,* 2005). *Barytelphusa cunicularis* possesses these criteria and is a sensitive indicator of heavy metal both at acute toxicity and at accumulation levels, indicating the possible use of this species in monitoring pollution. The contamination of aquatic resources with a wide range of pollutants has become a matter of concern over the past few decades and it affects the aquatic animals specially crabs vary widely. The objective of the present study is to evaluate the bioaccumulation of mercury in the ovary and spermatheca of the freshwater crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentration of mercuric chloride for 5 and 10 d of exposure.

Materials and methods

Crabs: The freshwater crabs, *Barytelphusa cunicularis* were collected from Pimpalwadi site (Jaikwadi Dam, Paithan) located at (19°29′6″N 75°22′12″E/19.485; 75.37) Aurangabad District. They were acclimatized to laboratory conditions under normal day/night of 11 L: 13 D illumination at 27±1ºC for about one week in plastic troughs (18 dia) containing sufficient tap water, so that crabs are submerged. Before experimentation, female crabs in intermoult stage (C_3) (Diwan, 1973) of approx. equal carapace width (45 to 50 mm) and body weight (50 to 55 g) were sorted.

Experimental design: For Hg accumulation testing, the crabs were split into 3 groups (Control, $HgCl_2$ treated-5 and 10 d treated) with similar biomass (n=6 for each group), each group being maintained in laboratory condition. The crabs were exposed to sublethal concentration of $HgCl_2$ 1/5 (0.208 ppm) for 5 and 10 d to analyze the bioaccumulation of Hg. Control animals were kept under the same conditions but without added metal. After their respective exposure period, the ovary and spermatheca were dissected out from both the control and experimental crabs to assess the concentration of accumulated Hg.

Table 1a. Percentage change in bioconcentration factor (BCF) of Hg in ovary and spermatheca of freshwater female crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentration of $HgCl_2$ (1/5th: 0.208 ppm) for 5 d.

Tissue		BCF		
	Group	Winter	Summer	Monsoon
Ovary	Control	0.068	0.037	0.017
	Experiment	2.31	2.33	2.51
	% Increase	3297%	6197.3%	14664.7%
Spermatheca	Control	0.056	0.044	0.039
	Experiment	1.63	3.08	1.86
	% Increase	2810.7%	6900%	4669.2%

Table 1b. Percentage change in bioconcentration factor (BCF) of Hg in ovary and spermatheca of freshwater female crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentration of $HgCl_2$ (1/5th: 0.208 ppm) for 10 d.

Tissue		BCF		
	Group	Winter	Summer	Monsoon
Ovary	Control	0.068	0.037	0.017
	Experiment	3.20	2.57	3.80
	% Increase	4605.8%	6845.9%	22252.9%
Spermatheca	Control	0.056	0.044	0.039
	Experiment	2.09	4.52	2.59
	% Increase	3632%	10172.7%	6541%

Determination of Hg accumulation: The samples were digested according to the methods described by Van Loon (1980) and Freez and Steyn (1992). The dissected tissues were dried for 72 h at 70°C in oven and 500 mg powder of dried tissue was taken in beaker and 10 mL conc. HNO_3 was added. After its complete evaporation, again added (2:1) mixture of HNO_3 and per chloric acid ($HClO_4$), mixed well and kept on hot plate and waited till the solution evaporates and the solution becomes colorless to brown color. About 10 mL of conc. HNO_3 was added for 3rd time again, mixed the content well and kept on hot plate for digestion till 5 mL remains in the beaker. It was cooled and made up to 25 mL with 2 M solution of conc. HNO_3 and samples were stored. PC based Atomic Absorptive Spectrophotometer (AAS-SL 163) hallow cathode lamp (mA-7, Sr. No. 509753) was used for the determination of Hg^{2+}. Each sample was analyzed in triplicate and the results were averaged and given as µg/g dry tissue and results were expressed as mean±S.E.

Results

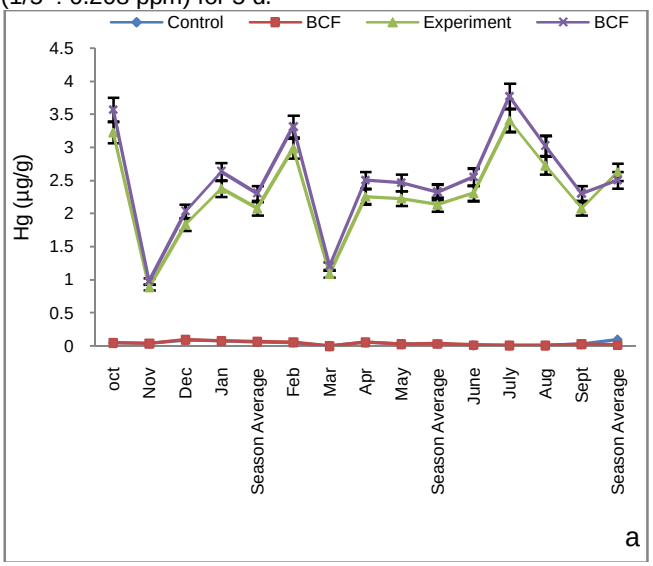
Bioconcentration factor (BCF) of Hg was studied in the ovary and spermatheca of freshwater crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentrations of $HgCl_2$ for 5 and 10 d of exposure. BCF is the physical property which characterizes the accumulation of chemicals, including pollutants, through chemical partitioning from the aqueous phase into an organic phase (Chiou, 2002). It is calculated as:

$$BCF = \frac{Concentration_{organism}}{Concentration_{Environment}}$$

The findings show that the rate of accumulation and percent of BCF were fluctuated from month to month. Tissues with BCF greater than 1,000 are considered high and less than 250 as low, with those between classified as moderate. Average value of Hg concentration in the freshwater of Pimpalwadi site (Jaikwadi Dam) is 0.9±0.001 µg/L. The crabs *Barytelphusa cunicularis* were exposed to sublethal concentrations (1/5th: 0.208 ppm) of $HgCl_2$. Value of bioaccumulation and BCF in ovary and spermatheca after 5 and 10 d exposure period are represented in Table 1a-b and Fig. 1a-d. Season average of BCF of Hg in the ovary of control crab was found to be 0.068 (winter), 0.037 (summer) and 0.017 (monsoon). Percentage increase in BCF in the ovary of experimental crab was found to be 3297% in winter, 6197.3% in summer and 14664.7% in monsoon after 5 d of exposure (Table 1a and Fig. 1a). Similarly an increase of 4605.8% in winter, 6845.9% in summer and 22252.9% in monsoon was found after 10 d of exposure (Table 1b and Fig. 1c).

Percentage increase of BCF of Hg in ovary was in the order: monsoon>summer>winter. The season average of BCF of Hg in the spermatheca of control crab was found to be 0.056 (winter), 0.044 (summer) and 0.039 (monsoon). Percentage increase in BCF in the spermatheca of experimental crab was found to be 2810.7% in winter, 6900% in summer and 4669.2% in monsoon after 5 d of exposure (Table 1a and Fig. 1b). Similarly, an increase of 3632% in winter, 10172.7% in summer and 6541% in monsoon was found after 10 d of exposure (Table 1b and Fig. 1c, d). Percentage increase of BCF of Hg in the spermatheca was in the order**:** summer>monsoon>winter.

Fig. 1a & b. Monthly Hg concentration (µg/g) and BCF in different tissues (a. ovary & b. Spermatheca) of the freshwater crab, *B. cunicularis* exposed to sublethal concentration of $HgCl_2$ ($1/5^{th}$: 0.208 ppm) for 5 d.



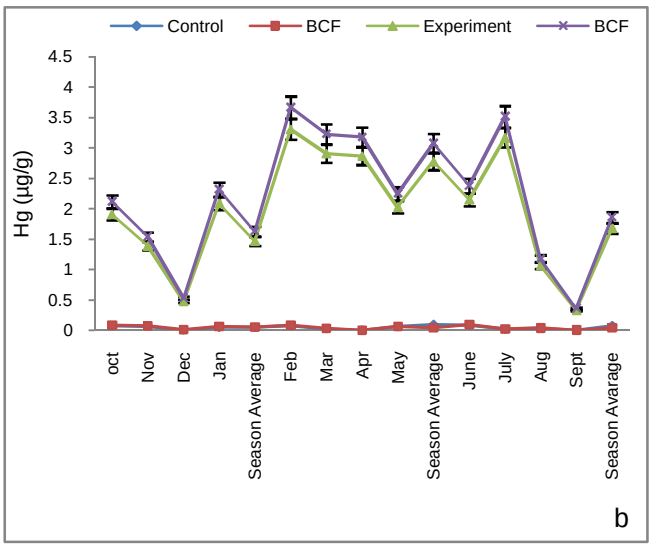
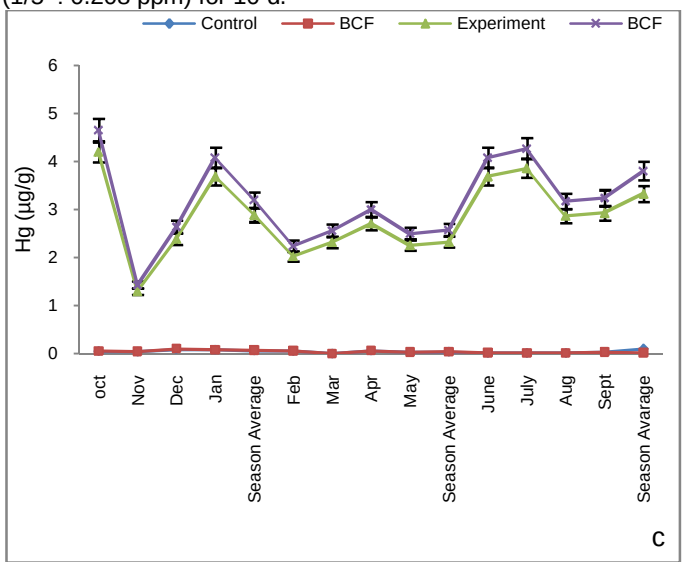
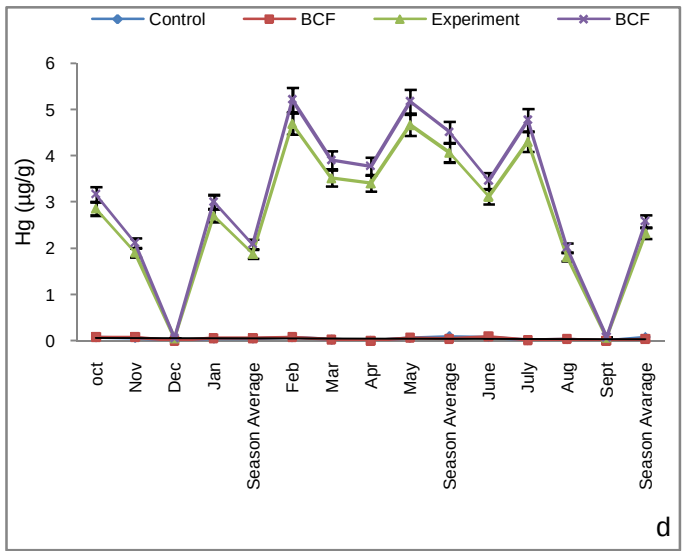


Fig. 1c & d. Monthly Hg concentration (µg/g) and BCF in different tissues (a. ovary & b. Spermatheca) of the freshwater crab, *B. cunicularis* exposed to sublethal concentration of $HgCl_2$ ($1/5^{th}$: 0.208 ppm) for 10 d.





Discussion

The term bioaccumulation is often a good integrative indicator of chemical exposures of organisms in polluted ecosystems and extent of occurrence or accumulation of trace metals by organisms in different tissues is dependent on the route of entry, i.e. either from surrounding medium or in the form of food or chemical form of material available in the media (Ghosh and Kshirsagar, 1973; Phillips and Rainbow, 1994). BCF is used as the criteria for identifying and classifying the bioaccumulation of substances that are hazardous to the aquatic environment (McGeer *et al.,* 2003). Past reviews on metal BCFs for aquatic biota, which account for water-only exposures, have shown that BCFs are often highly variable between organisms and generally inversely related to exposure concentration (DeForest *et al.,* 2007).

A high bioaccumulation potential of a chemical in biota increases the probability of toxic effects being encountered in aquatic and terrestrial organisms including humans and their environment so many existing regional and international regulatory classification schemes, guidelines and risk assessments use estimates of bioaccumulation to indicate whether chemicals may be hazardous to aquatic organisms, if their BCF exceeds designated threshold values (Geyer *et al.,* 2000). The results obtained indicate that bioaccumulation of Hg in the ovary and spermatheca of freshwater crabs, *Barytelphusa cunicularis* is increased with increasing concentration and also crabs ability to limit the bioaccumulation of Hg varied from organ to organ (Table 1a-b and Fig. 1c-d). In the experiment crabs, the BCF% of Hg ranged between 2002.9 to 22252.9 in ovary and 1632 to 10172.7 in spermatheca.

Bioconcentration factor (BCF) of Hg in different tissues was found in different order in winter, summer and monsoon season. BCF of Hg is in order of ovary>spermatheca in winter season, spermatheca>ovary in summer season and ovary>spermatheca in monsoon. Season may influence body burdens of heavy metals. This seasonal variability may results from either internal biological cycle of the organism or from changes in the availability of the metals in the environment of the organism (Yilmaz and Yilmaz, 2007; Olowoyo *et al.,* 2010). Several studies have been carried out on the determination of levels of heavy metals and their effects in aquatic organisms particularly in crab (Falusi and Olanipekun, 2007). The increase of Hg in the water body results in the excess accumulation of Hg by the aquatic animals like crabs, fishes, bivalves which are consumed by the people as their food, thus, posing human health risk; elevated levels of Hg in the crabs can also have ecologically significant effects, such as affecting reproduction (Wiener, 1995; Iliopoulou and Kotsanis, 2001). The nutritional implication is that consumers of these food materials may be exposed to heavy metal toxicity if bioaccumulation results due to regular consumption (WHO, 1972; Goyer, 1995; Ross and Morison 2002). The levels are far beyond the tolerable level of 0.001 µg/g (WHO, 1972). Though these food materials are processed (heating, cooking) before consumption, the effect of processing could be minimal, since the heavy metals are non-degradable. Mercury toxicity can occur after microbial degradation of Hg to dimethyl mercury. Human exposure to dimethyl mercury occurs through consumption of contaminated aquatic foods. Hg affects the central nervous system and brain due to its ability to cross the blood brain barriers (Goyer, 1995). Despite many studies carried out on heavy metals in crabs, scanty information is available on the BCFs of heavy metals in crab samples. Crustaceans, including crabs, are widely recognized as useful species for biomonitoring (Phillips and Rainbow, 1993).

Conclusion

The present study revealed that there was a significant difference in Hg BCF percent in ovary and spermatheca. The concentration rates of Hg in the tissues of freshwater crab vary significantly as a function of season and the pollution load of tissue.

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