



Study of interrelations between seasonal estuarine water parameters and cadmium chloride toxicity to estuarine clam, *Meretrix meretrix* (Linnaeus)

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Abstract

In the present investigation, acute static bioassays were conducted during summer, monsoon and winter season of 2019. During each season and before bioassay test, estuarine water samples were collected and analysed to quantify the cadmium. In April it was below detectable level. In July and December, it was 0.005 and 0.001 µg/ml respectively. Significant concentration of cadmium in monsoon was due heavy impounding of cadmium enriched soil, carried through rainy freshets. These values were considered during further experimental procedures. Static bioassay yielded different LC₀ and LC₅₀ values during three different seasons. During summer, calculated and observed LC₅₀ values were 1.7961 and 1.8 ppm respectively. The regression equation established is $Y = 2.8358 + 8.5506 \log X$. In monsoon, the calculated and observed LC₅₀ values were 2.1521 and 2.0 ppm respectively. The regression equation established is $Y = 0.5702 + 13.3074 \log X$. During winter, calculated and observed LC₅₀ values were 2.0946 and 2.1 ppm, respectively. Regression coefficient established is $Y = 1.0549 + 12.2856 \log X$. In the present study, it was found that, clams become more susceptible for cadmium toxicity in summer when water temperature and salinity was more. Increased water temperature, salinity and decreased river influx caused decrease in oxygen saturation in water. In monsoon, although water was impregnated with non-significant concentration of cadmium (0.005 µg/l) clams showed better resistance to cadmium because of optimum range of water parameters.

Keywords: *Meretrix meretrix*, Bhatye estuary, Cadmium toxicity, LC₅, LC₅₀, Acute toxicity

Introduction

For assessment of intensity and impact of contaminants on aquatic environment, Toxicity bioassay is proven and reliable method. During literature survey it was found that, numerous references are available related to this subject summarizing intensity and impact of contaminants like pesticides and heavy metals on organisms like crustaceans, mollusks and fishes. Many workers studied cadmium toxicity to estuarine mussels and fishes (Buikema and Cairns, 1982^[1]; Auffert, M. 1988^[2] Awari and Gaikwad, 1990^[3]; Chidambaram, N.1991)^[4] but scanty references are explaining cadmium toxicity to estuarine clams. Bhatye estuary is one of the most important and productive estuary along West coast of India. It is cradle bed for variety of edible crustaceans, mollusks fishes and shellfishes. Clams are abundantly found in Bhatye estuary and widely consumed by local people. Most of mussel, edible oyster and clam catch is transported and sold in adjoining states like Goa. Fishery of *M.meretrix* lasts for about 7 – 10 months. During lean period of open sea fishery, clam fishery provides cheap, protein rich food and livelihood to local population. Local population prefers *M.meretrix* as a cheap source of food, and thus, it is returning back to consumers. Considering its food value, economical value and comparatively stationary nature, it was decided to employ *M.meretrix* for detection of threshold limits of cadmium and its probable variance with seasonal hydromonics. Geochemistry of cadmium has been discussed by Eaton (1979)^[5] It is well established that although concentration of cadmium in surface water is negligible it increases many fold in sediments and still higher in marine biota. The concentration of heavy metals may exceed the

recommended threshold limit and pose a risk to humans and marine ecosystem (El-Gendy 2003)^[6]. Many workers have observed seasonal variations in heavy metal load in water, sediment and animals' bodies (Joseph and Shrivastav, 199^[7], Hamad Alyahya *et al.*, 2011)^[8]. In the present study, the seasonal estuarine water parameters like temperature, rainfall, salinity, pH and dissolved oxygen studied to unveil probable relations between hydromonics and sublethal, lethal thresholds for *M.meretrix* to cadmium chloride.

Material and Methods

The water samples were collected from five predecided sites from the Bhatye estuary, Ratnagiri. It is situated between 73°15'^[1] East and 16°51'^[1] North. It is formed due to merging of Kajali river in to Arabian sea near Ratnagiri. It has water spread of 2820 hectares and its mouth covers about 18 hectares. Its perennial opening permits an ideal estuarine zone for local fishermen. Physio-chemical parameters like temperature, salinity, pH and dissolved oxygen were determined for each season. Temperature of the aerated estuarine water used for holding the clams during experimentation in three different seasons was recorded at regular intervals. Salinity was recorded with the help of hand salinometer (Hanna instruments, Italy). pH of the estuarine water was measured by the Universal Indicator method in the field and the results were confirmed in the laboratory with digital pH meter. Dissolved oxygen was measured by Winkler's titrimetric method. Cadmium in estuarine water was analysed on Atomic Adsorption Spectrophotometer (Perkin Elmer Model 3030. USA). Clams of medium size measuring 4.0-4.8cms were selected and brought to the laboratory and stocked in plastic containers

containing filtered, aerated estuarine water for 48 h. During this period, water was changed thrice a day. While studying the toxicity, no food was given to the animals before or during the experimental period. The well acclimatized clams to laboratory conditions were grouped into 10 and kept in plastic containers containing 5 liter filtered estuarine water. Static bioassay tests were conducted for 96 h (acute) by using analytical grade cadmium chloride (CdCl_2 , Mole. Wt. 228.34, Atomic wt. 112.41), Cadmium chloride was dissolved in filtered estuarine water in appropriate volume to obtain the required stock solution. For every experiment a control group of clams was also run simultaneously. All the experiments were conducted in natural day light rhythm. The temperature, pH, dissolved oxygen and salinity of water used to hold the clams, were recorded during each experiment. The estuarine water of appropriate concentration of Cadmium chloride from plastic containers was renewed twice a day and mortality and behaviour of clams were recorded. The toxicity tests were repeated three times and the observed LC_{50} and LC_{50} values were determined. The regression equation between log concentrations (in ppm) (X) and the probity mortality (Y) was determined statistically for acute toxicity using the formula $Y = a + b \log X$, 95% fiducial limits were established (Finney, 1971)^[9]. Sensitivity of clams to cadmium chloride for three different seasons was statistically calculated according to Reed-Muench method of estimation of LD_{50} (Wolf, 1968)^[10]

Results and Discussion

Table 1: Seasonal estuarine water parameters of Bhatye Estuary, Ratnagirirashtra, Maharashtra during 2018 –2019

Experimental Period	Average water temperature. (0C)	Average Salinity (mg/l)	Average pH	Average Dissolved Oxygen (ml/l)
April-May.	26.5	35.1	8.2	3.9
July- August	24.6	23.94	7.1	5.6
Nov- Dec	25.7	33.4	8.3	4.7

While survey of literature it was noticed that, the independent variables like tides, river discharge, density, differences between water masses in the estuary caused by differences in salinity and temperature makes estuarine hydromonics very complex. Quasim, (1980)^[11] reported that, magnitude of variations in the environment depends on a large extent on the time of the year and the place of the observation. In the present study it was decided to focus on few estuarine parameters and unveil their probable relationship in intensifying or decreasing toxicity of cadmium chloride. It was observed that, average water temperature was minimum in winter (25.7). The maximum average water temperature was recorded in summer (26.5). Maximum average salinity was recorded in summer (35.1). It was mg/l in winter. Minimum average salinity was recorded in monsoon season (33.4). In the present study, it was observed that, salinity changes according to season. It was maximum in summer and minimum in monsoon. pH fluctuations was observed in all seasons. The average pH was 8.2, 7.1 and 8.3 in summer, monsoon and winter respectively.

The average dissolved oxygen was 3.9, 5.6 and 4.7 in summer, monsoon and winter respectively. During monsoon estuarine water showed maximum saturation of oxygen (5.6). It was minimum (3.9) in months of summer. Average pH during summer, monsoon and winter was 8.2, 7.1 and 8.3 respectively. Variations in pH did not show significant pattern, however, it was low in monsoon due to heavy influx of fresh water. Similar observations were noted by Chandran and Ramamorthy (1984)^[1, 2] in the Vellar estuary. According to skymet rainfall data average rainfall during monsoon was 2915mm, which was 53% more than previous year monsoon. During winter scanty rainfall was recorded. Summer received no rainfall. (Skymet Rainfall Data, 2019). In tropical countries rainfall is regular cyclic phenomenon. It brings profound changes in hydromonics of the estuarine environment (Quasim, 1980)^[11]. Heavy rainfall in monsoon reduces salinity, water temperature, pH values of estuary and increases turbidity and oxygen saturation. In the previous study author observed that, the hydromonics of Bhatye estuary is very labile and complete and it has profound effect on food, feeding behavior, metabolic and physiological processes in estuarine clams (Kumbhar, Sanjay 2001)^[13]. The hydromonics of an estuary in general is very complex.

Table 2: Distribution of cadmium in estuarine water of Bhatye Estuary, Ratnagiri, Maharashtra during 2018 –2019).

Month	Cadmium content ($\mu\text{g/l}$)
April	B.D.L.
July	0.005
December	0.001

It was decided to analyse preexistence of cadmium before conducting acute static bioassay. Unfortunately, data is not available stating cadmium concentration in Bhatye estuary. In the present study it was observed that, during summer, cadmium concentration in water was below detectable level. In monsoon and winter average concentration of cadmium was 0.005 and 0.001 $\mu\text{g/l}$ respectively. Maximum accumulation of cadmium was observed in July. At Ratnagiri, heavy rainfall is noted in monsoon (34.39 mm/day). Rainy freshets brought heavy load of soil that impregnated with heavy metals like cadmium. It resulted in increase in concentration of cadmium in monsoon. It also showed the rapid loss of cadmium during summer and winter. Fowler and Oregioni (1976)^[15] suggested that seasonal maximum concentration appeared in the spring was due to high water run-off which increased the amount of available metals. Philips (1973)^[14] proposed similar conclusion with zinc, cadmium, lead and copper. In the present investigation concentration of cadmium in estuarine water was considered while quantifying sublethal and lethal concentrations of cadmium chloride. S. Ray (1984)^[16] reported that, in open sea water cadmium occurs in very low concentrations, averaging about 40ng/l. Eaton and Boys *et al* (1987)^[17] observed 40 - 60 ng/l cadmium concentration in North Atlantic Ocean. S. Ray (1984) further stated that cadmium concentration in coastal environment is normally higher due to weathering and anthropogenic activities. In the present investigation concentration of cadmium in estuarine water was considered while quantifying sublethal and lethal concentrations of cadmium chloride.

Table 3: Acute toxicity of cadmium chloride to *M. meretrix* in different seasons.

Season	Period (hrs.)	Concentrations (in ppm)							
		1.1	1.3	1.4	1.5	1.7	1.8	1.9	2.1
Summer	12	-	-	-	-	-	-	1(d)	-
	24	-	-	-	-	-	-	-	-
	36	-	-	-	-	-	1(d)	1(d)	-
	48	-	-	-	-	1(d)	1(d)	1(d)	1(d)
	60	-	-	-	1(d)	1(d)	1(d)	1(d)	1(d)
	72	-	-	1(d)	1(d)	1(d)	1(d)	1(d)	2(d)
	84	-	-	-	1(d)	-	1(d)	-	1(d)
	96	-	1(d)	1(d)	-	1(d)	-	1(d)	2(d)
	M	0%	10%	20%	30%	40%	50%	60%	70%
Season	Period (hrs.)	Concentrations (in ppm)							
Monsoon	12	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-
	36	-	-	-	-	-	-	1(d)	-
	48	-	-	-	-	-	1(d)	1(d)	1(d)
	60	-	-	-	1(d)	1(d)	1(d)	1(d)	1(d)
	72	-	-	-	1(d)	1(d)	1(d)	-	1(d)
	84	-	-	-	1(d)	1(d)	1(d)	1(d)	1(d)
	96	-	1(d)	1(d)	-	1(d)	1(d)	2(d)	3(sd)
	M	0%	10%	20%	30%	40%	50%	60%	70%
Season	Period (hrs.)	Concentrations (in ppm)							
Winter	12	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	2(d)	1(d)
	36	-	-	-	-	-	1(d)	1(d)	1(d)
	48	-	-	-	-	-	1(d)	1(d)	1(d)
	60	-	-	-	1(d)	1(d)	1(d)	-	1(d)
	72	-	-	-	1(d)	1(d)	1(d)	1(d)	-
	84	-	-	1(d)	1(d)	1(d)	1(d)	-	1(d)
	96	-	1(d)	1(d)	-	1(d)	-	1(d)	2(d)
	M	0%	10%	20%	30%	40%	50%	60%	70%

0 = No mortality; (d) = No. of clams died; M= % mortality

Table 4: Regression equations, 95% fiducial limits with LC₅₀ values for *M. meretrix* exposed to cadmium chloride

Season	Y = α + β x	95% fiducial limits (ppm)	Calculated LC ₅₀ (ppm)	Observed LC ₅₀ (ppm)
Summer	Y = 2.8358+8.5506 x	0.7858 – 2.7961	1.7910	1.8
Monsoon	Y = 0.5702+13.3074 x	1.5170 – 2.7873	2.1521	2.0
Winter	Y = 1.0549+12.2856 x	0.8622 – 3.3269	2.0946	2.1

In the present investigation, it was found that, during summer, there was no mortality up to 1.3 ppm. There was 10% mortality in 1.4 ppm, whereas in 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 ppm; there was 20%, 30%, 40%, 50%, 60% and 70% mortality at the end of 96 h respectively. The calculated LC₅₀ value was 1.7961 ppm. The regression equation established is Y = 2.8358+8.5506 log X. The fiducial limit is 0.7858-2.7961 ppm for this season. It was observed that values for sublethal and lethal concentrations were low in summer. Water temperature and salinity was high during summer. The bioaccumulation and toxicity of metals increases with increase in temperature. The absorption and release of metals can also depend on temperature. This was established for mercury, methyl mercury and phenyl acetate in rainbow trout, *Salmo gairdneri*. (Mc Leod, 1973) [18]. Salinity is key trigger of other environmental factors. It depends on certain factors as

local precipitation, water influx, mixing of fresh water with sea water and evaporation. Hence, salinity is more labile parameter than any other estuarine water parameter. During summer salinity exceeds to its high(30.35mg/l) that minimized saturation of oxygen (3,7mg/l). Low saturation of oxygen enhanced oxygen consumption and energy utilization (Kumbhar, Sanjay, 2001) [13]. Temperature effects on chemical toxicity are complex because temperature alone may be lethal and toxicant may alter lethal thermal limits in unpredictable way (Habden, 1967) [19]. Less oxygen saturation and high salinity are other combined factors with high temperature and their combined effect might have intensified toxicity of cadmium. During monsoon, when the clams were subjected to different concentrations of cadmium chloride, there was no mortality up to 1.5 ppm. Mortality began to occur in 1.6 ppm (10% mortality), whereas in 1.7, 1.8, 1.9, 2.0, 2.1 and 2.2 ppm. There was 20%, 30%, 40%, 50%, 60% and 70% mortality respectively. The calculated LC₅₀ was 2.1521 ppm. The regression equation established is Y at the end of 96 h. =0.5702+13.3074 log X. The fiducial limit is 1.5170-2.7873 ppm. During monsoon saturation of oxygen was more (5.0ml/l). Salinity (5.2mg/l) and temperature (26.1 C) were comparatively low. Combined effect of these factors and abundance of food might have yielded high threshold limits for cadmium chloride. pH values were low during monsoon due to heavy influx of fresh water. During winter, clams showed no mortality in 1.7 ppm; whereas 20%, 30%, 40%, 50%, 60% and 70% mortality was found in 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, ppm respectively at the end of 96hrs. Regression coefficient established is Y= 1.0549+12.2856 log X. The fiducial limit is 0.8622 - 3.3269.ppm. Although having optimum salinity (30.36 mg/l) and pH (8.5) clams showed low threshold limits for cadmium. It might be due to high temperature (25.5 C) and comparatively low oxygen saturation(4.3ml/l) Many workers have contributed in cadmium bioassay (Mohan *et al*, 1986 [20]; Vincent *et al*. 1987 [22]; Awari and Gaekwad, 1994) [23], but no work done so far related with seasonal changes in lethal and sublethal concentrations of contaminants like cadmium.

Conclusion

In the present study, it was found that, physico-chemical estuarine water parameters play a key role in inducing toxicity of cadmium chloride to *M.meretrix*. Due to influence of estuarine water parameters acute toxicity bioassay yielded different sublethal and lethal concentration values for summer, monsoon and winter season. Clams were more susceptible to cadmium chloride toxicity during summer and comparatively less in winter and monsoon. This probably related to higher water temperature, salinity and less oxygen saturation of estuarine water. In monsoon and winter plentiful availability of food and favourable water parameters increased resistance of *M. meretrix* to cadmium chloride. All these parameters have very complex, interactive interactions with each other and they have profound impact on cadmium chloride toxicity to *M.meretrix*.

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