CHAPTER 2
BIOACCUMULATION STUDY

BIOACCUMULATION OF COPPER AND MERCURIC IN THE TISSUES OF A FRESHWATER FEMALE CRAB, BARYTELPHUSA CUNICULARIS [WESTWOOD].

“The man of science appears to be the only man who has something to say just now and the only man who does not know how to say it.”

-- Sir James Barrie
Chapter 2 Bioaccumulation Study

2.1 INTRODUCTION

Comprising over 70% of the Earth’s surface, water is undoubtedly the most precious natural resource that exists on our planet. Without the seemingly invaluable compound comprised of hydrogen and oxygen, life on Earth would be non-existent: it is essential for everything on our planet to grow and prosper. The impact of heavy metals in the environment has long been recognized as a risk factor for human health. The chemical reactivity of the metals depends on the particular environment or species in which it is present and its chemical form.

The problem of environmental pollution on account of essential industrial growth is, in practical terms, the problem of disposal of industrial water, whether solid, liquid or gaseous. All three types of wastes have the potential of polluting water. Polluted water, in addition to other effects, directly effects, soil not only in industrial areas but also in agricultural fields, as well as the beds of rivers, creating secondary sources of pollution [Barman, et al., 2000; Kisku, et al., 2000].

Aquatic animals include fishes, crabs and prawns etc. have their own way of life which makes it possible for them to live in water. Metal bioavailability to organisms involves at least two distinct phases. Firstly there must be a physicochemical desorption process driven by soil’s physical and chemical characteristics and this determines the supply of the metal to a potentially available pool [Peijenburg, 2002]. A physiologically driven uptake process must then follow. Since the environmental hazard of a chemical does not depend on its concentration in the environment it is essential to understand the pathways and the mechanisms through which a chemical enters the organism and also to evaluate the bioaccumulation potential of the chemical [Peijenburg et al. 1997].
Several researchers have revealed heavy metal content in crustaceans, the source of the heavy metals in them, and the toxic effect of the heavy metal content in crustaceans and in turn effects on man who consumes the crustacean [Baird and Ulanowics, 1999; Oluwu, et al., 2010]. Although many heavy metals are essential for animal tissue metabolism, the ranges between beneficial and toxic levels are very small. There is an increasing concern about the effect in human due to continuous consumption of food contaminated with heavy metals. The extent of this contamination depends on several complex factors, one of which is the specific metabolic and homeostatic mechanisms operating in the type of food and tissue considered [Chukwujindu, et al., 2008].

Agricultural activities also contributed to the pollution or the aquatic environment through run off which find their ways into the water bodies [Ikem, et al., 2003; Oyewo and Don-Pedro, 2003; Lisa and Klaus, 2005; Majolagbe and Bangbose, 2007; Jibiri and Adewuyi, 2008]. Increasing concentrations of the metals cause significant increases in the mortality in crabs and prawns. Increasing metal concentrations in permutation with varying salinities exhibit a significant negative influence on crustacean development [Adeniyi, et al., 2008].

**Current state of the science on bioaccumulation and bioavailability issues.**

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 [Dennis, et al., 2005; Soundarapandian, et al., 2010]. Indirect exposure occurs as dietary exposure when metals bioaccumulate in organisms at a lower trophic level are subsequently ingested by consumer organisms with the potential for effects or bioaccumulation. Even though direct and indirect exposure of
bioavailability and bioaccumulation are considered separately, this has only been done for practical reasons, because, in natural systems, these occur in unison.

The factors which contribute principally to the damaging effect of metal as environmental pollutants are, firstly, their inadequate biological degradation to inert metals and secondly, the trend of metals to accumulate and largely remain in the aquatic environment [Ikem, et al., 2003].

**Bioaccumulation of metals in aquatic biota.**

![Diagram of contaminant uptake by crab](image)

**Fig.1.** Uptake and bioaccumulation of contaminants by crab.

Direct uptake of metals from water occurs by either adsorption onto cell or organism surfaces or absorption across cell walls or body surfaces such as the gill and/or gut. Aquatic animals also accumulate metals through assimilation of ingested food [Fig 1]. The absorption of metals from water to organism surfaces is typically greater in smaller organisms since the role of surface area in total accumulation is of far less importance in larger species that have a low surface area to volume ratio [Phillips, 1980; Fowler, 1982].
In brief, uptake is generally nonlinear and often biphasic with an initial rapid component representing surface adsorption followed by a slower rate of metal bioaccumulation into internal tissues. The uptake rate generally decreases until a steady state is reached between the metal in the water and in organism tissues. In larger organisms, internal tissues are often isolated from the surrounding water, with longer equilibration times for surficial metal absorption from water [days to weeks] compared to small species such as plankton. The importance of the initial component of uptake depends to some extent on the surface characteristics of the organism. Hard shelled, calcareous animals can deposit appreciable amounts of metal in the shell during growth, whereas soft bodied organisms with no hard, external covering are able to equilibrate their internal tissues more rapidly.

What is bioaccumulation and Bioconcentration Factor [BCF]?

According to Smith, et al., [1988], “Bioaccumulation [or bioconcentration] is the uptake of organic compounds by biota from either water or food. Many toxic organic chemicals attain concentrations in biota several orders of magnitude greater than their aqueous concentrations, and therefore, bioaccumulation poses a serious threat to both the biota of surface waters and the humans that feed on these surface-water species.”

Bioaccumulation is the progressive increase in the amount of a substance in an organism or part of an organism which occurs because the rate of intake exceeds the organism’s ability to remove the substance from the body [International Union of Pure and Applied Chemistry, 1993].

U.S. Environmental Protection Agency [2000] defined bioaccumulation as the accumulation of chemicals in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, and pore water in the sediment. According to U.S. Geological Survey [2007], bioaccumulation is the biological sequestering of a substance at a higher
concentration than that at which it occurs in the surrounding environment or medium.

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, [U.S. Environmental Protection Agency, USEPA, 2010].

According to Chiou [2002], Bioconcentration Factor [BCF] is a major concern for environmental contamination is the extent to which pollutants concentrate from water into aquatic organisms such as fish. The extent of such concentration, termed the bioconcentration factor [BCF], is given by the ratio of the pollutant concentration in animals to that in water.”

The heavy metal contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by the organisms. The progressive and irreversible accumulation of these metals in various organs of marine as well as freshwater creatures ultimately leads to metal-related diseases in the long run because of their toxicity, thereby endangering the aquatic biota and the organisms therein [Rainbow, 1995; Mackey, 1996; Wu and Chen, 2004; Reddy, et al., 2007; Agoes, 2008].

The contamination of freshwater with wide range of pollutants has become a matter of great concern over the last decades. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities [Lenan et al., 1978; Mance, 1987; Kalay and Canli, 2000]. Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior [Heath, 1987; Allen, 1995].
Heavy metals may affect organisms directly by accumulating in their bodies or indirectly by transferring to the next trophic level of the food chain [Shah and Altindag 2005]. Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level [Kalay and Canli 2000].

Copper sulfate is one of the chemicals that are frequently used for the control of some fungal, parasite, and bacterial diseases of fish in the local environments. It is also used as an algaecide, molluscicide, and herbicide in aquaculture, irrigation, and municipal water treatment systems [Stoskopf, 1993]. However, industrial development has contributed to a continuous increase of copper in the aquatic environment. The Environmental Bureau in the United States has adopted the copper limits recommended by the Environmental Protection Agency [USEPA, 1984] for the protection of aquatic life. Copper is an essential metal for various physiological activities, but at higher concentration it tends to produce toxic effects [Maiti and Banerjee, 2000].

Most of the studies devoted so far to mercury contamination of aquatic animals have been concerned with the levels of metal accumulation in various species under different environmental conditions. Knowledge about the uptake, distribution, and persistence in tissues of heavy metals is well documented [Karuppasamy, 1999]. Toxic effects and the capacity of the metal for biomagnifications along food chains have also been well documented [Jackson 1991; Parkman & Meili 1993; Suedel *et al.*, 1994; Stein *et al.* 1996, 1998; Boudou & Ribeyre, 1998].

Heavy metals are considered serious contaminants of aquatic system due to their extended biological half-life inherent toxic nature at low concentration and high rate of bioaccumulation [Baby & Menon, 1986]. Heavy metal pesticide pollution is a major problem in the aquatic environment because of the toxicity,
their persistence, their tendency to accumulate in the organisms and undergo food chain amplification [Weis and Weis, 1977; Radike, et al., 2002; Reinecke, et al., 2003; Cornelis, et al., 2005; Swaileh and Sansur, 2006].

To better understand not only physiological effects but also toxicological and hygienic organism great attention is paid to hazardous elements such as mercury, lead, cadmium and arsenic [Alam, et al., 2002; Maffuci, et al., 2005]. An important negative characteristic of metals is their ability to accumulate in organs, especially in the liver, spleen, kidneys and gonads [Spurny, et al., 2002; Yilmaz, 2006; Andreji, et al., 2006b].

The uptake and accumulation of known pollutants by the whole organisms [in the case of crustaceans] can also provide useful data on the type and nature of toxicants [Hogstrand and Haux, 1991; Gill, et al., 1991; Fent and Hunn, 1993; Kaviraj and Das, 1995].

In crustacean species, it is also thought that there is a relationship between metal permeability, regulation and accumulation. If the metal uptake rate in organisms permeable to metals is higher than the excretion rate, then metal accumulation occurs. Metal accumulation in crustaceans seems to occur mainly in hepatopancreas and exoskeleton [Martinez, et al., 1993; Viarango and Nott, 1993]. The use of aquatic organisms to monitor trace metal bioavailability is well known [Glaven and Gandley, 1991; Amyot, et al., 1992; Kemal, et al., 1999].

An ideal bioindicator should be a net accumulator of the trace metal in question which does not in the body metal concentrations to relatively constant levels over a range of metal bioavailability, with a correlation between the metal content of the organism and the average metal concentration in surrounding water. 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 affecting the aquatic animals specially crabs very widely. The objective of the present study is the bioaccumulation of copper and mercury in the ovary, hepatopancreas, gills, thoracic muscle and spermatheca of the freshwater crab, *Barytelphusa cunicularis* [Westwood] exposed to sublethal concentration of copper sulphate and mercuric chloride for 5 and 10 days of exposure.

### 2.2. Material and Method

The freshwater crabs, *Barytelphusa cunicularis* were collected from Pimpalwadi site [Jaikwadi Dam Paithan] located at [19°29′6″N 75°22′12″E] Aurangabad District. They were acclimatized to laboratory conditions under normal day/night of 11 L : 13 D illumination at 27 ± 1°C for about one week in plastic troughs [18” diameter] containing sufficient tap water so that crabs are submerged. Before experimentation female crabs in intermoult stage [C3 Diwan, 1973] of approximately equal carapace width [45 to 50 mm] and body weight [50 to 55 gm] were sorted. For heavy metal accumulation testing the crabs were split into 3 groups [Control, CuSO₄ treated and HgCl₂ treated] with similar biomass [n = 6 for each group], each group being maintained in laboratory condition. The crabs were exposed to sublethal concentration of copper sulphate at 1/5 [56.4 ppm] and 1/10 [28.2 ppm] and mercuric chloride 1/5 [0.208 ppm] and 1/10 [0.104 ppm] for 5 and 10 days to analyzed the bioaccumulation of copper [Cu] and mercury [Hg]. Control animals were kept under the same conditions but without added metal. After their respective exposure period, the tissues like, ovary, hepatopancreas, gills, thoracic muscles and spermatheca were dissected out from both the control and experimental crabs to assess the concentration of accumulated heavy metals in the tissues.
Determination of Bioaccumulation of copper and mercury in the tissues of freshwater crab, *Barytelphusa cunicularis* [Westwood].

The samples were digested according to the methods described by Van Loon [1980] and Due Freez and Steyn [1992].

The dissected tissues were dried for 72 hr at 70 °C in oven. The dried tissue was powdered using mortar and pestle and 500 mg of dried tissue powder was taken in beaker and 10 ml concentrated nitric acid was added. The mixture was shaken well and kept on hot plate to evaporate the solution. After its complete evaporation again added [2:1] mixture of nitric acid [HNO₃] and per chloric acid [HClO₄], mixed well and kept on hot plate and waited till the solution evaporates and the solution becomes colorless. The dense white fumes from the beaker after brown fumes were an indication of completion of the process of digestion. No need to add per chloric acid again. Then third time again added 10 ml of concentrated nitric acid, mixed the content well and keep 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 concentrated nitric acid and samples were stored in properly washed bottles until the metal concentration could be determined. PC based Atomic Absorptive Spectrophotometer [AAS- SL-163] was used for the determination of heavy metals concentration of copper [Cu²⁺] and mercury [Hg²⁺] in ovary, hepatopancreas, gills, muscle and spermatheca tissue sample of crab. Copper [mA-15, Sr. No. 511047] and mercury [mA-7, Sr. No. 509753] hallow cathode lamp was used. Each sample was analysed in triplicate and the results were averaged and given as µg / g dry tissue. For each metal, stock solutions were used to prepare 4 different concentrations of analytical standards. Calibration of the spectrophotometer, for each metal individually, was subsequently conducted with the aid of these standards. Individual concentrations of digested tissue were then read against particular absorbencies defined the concentration of heavy metals present. The metal concentrations thus
2.3 OBSERVATION AND RESULTS

Bioaccumulation and bioconcentration factor [BCF] of heavy metals like copper and mercury were studied in various tissues of freshwater crab, *Barytelphusa cunicularis* [Westwood] exposed to sublethal concentrations of copper sulphate and mercuric chloride. Bioconcentration factor [BCF] is a physical property that characterizes the accumulation of chemicals, including pollutants, through chemical partitioning from the aqueous phase into an organic phase. Bioconcentration is the concentration of a particular chemical in a biological tissue per concentration of that chemical in water surrounding that tissue. That is, a dimensionless number representing how much of a chemical is in a tissue relative to how much of that chemical exists in the environment [Chiou, 2002].

\[
\text{BCF} = \frac{\text{Concentration}_{\text{Organism}}}{\text{Concentration}_{\text{Environment}}}
\]

The tissues like ovary, hepatopancreas, gill, thoracic muscle and spermatheca were analyzed to find the accumulation of copper and mercury after 5 and 10 days of exposure. The results show that the rate of accumulation and percent of bioconcentration factor were fluctuated from month to month. Tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate.

[A] COPPER [Cu^{2+}]:

The average value of copper concentration in the freshwater of Pimpalwadi site [Jaikwadi Dam] is 21.4 ± 0.004 µg / lit. The crabs *Barytelphusa cunicularis* were exposed to sublethal concentrations [1/5\(^{th}\): 56.4 ppm and 1/10\(^{th}\): 28.2 ppm] of copper sulphate. The value of bioaccumulation and bioconcentration factor could be subtracted from the concentrations determined for the digested 50 ml sample, using the following formula and obtained data was analyzed and results were expressed as mean ± S.E.
[BCF] in ovary, hepatopancreas, gills, thoracic muscles and spermatheca after 5 and 10 days of exposure period are represented in Table 1 to 8 and Fig. 1 to 8 [A-E].

1. OVARY: - The season average of bioconcentration factor [BCF] of copper in the ovary of control crab was found to be 0.018 [winter], 0.014 [summer] and 0.019 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5th: 56.4 ppm] of copper sulphate the season average of BCF in the ovary were found to be 0.41 [winter], 0.40 [summer] and 0.45 [monsoon] after 5 days of exposure [Table-1 and Fig- 1(A)] and 0.97 [winter], 0.90 [summer] and 1.04 [monsoon] after exposure for 10 days [Table-3 and Fig-3 (A)]. Percent increase in the BCF in the ovary of experimental crab was found to be 2177.8 % in winter, 2757.1 % in summer and 2268.4 % in monsoon after 5 days of exposure [Table-2]. Similarly an increase of 5311.1 % in winter, 6350 % in summer and 5352.6 % in monsoon was found after 10 days of exposure [Table-4].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10th: 28.2 ppm] of copper sulphate the season average of BCF in the ovary were found to be 0.31 [winter], 0.29 [summer] and 0.34 [monsoon] after 5 days of exposure [Table-5 and Fig-5 (A)] and 0.57 [winter], 0.59 [summer] and 0.60 [monsoon] after exposure for 10 days [Table-7 and Fig- 7 (A)]. Percent increase in the BCF in the ovary of experimental crab was found to be 1600 % in winter, 2014.3 % in summer and 1678.9 % in monsoon after 5 days of exposure [Table-6]. Similarly an increase of 3055.5 % in winter, 4150 % in summer and 3052.6 % in monsoon was found after 10 days of exposure [Table-8].

Percent increase of bioconcentration factor [BCF] of copper in the ovary was in the order: - summer > monsoon > winter.

2. HEPATOPANCREAS: - The season average of bioconcentration factor [BCF] of copper in the hepatopancreas of control crab was found to be 0.035 [winter], 0.048 [summer] and 0.049 [monsoon]. In experimental crab, exposed to the
sublethal concentration [1/5\textsuperscript{th}: 56.4 ppm] of copper sulphate the season average of BCF in the hepatopancreas were found to be 0.97 [winter], 0.91 [summer] and 0.82 [monsoon] after 5 days of exposure [Table-1 and Fig- (B)] and 2.20 [winter], 2.22 [summer] and 1.96 [monsoon] after exposure for 10 days [Table-3 and Fig-3 (A)]. Percent increase in the BCF in the hepatopancreas of experimental crab was found to be 2671.4 % in winter, 1795.8 % in summer and 1579.6 % in monsoon after 5 days of exposure [Table-2]. Similarly an increase of 6194.3 % in winter, 4525 % in summer and 3900 % in monsoon was found after 10 days of exposure [Table-4].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10\textsuperscript{th}: 28.2 ppm] of copper sulphate the season average of BCF in the hepatopancreas were found to be 0.68 [winter], 0.71 [summer] and 0.64 [monsoon] after 5 days of exposure [Table-5 and Fig-5 (B)] and 1.26 [winter], 1.61 [summer] and 1.62 [monsoon] after exposure for 10 days [Table-7 and Fig- (B)]. Percent increase in the BCF in the hepatopancreas of experimental crab was found to be 1900 % in winter, 1375 % in summer and 1197.9 % in monsoon after 5 days of exposure [Table-6]. Similarly an increase of 3514.3 % in winter, 3268.7 % in summer and 3214.3 % in monsoon was found after 10 days of exposure [Table-8].

Percent increase of bioconcentration factor [BCF] of copper in the hepatopancreas was in the order: - **winter>summer>monsoon**.

**3. GILLS:** - The season average of bioconcentration factor [BCF] of copper in the gills of control crab was found to be 0.007 [winter], 0.015 [summer] and 0.009 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5\textsuperscript{th}: 56.4 ppm] of copper sulphate the season average of BCF in the ovary were found to be 0.38 [winter], 0.28 [summer] and 0.33 [monsoon] after 5 days of exposure [Table-1 and Fig- 1 (C)] and 0.46 [winter], 0.40 [summer] and 0.70 [monsoon] after exposure for 10 days [Table-3 and Fig-3 (C)]. Percent increase in the BCF in the gills of experimental crab was found to be 5185.7 % in winter, 1766.7 % in
summer and 3566.7 % in monsoon after 5 days of exposure [Table-2]. Similarly an increase of 6512.3 % in winter, 2563.3 % in summer and 7722.2 % in monsoon was found after 10 days of exposure [Table-4].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10\textsuperscript{th}: 28.2 ppm] of copper sulphate the season average of BCF in the gills were found to be 0.26 [winter], 0.23 [summer] and 0.25 [monsoon] after 5 days of exposure [Table-5 and Fig-5 (C)] and 0.45 [winter], 0.34 [summer] and 0.36 [monsoon] after exposure for 10 days [Table-7 and Fig-7 (C)]. Percent increase in the BCF in the gills of experimental crab was found to be 3657.1 % in winter, 1486.7 % in summer and 2711.1 % in monsoon after 5 days of exposure [Table-6]. Similarly an increase of 6300 % in winter, 2193.3 % in summer and 3877.8 % in monsoon was found after 10 days of exposure [Table-8].

Percent increase of bioconcentration factor [BCF] of copper in the gill was in the order: - monsoon > winter > summer.

4. THORACIC MUSCLE: - The season average of bioconcentration factor [BCF] of copper in the thoracic muscle of control crab was found to be 0.011 [winter], 0.009 [summer] and 0.013 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5\textsuperscript{th}: 56.4 ppm] of copper sulphate the season average of BCF in the ovary were found to be 0.39 [winter], 0.38 [summer] and 0.44 [monsoon] after 5 days of exposure [Table-1 and Fig-1 (D)] and 0.59 [winter], 0.61 [summer] and 0.48 [monsoon] after exposure for 10 days [Table-3 and Fig-3 (D)]. Percent increase in the BCF in the thoracic muscle of experimental crab was found to be 3445.4 % in winter, 4650 % in summer and 3284.6 % in monsoon after 5 days of exposure [Table-2]. Similarly an increase of 5300 % in winter, 7487.5 % in summer and 3576.9 % in monsoon was found after 10 days of exposure [Table-4].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10\textsuperscript{th}: 28.2 ppm] of copper sulphate the season average of BCF in the thoracic
muscles were found to be 0.30 [winter], 0.32 [summer] and 0.25 [monsoon] after 5 days of exposure [Table-5 and Fig-5 (D)] and 0.40 [winter], 0.45 [summer] and 0.48 [monsoon] after exposure for 10 days [Table-7 and Fig-7 (D)]. Percent increase in the BCF in the thoracic muscle of experimental crab was found to be 2690.9% in winter, 3887.5% in summer and 1853.8% in monsoon after 5 days of exposure [Table-6]. Similarly an increase of 3563.6% in winter, 5525% in summer and 3592.3% in monsoon was found after 10 days of exposure [Table-8].

Percent increase of bioconcentration factor [BCF] of copper in the thoracic muscle was in the order: summer > winter > monsoon.

5. SPERMATHECA: - The season average of bioconcentration factor [BCF] of copper in the spermatheca of control crab was found to be 0.004 [winter], 0.010 [summer] and 0.006 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5th: 56.4 ppm] of copper sulphate the season average of BCF in the ovary were found to be 0.21 [winter], 0.25 [summer] and 0.23 [monsoon] after 5 days of exposure [Table-1 and Fig-1 (E)] and 0.33 [winter], 0.37 [summer] and 0.31 [monsoon] after exposure for 10 days [Table-3 and Fig-3 (E)]. Percent increase in the BCF in the spermatheca of experimental crab was found to be 5275% in winter, 2360% in summer and 3750% in monsoon after 5 days of exposure [Table-2]. Similarly an increase of 8075% in winter, 3640% in summer and 5116.7% in monsoon was found after 10 days of exposure [Table-4].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10th: 28.2 ppm] of copper sulphate the season average of BCF in the spermatheca were found to be 0.12 [winter], 0.16 [summer] and 0.14 [monsoon] after 5 days of exposure [Table-5 and Fig-5 (E)] and 0.29 [winter], 0.31 [summer] and 0.26 [monsoon] after exposure for 10 days [Table-7 and Fig-7 (E)]. Percent increase in the BCF in the spermatheca of experimental crab was found to be 3025% in winter, 1510% in summer and 2083.3% in monsoon after 5 days of
exposure [Table-6]. Similarly an increase of 7150 % in winter, 3040 % in summer and 4350 % in monsoon was found after 10 days of exposure [Table-8].

Percent increase of bioconcentration factor [BCF] of copper in the spermatheca was in the order: - winter>monsoon>summer.

[B] MERCURY [Hg^{2+}]:

The average value of mercury concentration in the freshwater of Pimpalwadi site [Jaikwadi Dam] is 0.9 ± 0.001 µg / lit. The crabs Barytelphusa cunicularis were exposed to sublethal concentrations [1/5<sup>th</sup>: 0.208 ppm and 1/10<sup>th</sup>: 0.104 ppm] of mercuric chloride. The value of bioaccumulation and bioconcentration factor [BCF] in ovary, hepatopancreas, gills, thoracic muscles and spermatheca after 5 and 10 days exposure period are represented in Table 9 to 16 and Fig 1 to 16 [A-E].

1. OVARY: - The season average of bioconcentration factor [BCF] of mercury in the ovary of control crab was found to be 0.068 [winter], 0.037 [summer] and 0.017 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5<sup>th</sup>: 0.208 ppm] of mercuric chloride the season average of BCF in the ovary were found to be 2.31 [winter], 2.33 [summer] and 2.51 [monsoon] after 5 days of exposure [Table-9 and Fig-9 (A)] and 3.2 [winter], 2.57 [summer] and 3.80 [monsoon] after exposure for 10 days [Table-11 and Fig-11 (A)]. Percent increase in the 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 days of exposure [Table-10]. Similarly an increase of 4605.8 % in winter, 6845.9 % in summer and 22252.9 % in monsoon was found after 10 days of exposure [Table-12].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10<sup>th</sup>: 0.104 ppm] of mercuric chloride the season average of BCF in the ovary were found to be 1.43 [winter], 1.13 [summer] and 1.68 [monsoon] after 5 days of exposure [Table-13 and Fig-13 (A)] and 2.81 [winter], 2.59 [summer] and 3.17
Percent increase of bioconcentration factor [BCF] of mercury in the ovary was in the order: - monsoon>summer>winter.

2. HEPATOPANCREAS: - The season average of bioconcentration factor [BCF] of mercury in the hepatopancreas of control crab was found to be 0.077 [winter], 0.03 [summer] and 0.036 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5th: 0.208 ppm] of mercuric chloride the season average of BCF in the hepatopancreas were found to be 2.85 [winter], 2.31 [summer] and 2.72 [monsoon] after 5 days of exposure [Table-9 and Fig-9 (B)] and 3.71 [winter], 3.19 [summer] and 3.65 [monsoon] after exposure for 10 days [Table-11 and Fig-11 (B)]. Percent increase in the BCF in the hepatopancreas of experimental crab was found to be 3601.2 % in winter, 7600 % in summer and 7455.5 % in monsoon after 5 days of exposure [Table-10]. Similarly an increase of 4718 % in winter, 10533.3 % in summer and 10038.9 % in monsoon was found after 10 days of exposure [Table-12].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10th: 0.104 ppm] of mercuric chloride the season average of BCF in the hepatopancreas were found to be 1.72 [winter], 1.95 [summer] and 1.65 [monsoon] after 5 days of exposure [Table-13 and Fig-13 (B)] and 3.10 [winter], 2.78 [summer] and 3.38 [monsoon] after exposure for 10 days [Table-15 and Fig-15 (B)]. Percent increase in the BCF in the hepatopancreas of experimental crab was found to be 2133.7 % in winter, 6400 % in summer and 4483.3 % in monsoon after 5 days of exposure [Table-14]. Similarly an increase of 3925.9 % in winter, 9166.7 % in summer and 9288.9 % in monsoon was found after 10 days of exposure [Table-16].
Percent increase of bioconcentration factor [BCF] of mercury in the hepatopancreas was in the order: - **summer>monsoon>winter**.

3. **GILLS**: - The season average of bioconcentration factor [BCF] of copper in the gills of control crab was found to be 0.049 [winter], 0.007 [summer] and 0.014 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5\textsuperscript{th}: 0.208 ppm] of mercuric chloride the season average of BCF in the gills were found to be 2.57 [winter], 2.73 [summer] and 1.21 [monsoon] after 5 days of exposure [Table-9 and Fig-9 (C)] and 3.39 [winter], 3.03 [summer] and 1.69 [monsoon] after exposure for 10 days [Table-11 and Fig-11 (C)]. Percent increase in the BCF in the gills of experimental crab was found to be 5144.8 % in winter, 38900 % in summer and 8542.8 % in monsoon after 5 days of exposure [Table-10]. Similarly an increase of 6818.4 % in winter, 43185.7 % in summer and 11971.4 % in monsoon was found after 10 days of exposure [Table-12].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10\textsuperscript{th}: 0.104 ppm] of mercuric chloride the season average of BCF in the gills were found to be 1.73 [winter], 1.97 [summer] and 0.88 [monsoon] after 5 days of exposure [Table-13 and Fig-13 (C)] and 3.31 [winter], 2.83 [summer] and 1.66 [monsoon] after exposure for 10 days [Table-15 and Fig-15 (C)]. Percent increase in the BCF in the gills of experimental crab was found to be 3430.6 % in winter, 28042.8 % in summer and 6185.7 % in monsoon after 5 days of exposure [Table-14]. Similarly an increase of 6655 % in winter, 40328.6 % in summer and 11757 % in monsoon was found after 10 days of exposure [Table-16].

Percent increase of bioconcentration factor [BCF] of mercury in the gills was in the order: - **summer>monsoon>winter**.

4. **THORACIC MUSCLE**: - The season average of bioconcentration factor [BCF] of copper in the thoracic muscle of control crab was found to be 0.051 [winter], 0.038 [summer] and 0.046 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5\textsuperscript{th}: 0.208 ppm] of mercuric chloride the season
average of BCF in the thoracic muscles were found to be 1.72 [winter], 2.85 [summer] and 2.27 [monsoon] after 5 days of exposure [Table-9 and Fig-9 (D)] and 2.39 [winter], 2.98 [summer] and 3.21 [monsoon] after exposure for 10 days [Table-11 and Fig-11 (D)]. Percent increase in the BCF in the thoracic muscle of experimental crab was found to be 3272.5 % in winter, 7400 % in summer and 4834.8 % in monsoon after 5 days of exposure [Table-10]. Similarly an increase of 4586.3 % in winter, 10373.7 % in summer and 6878.3 % in monsoon was found after 10 days of exposure [Table-12].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10th: 0.104 ppm] of mercuric chloride the season average of BCF in the thoracic muscles were found to be 1.16 [winter], 2.24 [summer] and 1.83 [monsoon] after 5 days of exposure [Table-13 and Fig-13 (D)] and 2.1 [winter], 2.89 [summer] and 3.18 [monsoon] after exposure for 10 days [Table-15 and Fig-15 (D)]. Percent increase in the BCF in the thoracic muscle of experimental crab was found to be 2174.5 % in winter, 5794.7 % in summer and 3878 % in monsoon after 5 days of exposure [Table-14]. Similarly an increase of 4017.6 % in winter, 7505 % in summer and 6813 % in monsoon was found after 10 days of exposure [Table-16].

Percent increase of bioconcentration factor [BCF] of mercury in the thoracic muscle was in the order: - summer>monsoon>winter.

5. SPERMATHECA: - The season average of bioconcentration factor [BCF] of copper in the spermatheca of control crab was found to be 0.056 [winter], 0.044 [summer] and 0.039 [monsoon]. In experimental crab, exposed to the sublethal concentration [1/5th: 0.208 ppm] of mercuric chloride the season average of BCF in the spermatheca were found to be 1.63 [winter], 3.08 [summer] and 1.86 [monsoon] after 5 days of exposure [Table-9 and Fig-9 (E)] and 2.09 [winter], 4.52 [summer] and 2.59 [monsoon] after exposure for 10 days [Table-11 and Fig-11 (E)]. Percent increase in the 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 days of exposure [Table-10]. Similarly an increase of 3632 % in winter, 10172.7 % in summer and 6541 % in monsoon was found after 10 days of exposure [Table-12].

Similarly in the experimental crab, exposed to the sublethal concentration [1/10th: 0.104 ppm] of mercuric chloride the season average of BCF in the spermatheca were found to be 0.97 [winter], 1.87 [summer] and 1.16 [monsoon] after 5 days of exposure [Table-13 and Fig-13 (E)] and 2.08 [winter], 2.63 [summer] and 2.27 [monsoon] after exposure for 10 days [Table-15 and Fig-15 (E)]. Percent increase in the BCF in the spermatheca of experimental crab was found to be 1632 % in winter, 4150 % in summer and 2874 % in monsoon after 5 days of exposure [Table-14]. Similarly an increase of 3614 % in winter, 5877 % in summer and 5720.5 % in monsoon was found after 10 days of exposure [Table-16].

Percent increase of bioconcentration factor [BCF] of mercury in the spermatheca was in the order: - **summer>monsoon>winter**.

### 2.4. DISCUSSION

The term bioaccumulation is often a good integrative indicator of chemical exposures of organisms in polluted ecosystems [Phillips and Rainbow, 1994]. The process by which a certain quantity of pollutant enters the body of organisms, through gills and body surface, as well as through food and finally accumulating in various tissues in measurable quantities.

The extent of occurrence or accumulation of trace metals by organisms in different tissues is dependent on the route of entry, that is, either from surrounding medium or in the form of food or chemical form of material available in the media [Ghosh and Kshirsagar, 1973]. The bioconcentration factor [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].
Bioconcentration factor [BCF] is the ratio of a chemical concentration in an organism to the concentration in water, where the chemical concentration in the aquatic organism, usually determined in laboratory studies, results from exposure to waterborne chemical [Gobas and Morrison, 2000]. Bioconcentration is the result of direct uptake of a chemical by an organism only from water. Experimentally, the result of such a process is reported as the bioconcentration factor [BCF]. 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].

Current aquatic hazard identification procedures for chemical contaminants, such as those developed by the OECD, are based on persistence, bioaccumulation, and toxicity [PBT]. The basic premise behind these procedures is that substances which are toxic cause greater hazard when they are both persistent and bioaccumulative. For metals, however, defining critical PBT levels is problematic because (1) metals are naturally persistent in the environment; (2) both essential and non-essential metals are naturally bioaccumulated and internally regulated using different strategies (e.g., active excretion, storage); and (3) the toxicity of metals is highly influenced by geochemical factors that influence metal bioavailability [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. Therefore, many proposed and 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 bioconcentration factor [BCF] exceeds designated threshold values [Geyer, et al., 2000].
The experimental and field studies showed that the concentrations of the metals in the organisms depend mainly on their environmental levels [Amiard et al., 1987]. Invertebrates, particularly crustaceans, were very sensitive to heavy metals [Thor and Lake, 1974].

The metal [copper and mercury] concentrations in various tissues of the freshwater female crab, *Barytelphusa cunicularis* during the winter, summer and monsoon seasons were studied. The crabs were exposed to sublethal concentration 1/5\textsuperscript{th} [56.4 ppm] and 1/10\textsuperscript{th} [28.2 ppm] of 24 h lethal concentration of copper sulphate and 1/5\textsuperscript{th} [0.208 ppm] and 1/10\textsuperscript{th} [0.104 ppm] of mercuric chloride for 5 and 10 days.

The results obtained indicate that bioaccumulation of copper and mercury in the ovary, hepatopancreas, gills, thoracic muscles and spermatheca of freshwater crabs, *Barytelphusa cunicularis* is increased with increasing concentration, and also crabs ability to limit the bioaccumulation of copper and mercury varied from organ to organ and represented in Table 1 to 16 and Fig-1 to 16 [A-E].

Moloukhia and Sleem [2011] observed that the accumulations of Cd and Cr in the soft parts of the molluscs increase as their concentrations in their medium increase at various immersions time intervals. This is in agreement with the findings of EL-Deek et al. [1994] on fishes, Hook and Fisher [2002] on copepods and Buschiazzo et al. [2004] on oysters. The enhanced uptake of metals with increase in water concentrations was reported earlier by Eaton [1974].

In the ovary of experiment crab the BCF percent of copper and mercury range between 1600 to 6350 and 2002.9 to 22252.9 respectively in the season winter, summer and monsoon. The percent increased in bioconcentration factor [BCF] of copper in the ovary of experimental crab was more in the summer season followed by monsoon and winter. Similarly the percent increased of bioconcentration factor [BCF] of mercury in ovary was more in the monsoon season followed by summer and winter.
Bryan [1964] has reported that the role of hepatopancreas appears to be like that of a “sponge” to mop up excess heavy metals from the blood and keep the level of heavy metal in blood fairly normal. The hepatopancreas tissue is highly active in the uptake and storage of heavy metals. The BCF percent of copper and mercury in the hepatopancreas of experimental crabs range between 1197.9 to 6194.3 and 2133.7 to 10533.3 respectively in the season winter, summer and monsoon. The percent increased of bioconcentration factor [BCF] of copper in the hepatopancreas was more in the winter season followed by summer and monsoon. Similarly the percent change of bioconcentration factor [BCF] of mercury in the hepatopancreas is more in the summer season followed by monsoon and winter.

Gill surfaces are the first target of water-born metals [Spicer and Weber, 1991]. The gill was also a tissue in which active and passive exchanges occurred between the animal and the aquatic environment. The gills function as the major route for uptake of heavy metals. Uptake of heavy metals from the medium by the gills surface by mucous layer and probably on the properties of a saturable carrier in the cell wall [Vijayaraman, 1994]. The BCF percent of copper and mercury in the gills of experimental crabs range between 1486.7 to 7722.2 and 3430.6 to 43185.7 in the season winter, summer and monsoon. The percent change of bioconcentration factor [BCF] of copper in the gills is more in the monsoon season followed by winter and summer. Similarly the percent increased of bioconcentration factor [BCF] of mercury in the gills is more in the summer season followed by monsoon and winter.

Crustaceans can potentially accumulate heavy metals by absorption through gills or by consumption of contaminated sediments. The concentrations of metals in gills reflect the concentrations of metals in waters [Romeo et al., 1999].

Muscle is the major tissue of interest under routine monitoring of metal contamination because it is consumed by people. Muscle of *Barytelphusa cunicularis* in the present study concentration of copper and mercury shows
maximum in the thoracic muscles of experimental crab. The percent change of bioconcentration factor [BCF] of copper and mercury in the thoracic muscles of experimental crabs range between 1853.8 to 7484 and 2174.5 to 10373.7 respectively in the season winter, summer and monsoon. The percent increased of bioconcentration factor [BCF] of copper in the thoracic muscles was more in the summer season followed by winter and monsoon. Similarly the percent increased of bioconcentration factor [BCF] of mercury in the thoracic muscles was more in the summer season followed by monsoon and winter.

The BCF percent of copper and mercury in the spermatheca of experimental crabs range between 1510 to 8070 and 1632 to 10172.7 respectively in the season winter, summer and monsoon. The percent increased of bioconcentration factor [BCF] of copper and mercury in the spermatheca was more in summer season followed by monsoon and winter.

In the present study the bioaccumulation of copper and mercury in different tissues of the freshwater female crab, *Barytelphusa cunicularis* [Westwood] in the order of hepatopancreas>ovary>gills>thoracic muscles>spermatheca in the season winter, monsoon and summer. But the bioconcentration factors [BCF] of copper and mercury in the different tissue were found in different order in winter, summer and monsoon season respectively. The BCF of copper concentration in the order of spermatheca > gills > thoracic muscles > hepatopancreas > ovary in the winter season, Thoracic muscle> ovary> spermatheca> hepatopancreas> gills in the summer season and spermatheca> gills> thoracic muscle> ovary> hepatopancreas in the monsoon season.

Similarly the BCF of mercury concentration is in the order of gill > hepatopancreas > ovary > thoracic muscle > spermatheca in the winter season, gills > hepatopancreas > thoracic muscles > spermatheca> ovary in the summer season and ovary > gills > hepatopancreas > thoracic muscles > spermatheca in the monsoon season.
Yilmaz and Yilmaz [2007] observed the seasonal changes in heavy metal concentrations in the tissues of tiger shrimp in the order of gill>gonads>hepatopancreas>muscle in both male and female.

Bjerrengaard, et al., [1982] reported the order of accumulation of heavy metals in *Carcinus maenas* as hepatopancreas>ovary>muscles. Narayanan et al., [1988] reported maximum levels of copper and zinc during monsoon and minimum during summer in the estuarine crab, *Thalamita crenata* in order of hepatopancreas>ovary>muscle.

Kaoud, et al., [2011] observed highest bioaccumulation of mercury in the hepatopancreas followed by gills and muscles in the freshwater giant shrimp, *Macrobrachium rosenbergii* when exposed to heavy metal intoxication. The maximum accumulation of cadmium in the hepatopancreas of *Penaeus duorarum* exposed to cadmium chloride [Nimmo, et al., 1977].

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]. Steenkamp, et al., [1994] reported that significant bioaccumulation of manganese was detected in various months for most of the tissue in crab, *Potamonautes warren* [Calman]. Joseph and Srivastava [1992] showed that in prawn *Penaeus indicus*, heavy metals exhibited seasonality.

The fleshy tissues of the animal are consumed by man as protein supplement when cooked. However, fleshy tissues of crabs are good accumulators of heavy metals and the nutritional implication of this is that consumers of the animal may be exposed to heavy metal toxicity if bio-accumulation results due to regular consumption [Goyer, 1995].

Several different studies have been carried out on the determination of levels of heavy metals and their effects in aquatic organisms [Ogindo, 2001; Olaifa et al, 2004; Ako and Salihu, 2004] and particularly in crab [Krishnan, 1992; Mortimer
and Miller, 1994; Heslin, 1995; Mremi and Machiwa, 2003; Otchere, 2003; Falusi and Olanipekun, 2007].

Kargin et. al. [2001] studied the distribution of heavy metals in different tissues of the shrimp, *Penaeus semiculatus* and *Metapenaeaus monocerus* from the Iskenderun Gulf. Deshpande [2007] has found concentration of mercury and copper increasing as the exposure period increased in the freshwater leech, *Poecillobdela viridis* exposed to mercuric chloride and copper sulphate.


Concentration of heavy metal in tissues of crabs and shrimp is well known [Vazquez et al., 2001; Chou et al., 2002; Pourang et al., 2005; Turkmen et al., 2006; Firat et al., 2008]. Studies show that in crustaceans copper and mercury regulation is a characteristic of species belong to the order Decapoda, but not observed in the other crustacean orders [Rainbow, et al., 1989].

Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. People with Wilson’s disease are at greater risk for health effects from over exposure to copper. Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth.

Mercury and its compounds are widely distributed in the environment as a result of both natural and man-made activities. The utility, and the toxicity, of mercury have been known for centuries. New evidence demonstrates that even low levels of mercury exposure may be hazardous. Mercury can combine with a methyl group to become methyl mercury. This form of mercury is found in a variety of environmental pollution situations and can produce a range of toxic hazards like Minamata disease [Harada, 1995].
The increase of the mercury in the water body results in the excess accumulation of mercury by the aquatic animals like crabs, fishes, bivalves which are consumed by the people as their food, thus, posing a human health risk; elevated levels of mercury in the crabs can also have ecologically significant effect, such as affecting reproduction [Wiener, 1995; Iliopoulou and Kotsanis, 2001]. Mercury accumulation in the different tissues of prawn, lobster and fish was studied by Abdul Hamed, [1995].

The Bureau of Indian Standards [BIS] has laid down safety limits for drinking water at 0.05 mg of copper and 0.001 mg of mercury per liter. A number of samples of groundwater in some industrial belts have shown concentrations of mercury higher than safe standards. However, in a country like India where a large percentage of the population eat crabs and fishes as a staple food, no provisions for daily or weekly mercury intake levels have been set down.

Aquatic organisms were reported to be selective in metal accumulation due to toxicity effects [Ayodele and Abubakar, 2002]. The mode of action of heavy metals on biological systems is thought to be enzymes systems, although extraordinary concentrations may result in direct tissue damage [Abubakar and Garba, 2006]. Regulation of metal body burden by aquatic organisms can be through three principal mechanisms, via gut, urine and diffusion through the body surface. Crustaceans excrete Zn, Cu, Co, Mn, and Hg in the urine [Bernard and Lane, 1961].

High levels of copper are highly toxic to aquatic animals, especially freshwater invertebrates. Through animal experiments, it has been shown that exposure to copper may cause a wide variety of symptoms, including e.g. a loss of cellular adhesion in the gills, cell necrosis, retarded growth and a lowered rate of reproduction and egg survival, behavioural changes such as decreased degree of concealment and ability to orientate may also occur [Moore and Ramamoorthy, 1984].
Naturally aquatic invertebrates accumulate high amounts of heavy metals. The benthic crab, *Dorippe granulate* accumulates copper, zinc and cadmium from natural aquatic environment [Depledge et al., 1993]. Many of these are causing concern due to the contamination by human expanded activities. The effects of these heavy metals on the normal function of cells, tissues and organs are hazardous when accumulated beyond the trace level even though they are the essential metals found in the system of all living organism.

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 [Clark, et al., 1997].

Habitat of *Barytelphusa cunicularis* are close to the mouth of the river which is the area affected by heavy pollution. It can be used as bio-indicator as it contains variable levels of the metals analysed with high enrichment of copper and mercury observed. In order to reduce excessive discharge of metals into the reservoir there should be reduction of farming activities around the aquatic area.

The heavy metal accumulation in the tissues, water and sediment increased as the exposure time increased. If this continues, heavy metal will reach the tissues of human beings through the food chain. As there have been great increases in the level of expenditure for pollution control in both the public and the private sector, the industrialists take no care of the environment. As this time, when the policies which will control, future environmental management are still being
formulated, it is important that clear objectives be set, and the strategies be selected which will produce significant improvements at a reasonable cost.

Despite the many studies carried out on heavy metals in crabs, scanty information is available on the bioconcentration factors [BCF] of heavy metals in crab samples. Crustaceans, including crabs, are widely recognized as useful species for biomonitoring [Phillips and Rainbow, 1993].

The present study revealed that the bioaccumulation of copper and mercury in the tissues of freshwater crab, *Barytelphusa cunicularis* [Westwood] is observed as follows: hepatopancreas>ovary>gills>thoracic muscles>spermatheca in the season winter, summer and monsoon. The significant differences in percent increased are found in different tissues in view of bioaccumulation and bioconcentration of copper and mercury. The concentration rates of copper and mercury in the tissues of freshwater crab vary significantly as a function of season and the pollution load of tissue.
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"Toxicity, Bioaccumulation and Detoxification of Heavy Metal Pesticides in a Freshwater Female Crab, Barytelphusa cunicularis (Westwood) and Impact Assessment on its Reproduction".


Thesis Submitted By - Atulkumar Ramakant Chourpagar to Dr. Babasaheb Ambedkar Marathwada University, Aurangabad


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111
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