INTRODUCTION

The term, trace amount identifies a large group of metallic elements which are present fractionally in nature as well as in biota. Heavy metals are among the major problem in concern with waste water treatment [50]. High metal concentration in water increases its bioaccumulation in animal tissue leading to pathological condition [10, 9]. Heavy metals often found in high concentration in river sediments because of industrial discharge [4]. According to [51], heavy metal contamination in water bodies, deserve a special mention as it forms a repository for the industrial effluents and city sewage. Increased population, heavy industrialization, indiscriminate dumping and discharged of industrial effluent’s with petroleum waste contaminated the aquatic media [58]. Copper found to be essential for metabolism; consequently, if concentration enhanced may cause tissue damage [5].

Gastropods proved useful and were employed for monitoring of metal pollution, as they have importance in food chains and broad geographical range [40, 53-57, 39, 8, 17, 49, 11]. Mortality is nothing but the death of an organism at a particular time. The death rate found increased tremendously mainly due to environmental contamination with alterations in temperature, pH, Salinity, humidity, etc.

Environmental pollution has become global problem because of undesirable changes in air, land and water, which has threatened animal life [34]. Widespread use of synthetic pesticide, have been found to affect water bodies due to their high toxicity, bioaccumulation and long term persistence [32, 52]. Hazardous nature of synthetic pesticides has sensitized researchers to find out least disruptive options in the field of pest control management. Molluscicides of plant origin are being widely used, because of the fact that, the toxicity of these products found very high, easily biodegradable in nature and are safer for the users along with their low cost [28]. Agricultural productivity over the past century has significantly increased due to more efficient and economical pest control. However, there is continuing and growing social and legislative pressure to reduce the ecotoxicological risk of pesticide. One of the most important concerns about a novel type of pesticide, that it should be specific for targeted organism [14]. Pesticides are helpful, when they used in proper way. But due to indiscriminate use of these pesticides, it get accumulated in air, water and soil to pollute the environment. In agricultural practices, in order to take more yield, large amount of chemical fertilizers, pesticides, herbicides and molluscicides were used to control the pests. Toxic content through runoff find its way to water bodies [2]. All the contaminant accumulated in animal can be stored without excretion (accumulator) or maintained at low concentration (regulators) by balancing the uptake with controlled rates of excretion [46]. Hence, there is need of more advanced knowledge for assessment of heavy metal pollution. By accounting the above problem, we have decided to find out mortality rate of freshwater snail Bellamya bengalensis against toxicity of copper sulphate and A. sinuata. The rate of bioaccumulation and bioconcentration in the tissue level...
was also determined to discuss it with digestive physiology in the experimental of snail *Bellamya bengalensis*.

**MATERIAL AND METHOD**

**Experimental animals:** The freshwater snails *Bellamya bengalensis* (L) were collected from Rajaram tank, near Shivaji University Kolhapur, Maharashtra (India). Snails were brought to the laboratory, acclimatized for a week. Provided with proper food and ventilation. Healthy snails (23-26 mm shell height and 2.8-3.5 gm weight) were selected for the experimentation. Mortality rate was calculated against both toxicants (Plate No.1).

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**Fig. 1:** Regression equation of heavy metal copper sulphate for freshwater snail *Bellamya bengalensis*

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**Fig. 2**

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**Fig. 3**

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**Fig. 4**

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**Fig. 5**

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**Fig. 6**

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**Fig-1:** Collection Sites at the Rajaram tank, Kolhapur, (Maharashtra), 2- Freshwater Snail *Bellamya bengalensis*, 3- Digestive Tract of *Bellamya bengalensis*, 4- Plant of *Acacia Sinuata*, 5- Pod extract powder, 6- Copper sulphate
Fig. No.1: Regression equation of pod extract of *Acacia sinuata* for freshwater snail *Bellamya bengalensis*
Preparation of aqueous extract of pod of Acacia sinuata: Acacia sinuata is used as a medicinal plant. Pods are a part of plant, and highly toxic to the snail. Therefore, used as a molluscicide. The pod of A. sinuata was oven dried. The powder of pod in 50 gm was soaked in 200 ml of alcohol for 48 hours. The mixture was stirred continuously by using magnetic stirrer for 6 hours, filtered through cheesecloth, followed by Wattman filter paper. Alcohol content was removed under reduced pressure by using flash evaporator to concentrate the material. Residue dissolved in 100 ml of distilled water and considered as stock solution. The stock solution was further diluted into acetone to get desired concentration in ppm and used against B. bengalensis by static bioassay method.

Intoxication:
Experimental snails were kept in plastic troughs (5 liters capacity). Heavy metal copper sulphate and pod extract of A. sinuate used for intoxication for mortality study. Five sets were prepared as 2, 3, 4, 5 ppm and 200, 300, 400, 500 ppm concentration with control. Out of five troughs, one used for the control group of snails. In each trough, for all the sets 10 snails were used. For each metallic and molluscicidal concentration experimental animals were exposed for 24 hrs., 48 hrs., 72 hrs. and 96 hrs. The experiments were repeated thrice to confirm the mortality and LC50 concentration by Probit analysis [16]. Experimental snails were exposed to heavy metal copper sulphate at 0.56 ppm and A. sinuata pod extract for 2.32 ppm (pre-determined LC50 concentration). The LC50 concentration of copper sulphate was used for the bioaccumulation study. After completion of exposure period of 96 hrs live snails from each group were immediately dissected out for digestive system, selected salivary gland, oesophagus, intestine, stomach and hepatopancreas were cut out and processed for bioaccumulation study.

Bioaccumulation study
For bioaccumulation study, both control and experimental animals (intoxicated with mean LC50 concentration of CuSO4, 5H2O up to 96 hrs.) were processed. Animals were sacrificed organ as for desired digestive organs salivary gland, oesophagus, intestine, stomach and hepatopancreas. Known weights of wet tissues were kept in oven at 60° C for complete dehydration. Completely dehydrated tissue was crushed in mortal pistol and transferred to Muffle furnace by adjusting at 550° C for 6-8 hr. for ash preparation. 100 mg powder was acidified with 20 ml of 6 N HNO3 solution. Filtered and diluted in 100 ml of double glass distilled water. The concentration of copper sulphate in targeted organ was analyzed by Atomic Absorption Spectrophotometer (AAS Chemito AA 201). The metal concentration in tissue was expressed in mg/gm dry tissue weight.

Calculation of safe concentration [22]:

\[
C = \frac{48 \text{ hrs.TLM} \times 0.3}{S2} \\
S = \frac{24 \text{ hrs. TLM}}{48 \text{ hrs. TLM}}
\]

Where, C = Safe concentration
TLM = Median tolerance limit or LC50 value for that

exposure period.

RESULTS
Toxicity study:
Toxicity of metal and molluscicide was comparatively assessed for mortality study and bioaccumulation in the targeted organs of freshwater snail B. bengalensis. Mortality rate by both intoxication was differentiated with control group. Copper sulphate induced mortality in experimental animal for 2 ppm was noted at 24 hrs. and found 6 %, 23% at 48 hrs., 72 hrs. for 36% and at 70% mortality at 96 hrs. 3 ppm induced concentration has percent mortality of 20, 30, 46 and 80 for 24, 48, 72 and 96 hrs. respectively. After induction of 4 ppm concentration to mortality recorded at 24 hrs. 33%, at 48 hrs. 40%, at 72 hrs. 60% and at 96 hrs. 86%. For 5 ppm concentration, percent mortality was at 24 hrs. 43, 48 hrs. 50, at 72 hrs. 60 and 96 hrs. 93. During the experiment, 100% mortality was not recorded and so considered for calculation of mean LC50 and for safe concentration (Table No. 1).

Table No. 1: Percent mortality of freshwater snail B. bengalensis during intoxication of heavy metal copper sulphate at different time intervals.

<table>
<thead>
<tr>
<th>Concentration in ppm</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>96 hrs.</th>
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<td>43</td>
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<td>93</td>
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</tbody>
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Table No. 2: Percent mortality of freshwater snail B. bengalensis during intoxication of heavy metal A. sinuata at different time intervals.

<table>
<thead>
<tr>
<th>Concentration in ppm</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>96 hrs.</th>
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<tbody>
<tr>
<td>Control</td>
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Pod extract of A. sinuata by its intoxication to the experimental animal at 200 ppm concentration of molluscicide for 24 hrs., 48 hrs., 72 hrs., 96 hrs., 10%, 26%, 36%, 43% mortality recorded. At the concentration of 300 ppm mortality at 24 hrs. was 20%, at 48 hrs. 33%, at 72 hrs. 40% and at 96 hrs. it was 46% recorded. 400 ppm concentrated pod extract caused 23% at 24 hrs., 43% at 48 hrs., 46% at 72 hrs. and 53% at 96 hrs. of mortality. After Induction of 500 ppm dose to the experimental animal at 40% 24hrs., 50% at 48 hrs., 60% at 72 hrs. and at 66% 96 hrs. mortality was observed. Results indicated that, induction of pod extract of A. sinuata not showing 100% of mortality for respective exposure period and so, taken for the calculation of LC50 concentration (Table No. 2). The concentrations of toxicants with respect to mortality for control and experimental group compared and were used for calculation of LC50 and safe concentration. It was observed that for the 24, 48, 72 and 96 hrs. experimental snails were showed mortality, but not 100%. So the calculated mean LC50 concentration of copper sulphate was
carbonate would be cumulative (Table No. 2) and its shell contained was metal rate of biomagnifications of heavy metals in the cells found. Researchers found that, the rate of bioaccumulation and increased after that period. Compare to control group up to fifteen weeks, but found cadmium induced mortality of Hemelraad et al. (1986, 1990) [29] molluscs long been known to accumulate both proteins in the tissues [29]. Lying in the second tropic level, Metal accumulation found to be more rapid digestive organ found highly metal ac secrets the enzymes and digest the food material, therefore that, hepatopancreas and stomach was the digestive part it seems to be targeted for temporary storage and safe concentration, base level concentration in all the targeted tissues from control group analysed and found 0.120 mg/gm in salivary gland, 0.328 mg/gm in oesophagus, 0.410 mg/gm intestine, 0.375 mg/gm in stomach and 0.385 mg/gm in hepatopancreas. LC50 concentration of copper sulphate induction showed high content of metal i.e. increased rate of bioaccumulation in all tissues, but comparatively stomach and hepatopancreas was found 1.057 mg/gm maximum concentration. Intestine noted minimum concentration as 0.710 mg/gm. Oesophagus showed 0.630 mg/gm concentration and salivary gland showed 0.530 mg/gm concentration at 96 hrs. In above observations concluded that, hepatopancreas and stomach was the digestive part it secrets the enzymes and digest the food material, therefore digestive organ found highly metal accumulated (Table No. 5).

**DISCUSSIONS**

Metal accumulation found to be more rapid than its elimination, probably due to the presence of metal binding proteins in the tissues [29]. Lying in the second tropic level, molluscs long been known to accumulate both essential and non-essential trace elements from aquatic ecosystems [45]. Hemelraad et al. (1986, 1990) [23,24] reported that cadmium induced mortality of Anodonta cygnea was less as compare to control group up to fifteen weeks, but found increased after that period. Researchers found that, the rate of bioaccumulation and biomagnifications of heavy metals in the cells found dependent on its uptake and elimination in the tissue [21]. Gomaa et al. (1995) [18] reported that, bivalve molluscs were to withstand remarkably high metal concentrations in their environment. Cadmium, zinc, Nickel, Copper, Lead and Mercury has high rate of ion accumulate of tissue levels in both freshwater and marine mussels [59, 24-25]. Elangoven et al. (1997) [15] noted that, the digestive gland seems to be targeted for temporary storage and bioaccumulation of heavy metals. Further more, soft tissues of different gastropods contain various subcellular metal containing granules in the targeted cells [7]. Hopkin (1989) [28]; Dallinger et al. (2000) [13] have demonstrated relatively higher toxicity of copper derivatives than lead, tested against Helix aspera and reported higher toxicity of copper induced due to its ability to form complexes with anions. They noted Cu (II) induced oxidation of quinone and hydroquinone in target cell. According to Hopkin (1989) [28], Cu can be deposited as insoluble intracellular membrane-bound granules in the hepatopancreas of most terrestrial invertebrates. Metal (Cu), transiently detoxified through synthesis of low-molecular-weight protein e.g. metallothioninen, which binds the metal ions [30]. Adewunmi et al. (1996) [1] noted that, Cu, Pb and Cd were the metals having high rate of bioaccumulation in tissues of freshwater snails collected from dams and rivers at southwest Nigeria. Hoang and Rand (2009) [26] demonstrated, the potential toxicity of Cu carbonate to snails dissociated through biological and chemical reactions. Carbonate would be available for shell development, Cu found to be accumulated in soft tissue. Hoang et al. (2008) [27] reported, in juvenile apple snail Pomacea paludasa Cu found bioaccumulated in soft tissue (about 60% in the viscera and 40% in the foot) and its shell contained was <4% of total accumulated copper. However, in comparison uptake rate of metals aquatic organisms showed bioaccumulation order as Ag>Zn>Cd>Cu>Co>Cr>Se [31]. Mortality rate of E. alba found, treated due to floral content.
wedelolactone [20], while in *B. aegyptiaca* due to steroidal saponin Diosgenin content [21] mixture of deltonin, 25 isodeltonin [6] above results showed, molluccidal activity [3]. Mustafec (1999) [37] and Matsmura (1972) [33] observed, use of pesticides were contaminate freshwater ecosystem and causes unfolded hazards to several non-target aquatic invertebrate and vertebrates. Chaudhary et al. (1988) [12] studies, the effect of toxicity of organophosphorus pesticides like cython, zolene and roger on freshwater snail, *Lymnea acuminata*, *Thiara scabra* and *Thiara lineata*. Panwar et al. (1982) [38] have studied, the toxicity of pesticides on *V. bengalensis* and reported that, organophosphorus pesticides were more toxic than Chlorinated hydrocarbons. Mule and Lomte (1992 and 1994) [35-36] have studied, the mortality effect due to Cypermethrin less toxic than Copper sulphate toxicity to *Thiara tuberculatus*.

In the above investigation, it was found that, copper sulphate induction to the snail was more toxic than intoxication of pod extract of *A. sinuata*. It was found both toxicants have direct effect over the digestive system. As a result, during experiment snails were secreting more mucus and became sluggish or immotile. Behavioral changes found direct relation with mortality. In comparison, it was observed that metallic content was more in the targeted tissue showing more cellular pathology due to copper induction as that of intoxication of *A. sinuata*. Results indicated that mean LC50 of copper sulphate was 0.56 ppm and *A. sinuata* 2.32 ppm. With respect to this calculated safe concentration of *A. sinuata* was found more than copper sulphate concentration, indicating its less toxicity as compare to the heavy metal copper sulphate over experimental snail *B. bengalensis*.

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**REFERENCES**


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