Uttar Pradesh J. Zool. 30(3) : 399-405, 2010 ISSN 0256-971X

STUDY OF COPPER SULPHATE INDUCED MORTALITY TO FRESHWATER SNAIL, *BELLAMYA BENGALENSIS* (LAMARCK).

N.A. KAMBLE AND V.V. POTDAR
DEPARTMENT OF ZOOLOGY, SHIVAJI UNIVERSITY,
KOLHAPUR-416012, INDIA
(e-mail : drknitinkumar@yahoo.in)

Induced toxicity of copper sulphate to freshwater snail *Bellamya bengalensis* showed mortality, which increased with increase in concentration and time of exposure to intoxicant. Experimental group of snails were intoxicated by using 1, 2, 3, 4 and 5 ppm concentrations of copper sulphate for 24, 48, 72 and 96 hrs of exposure. Mean LC50 value observed 0.36 ppm and safe concentration was 0.056 ppm for snail *Bellamya bengalensis*.

Key words : Copper sulphate, mortality, *Bellamya bengalensis*.

INTRODUCTION

Survivality of aquatic flora and fauna in balanced ecosystem is under threat due to contamination of toxic pollutant in media. Among the pollutants, heavy metals are conservative contaminants which enter the aquatic environment through a number of ways (Bryan, 1976; Forstner & Wittmann, 1979; Langston & Spence, 1994). Residues of toxic chemical substances, if accumulated cause adverse effects on the organism. Toxicity of chemicals results in harmful effects on living organisms and it generally referred to as dose- dependent (Kamble *et al.*, 2009). The toxic metals cause serious effects to aquatic ecosystem, as it almost forms a repository for the industrial effluents and city sewage (Satyanarayana *et al.*, 1985). The freshwater invertebrates in general and molluscs in particular are sensitive to heavy metals. Marine invertebrates, especially molluscs, therefore have been widely used as monitors of trace metal pollution. (Phillips 1977, 1980; Bryan *et al*, 1980 & 1985.) High concentration of heavy metals often found in aquatic ecosystem responsible for mortality of snail (Bhosale & Shahu, 1991). Morley *et al.* (2003) found that the increased mortality of infected snails *Lymnaea peregra* and *L. stagnalis* exposed to heavy metals may have a number of additional consequences for host-parasite interaction. Few investigators have evaluated the toxicity of some toxicants on some of the molluscan species. Toxicity of methyl parathion and thimet in freshwater molluscs *Lymnaea stagnalis* (Bhide & Sharma, 1998) and *Viviparus bengalensis* (Panwar *et al.*, 1982) have been reported. The sensitization during pre-exposure to Cu in freshwater implies that induction of protein and additional tolerance did not occur, and some deleterious effects of pre- exposure was carried over, contributing to the impact of the subsequent lethal concentration. Prolonged exposure of copper (without acclimation), especially at lower salinities, would incapacitate aquatic organisms to perform normal metabolic functions. (Prabhudeva & Menon, 1992; Ahsanullah & Ying, 1995).

Perusal of literature reveals that scanty information is available on heavy metal toxicity to the molluscan snail, therefore in present problem we decided to evaluate the effect of heavy metal copper sulphate on the mortality of snail *Bellamya bengalensis*.

MATERIALS AND METHODS

The freshwater snail, *Bellamya bengalensis* was selected for the present study, since it is locally available throughout the year. Snails were collected from 'Rajaram tank' near Shivaji University, Kolhapur. Healthy snails having same size and weight (23-26 mm. shell height and 2.8-3.5 g weight) were selected for experiment. Experiments were carried out in plastic troughs having 5 liters capacity. Water miscible Copper sulphate ($3CuSO_4.5H_2O$) was used as intoxicant. In one experiment snails were divided into six groups. First as control group of snail, remaining five groups of snails were intoxicated with 1, 2, 3, 4 and 5 ppm of copper sulphate for 24, 48, 72, and 96 hrs of exposure. In each group 10 snails were used after completion of exposure period percent mortality was calculated and graphically presented. The whole experiment was repeated thrice, for the correct evaluation and calculation.

A) *Calculation of LC_{50} values :* The per cent mortality during intoxication of copper, the probit values of mortality (Y) and concentration of metals (X), LnX, LnX^2, LnXY, ΣY, ΣLnX^2, and $\Sigma LnXY$ were calculated.

$$LC_{50} = \frac{5 - a}{b}$$

The values of 'b' and 'a' of the above equation were calculated by the following formula:

$$b = \frac{\Sigma LnXY - \dfrac{\Sigma Ln\, x.\Sigma Y}{n}}{\Sigma LnX^2 - \dfrac{(\Sigma LnX)^2}{n}}$$

n is total number of concentrations used.

$$a = Y - bLnX$$

where, X is concentration of heavy metal and Y is mortality.

B) *Calculation of safe concentration* : The safe concentration of each heavy metal was calculated by the formula proposed by Hart *et al.* (1945) as follows :

$$c = \frac{48 \text{ hrs TLM} \times 0.3}{S^2}$$

where, c = Safe concentration

$$S = \frac{24 \text{ hrs. TLM}}{48 \text{ hrs. TLM}}$$

TLM = Median tolerance limit or LC_{50} value for that exposure period.

RESULTS

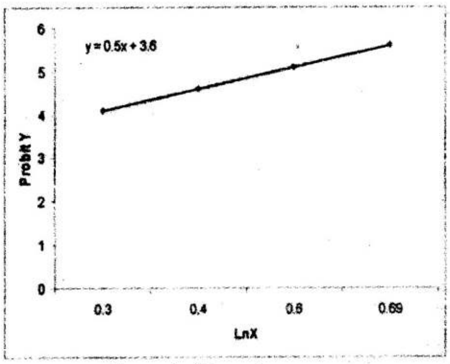
(a) ***Mortality at 1 ppm*** : 12% of mortality was observed after 24 h, 22% after 48 h, 27% after 72 h, and 32% after 96 h. of intoxication. 100% mortality was not found upto 96 h.

(b) ***Mortality at 2 ppm*** : 20% mortality was recorded after 24 h, of exposure, 35% after 48 h., 42% after 72 h. and 47% after 96 h. 100% mortality was not recorded unto 96 h.

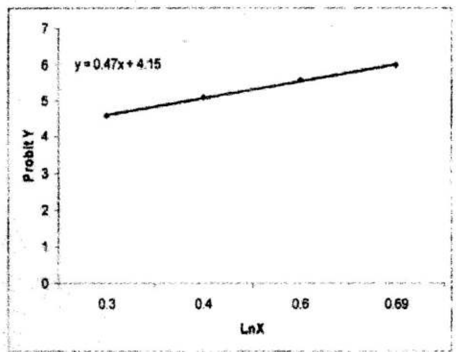
(c) ***Mortality at 3 ppm*** : At 3 ppm. 35% mortality was recorded after 24 h, 55% after 48 h, 62% after 72 h, and 65% after 96 h. 100% mortality was not up to 96 h. of exposure.

(d) ***Mortality at 4 ppm*** : At 4 ppm 57% mortality was observed after 24 h, 75% after 48 h, 77% after 72 h and 77% after 96 h. 100% mortality was not observed up to 96 h.

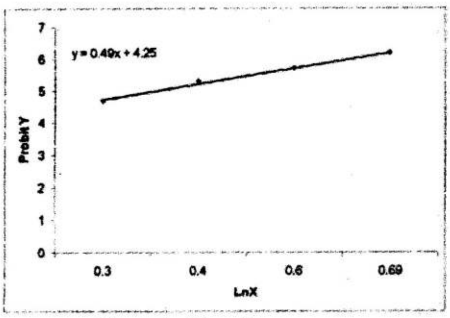
(e) ***Mortality at 5 ppm*** : At 5 ppm 75% mortality was observed after 24 h, 85% after 48 h, 90% after 72 h, and 95% after 96 h of intoxication. 100% mortality was not observed even after 96 h of intoxication. All the data are represented in Table No. I, II, III & IV, Graph No. 1, 2, 3 & 4.



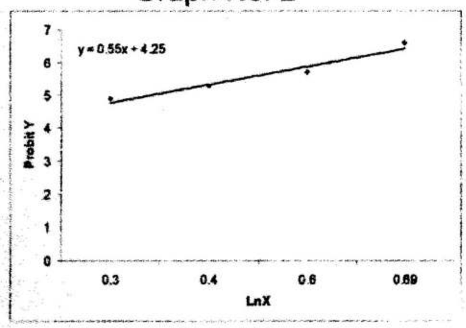
Graph No. 1



Graph No. 2



Graph No. 3



Graph No. 4

DISCUSSION

Mortality study is an important criterion in toxicological analysis. Mortality is one of the most noticeable effects of any chemical to the animal. Toxicity depends on internal cellular structure, its metabolism and also on several factors such as its availability and occurrence, complexation of other chemical reaction and also absorption potential by species to the toxicant. To support this Bryan (1976), has listed a series of factors influencing toxicity of heavy metal to aquatic animals. Which include source of metal, concentration of metal, food and feeding mechanism of animals, internal immunity of organism and also tolerance capacity of animal to the toxicant. The survivality and mortality of aquatic organism, under toxic conditions depends upon temperature, salinity, dissolved oxygen, pH, hardness, and also on genetic diversity of species in aquatic body (Mc Kee & Wolf, 1963; Holden, 1973; Brungs *et al.*, 1977). Seth *et al.* (1990) observed mortality to *Viviparous bengalensis* (Swainson) when exposed to 2-methoxy ethyl mercuric chloride and copper sulphate and found that both are toxic to snail depending on concentration and time of exposure. Similarly mortality effect of mercury was studied on *Crepidula formicate* by Thian (1984). Martin (1979) reported 59.9 μg l^{-1} of copper accumulation in the body of *Mytillus edulis* which was lethal to the snail causing

Table I : Mortality percentage of *Bellamya bengalensis* during intoxication of copper at different time intervals (24-96 h.)

Cu. conc. (ppm)	Intoxication period			
	24 h	48 h	72 h	96 h
Control	0.0	0.0	0.0	0.0
1	12	22	27	32
2	20	35	42	47
3	35	55	62	65
4	57	75	77	77
5	75	85	90	95

Table II : Numerical data of 'b' and 'a' for the calculation of LC_{50} values of copper for *Bellamya bengalensis* .

Exposure time in (h.)	Values of 'b' and 'a' with reference to snail	
	'b'	'a'
24	2.84	3.49
48	2.87	3.93
72	2.89	4.09
96	3.ol	4.19

Table III : Numerical data for the calculation of LC_{50} values of Copper for *Bellamya bengalensis* .

Exposure time in (h)	LC_{50} Values, ppm	Mean LC_{50} Value, ppm
24	0.52	0.36
48	0.37	
72	0.31	
96	0.26	

Table IV : Numerical data for estimation of 'b' and 'a' in relation to mortality due to copper in *Bellamya bengalensis* .

24 h						48 h					
% mortality	**Probit (Y)**	**Conc ppm (X)**	**LnX**	**LnX^2**	**LnXY**	**% mortality**	**Probit (Y)**	**Conc ppm (X)**	**LnX**	**LnX^2**	**LnXY**
12	3.82	1	0	0	0	22	4.22	1	0	0	0
20	4.15	2	0.30	0.09	1.251	35	4.61	2	0.30	0.09	1.389
35	4.61	3	0.47	0.22	2.201	55	5.12	3	0.47	0.22	2.445
57	5.17	4	0.60	0.36	3.116	75	5.67	4	0.60	0.36	3.416
75	5.67	5	0.69	0.47	3.965	85	6.03	5	0.69	0.47	4.218
	ΣY = 23.44 Y= 4.68		ΣLnX = 2.079 LnX = 0.416	$ΣLnX^2$ = 1.14	ΣLnXY = 10.53		ΣY = 25.67 Y= 5.13		ΣLnX = 2.079 LnX = 0.416	$ΣLnX^2$ = 1.14	ΣLnXY = 11.46
72 h						**96 h**					
% mortality	**Probit (Y)**	**Conc ppm (X)**	**LnX**	**LnX^2**	**LnXY**	**% mortality**	**Probit (Y)**	**Conc ppm (X)**	**LnX**	**LnX^2**	**LnXY**
27	4.38	1	0	0	0	32	4.53	1	0	0	0
42	4.79	2	0.30	0.09	1.444	47	4.92	2	0.30	0.09	1.482
62	5.30	3	0.47	0.22	2.531	65	5.38	3	0.47	0.22	2.569
77	5.73	4	0.60	0.36	3.454	77	5.73	4	0.60	0.36	3.454
90	6.28	5	0.69	0.47	4.390	95	6.64	5	0.69	0.47	4.644
	ΣY = 26.51 Y= 5.30		ΣLnX = 2.079 LnX = 0.416	$ΣLnX^2$ = 1.14	ΣLnXY = 11.82		ΣY = 27.22 Y= 5.44		ΣLnX = 2.079 LnX = 0.416	$ΣLnX^2$ = 1.14	ΣLnXY = 12.15

mortality. Awati *et al*, (2004) have investigated the mortality of Thimet 10-G on snail *Bellamya bengalensis* for the different concentrations and found lethal dose to the snail. They also observed that the mortality of snail was time dependent and also concentration of toxicant intoxicated to the snail.

In present investigation mortality has presumably resulted from a failure to balance rate of incorporation of copper into detoxification pathways resulting in increased rate of uptake of Copper under high exposure and concentrations. The rate of mortality in snail *Bellamya bengalensis* varies to different time intervals. Percent mortality increased with increase in concentration of Copper and also increased with increase in the exposure period. The LC_{50} values of copper for different time interval 24, 48, 72 and 96 hrs were: 0.52, 0.37, 0.31 and 0.26 ppm respectively. The mean LC50 concentration of copper to the snail was 0.36 ppm. of copper sulphate is toxic to snail. Mortality of snail is depending upon concentration and time of exposure etc. The LC_{50} value determined by calculation and by graphical method coincided with each other with negligible difference.

Therefore, the results of the present investigation provide concrete proof for the correctness of LC_{50} value of copper sulphate to the snail *Bellamya bengalensis.* Depending on calculated results safe concentration of copper to snail *Bellamya bengalensis* was 0.056 ppm.

As the mortality occurred due to intoxication of different concentration ot copper at different time intervals, results its toxicity to the snail *Bellamya bengalensis.* Lethality occurred due to intoxication of copper in snail *Bellamya bengalensis* required further histological and biochemical investigations.

ACKNOWLEDGEMENT

Authors are thankful to the U.G.C. New Delhi, for providing financial support under Major Research Project, also to the Head, Department of Zoology, Shivaji University, Kolhapur, for kind encouragement in the progress of work.

REFERENCES

AHSANULLAH, M. & YING, W. 1995. Toxic effects of dissolved copper on *Penaeus merguiensis* and *Penaeus monodon. Bull. Environ. Contam. Toxicol.* **55** : 81-88.

AWATI, A.A. 2004. Comparative studies on toxic effects of Synthetic and natural molluscicide on respiratory and reproductive organs of aquatic snail *Viviparus bengalensis. Ph.D. Thesis, Shivaji University, Kolhapur* (India).

BHIDE, M. 1998. Effect of nuvan, methyl parathion and thimet on the mortality, behaviour and reproductive performance of freshwater molluscs, *Lymnaea stagnalis. J. Environ. Biol.* **19** : 325-332.

BHOSALE, U. & SHAHU, K.C. 1991. Heavy metal pollution around the Island city of Bombay, India, Part II: Distribution of heavy metals between water suspended particles and sediments in polluted aquatic regime. *Chemical Geology.* **90** : 285-305.

BRUNGS, W.A., SPEHAR, C.E. & STOKES, G.N. 1977. Effects of pollution on freshwater fish. *J. Water. Pollut. Cont. Fed. Washington.* **49** :1425-1439.

BRYAN, G.W. 1976. Heavy metal contamination in the sea. In : *Marine pollution* (Johnston, R. Ed.). Academic Press. London, pp. 185-302.

BRYAN, G.W., LANGSTON, W.J. & HUMMERSTONE, L.G. 1980. The use of biological indicators of heavy metal contamination in estuaries. Occ. Publ. Mar. Biol. Ass. U.K. pp. 11-73.

BRYAN, G.W., LANGSTON, W.J., HUMMERSTONE. L.G. & BURT, G.R. 1985. A guide to the assessment of heavy metal contamination in estuaries using biological indicators. Occ. Publ. Mar. Biol. Ass. U.K. **4** : 1-92.

FORSTNER, U. & WITTMANN, G.T.W. 1979. *Metal Pollution in the Aquatic Environment.* Springer-Verlag, New York, USA.

HOLDEN, A.V. 1973. Effect of pesticides. In : *Environmental Pollution by Pesticides* (Edward, C.A. Ed.). Plenum Press. pp 213-253.

KAMBLE, N.A. 2009. Cadmium sulphate induced toxicity effect on mortality of freshwater snail, *Bellamya bengalensis* (Lamarck). *Geobios.* **36**(1) : 21-24

LANGSTON W.J. & SPENCE, S.K.1994. 'Metal analysis ' . In : *Handbook of Ecotoxicology* (Calow, P. Ed.). Blackwell Scientific, Oxford, UK. pp. 45-78.

MARTIN, J.L.M. 1979. Schema of lethal action of copper on mussels. *Bull. Environ. Contam. Toxicol.* **21** : 808-814.

MC KEE, J.E. & WOLF, H.W. 1963. Water quality criteria : A report for the State of California. Water Poll. Cont. Bd. Wash, D.C.

MORLEY, N.J. 2003. Cadmium toxicity and snail-digenean interactions in a population of *Lymnaea* spp. J. *Helminthology.* **77** : 49-55

PANWAR, R.S., GUPTA, R.A. & MHAMED, M.P. 1982. Toxicity of some chlorinated hydrocarbon and organophosphorus insecticides to gastropod, *Viviparus bengalensis* (Swainson). *J. Environ. Biol.* **3** : 31-36.

PHILLIPS, D.J.H. 1977. The use of biological indicator organisms to Monitor traces metal pollution in marine and estuarine environnants - A review. *Environ. Pollut.* **13** : 281-217

PHILLIPS, D.J.H. 1980. *Quantitative aquatic biological indicators. Their use to monitor trace metal and organochlorine Pollution.* Applied Science Publ. Ltd, London.

PRABHUDEVA, K.N .& MENON, N.R. 1992. *Aquatic living Resources* (New York: Springer Verlag).

SATYANARAYANA, D., RAO, I.M. & REDDY, B.R.1985.Chemical oceanography of harbor and coastal environment of Visakhapatnam (Bay of Bengal). Part I : Trace metals in water and particulate matter. *Indian J. Mar. Sci.* **14** :139-146.

SETH, R.N., TYAGI, R.K. & PANWAR, R.S. 1990. Toxicity of 2-methoxy ethyl mercuric chloride, copper sulphate and mercuric chloride to freshwater snail, *Viviparus bengalensis* (Swainson), *J. Environ. Biol.* **11** : 263-267.

THIAN, J.E. 1984. Effect of mercury on the prosobranch mollusc *Crepidula fornicata*: acute lethal toxicity and effects on growth and reproduction of chronic exposure. *Marine Environ. Recs.* **12** : 285-309.