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Effect of heavy metal toxicity on Zooplankton population based on dyes and printing industries in Jodhpur (Rajasthan)

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ABSTRACT

Industrial effluent based on printing and dying factories contain heavy metals. Present study reveals with bioassay test on fresh water zooplankton with special reference to Daphnia magna. Planktons are indicator species for biomonitoring of a few water pollutants. Bioassay experiments were conducted to find out lethal concentration (LC50) values of test chemical $CuSO_4$ and $Pb(NO_3)_2$. Sublethal concentration of Cu was tested in the range of .05 to .150 mg/l. Experiment with Pb was conducted at concentrations ranging from .110 to .300 mg/l. Neonates less than 24 h old were used for toxicity test. Mortality was assessed at interval of 24h, 48h and 72h. The LC50 values of Cu for 24h, 48h and 72h to D. magna were as follows: 0.130, 0.080 and 0.074 mg/l respectively. In case of Pb the LC50 values for 24h, 48h and 72h were 0.250, 0.235 and 0.200 mg/l respectively.

Keywords: Textile effluent, Heavy metal, Zooplankton, *Daphnia magna*, Bioassay, Bioindicator.

INTRODUCTION

The aquatic ecosystems are being threatened by the discharge of untreated sewage wastes and industrial effluent. The aquatic ecosystem is final sink for all potentially toxic metals in the environment via transfer through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes (Tarvainen *et al*., 1997; Stephen *et al*., 2000).

The presence of heavy metals in the aquatic ecosystem affecting the aquatic life (Olomukoro *et al*., 2011) and fish, the major component of the food chain leading to humans (Mason, 1996) and it may transform into the persistent metallic compounds with high toxicity, which can be bio accumulated in the organisms, magnified in the food chain, thus threatening human health. Heavy metal pollution generally represents high levels of Hg, Cr, Pb, Cd, Cu, Zn, Ni etc. in water system (Liang, 2004). Some metals are essential and others are non-essential.

Lead is one of the non-essential and non-biodegradable heavy metals and it is highly toxic to many organisms even at low concentrations (Biesinger *et al*., 1972; LeBlanc, 1982) and it can accumulate in different trophic level of aquatic ecosystems. Due to lead poisoning causes anaemia, encephalopathy, weight and coordination loss, abdominal pain, vomiting, constipation, and insomnia (Khallaf *et al*., 1998).

Cu is probably a functional constituent of all cells. Toxicity can result from excessive intake, which results in gastrointestinal disturbance, headache, cirrhosis, necrosis, and liver failure (Bahnasawy, 2011).

The best way to calculate effluent toxicity effect is to use biotoxicity test (Metcalf and Eddy, 2003). Cladoceran species are important aquatic organisms because they transfer energy and materials from primary producers to higher trophic level. They are also one of the most sensitive bioindicator species to toxic chemicals [Hanazato, 2001].

In the present study Cladoceran species *Daphnia magna* was selected in toxicological tests in wastewater treatment, due to extremely fast growth rates, high reproductive rates and short life cycles, high sensitivity, and simplicity; therefore, it was used as an indicator in this study (APHA, 1992; USEPA, 2000). Aim of this

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study to evaluate the LC_{50} value of metals. The present study is an attempt to assess the influence of heavy metals on zooplankton and calculate the lethal concentration.

MATERIALS AND METHODS

Sample collection:

The industrial waste water effluent samples were collected monthly from basani industrial area of Jodhpur. The samples were collected every month from July 2014 to June 2015 in polythene bottles of 1 L and 2L. Prior to sampling each sampling bottles washed with double distilled water and again washed with waste water to be sampled.

For heavy metal analysis effluent sample were filtered by whatman paper no.40 and then filtrate (500 ml) acidified with 2ml nitric acid. Metal ions were determined by atomic absorption spectrophotometer.

Culture preparation:

5 gm of dried cow dung was mixed with 25 gm of soil garden. Then, one liter of pond water was added to the mixture and allowed to stand for 2 days then strained through a plankton cloth. For the preparation of the final culture medium, one volume of filtered liquid was mixed with 6 to 8 volumes of pond water (Davis and Ford, 1992; Verma *et al.,* 2013).

Propagation and culture of Daphnia:

Zooplankton required for the toxicity evaluation studies were collected from a frogery unit and fish hatchery unit of department of Zoology, JNVU Jodhpur. The test species *Daphnia magna* was isolated for culture in isolation culture jar. In the next step, the recultured daphnids were used to prepare the final culture. For this reason, 100 ml of the final culture was poured into special bottles. Then, one single *Daphnia was* added to each bottle. To support the growth of *Daphnia* during the day after the initial culture, one mg of dry yeast was added to each bottle, every other day. Then the organism were separated from the culture flask and used for toxicity evaluation (Movahedian, 2005; Verma *et al.,* 2013).

Bioassay:

Bioassay test were conducted for Cu and Pd using the following test substances: $CuSO_4$ and $Pb(NO_3)_2$. The water used for dilution was chlorine free tap water, having physical and chemical characteristics within the limits suggested by APHA (1992). Bioassay test were carried out in 250 ml glass beakers with 200 ml test solution containing 10 neonates of 48h old obtained from the original culture, were introduced into test containers having different concentrations of heavy metals. There was no feeding during the test and the containers were slightly aerated (not to disturb daphnids with air bubles). The LC_{50} value is expressed by the concentration at which 50% of the test animals survived after a specific period of exposure under the prescribed experimental conditions. The number of dead zooplankton in each container was recorded at an interval of 24 h till 72 h and after gently shaking the glass containers, the ones that could not move were regarded as immobile. Dead ones were removed from the container. The experiments were repeated thrice and the mean values of the median lethal concentration (LC_{50}) were used for statistical analysis.

RESULTS AND DISCUSSION

In present investigation, Cu content was found to very between 1.00 to 2.25 mg/l whereas permissible limit is 1.5 mg/l (BSI). The Pb content was found to very in the range of 0.78 to 1.70 mg/l. The values reported were above the permissible limit of 0.1 mg/l set by BSI. Similar findings with higher values of heavy metals in industrial effluent have been reported by Singare *et al.,* 2014.

Table-1. Showing the concentration of trace heavy metal in industrial effluent observed during the July 2013 to June 2014.

Month	Cu Concentration (mg/l)	Pb Concentration (mg/l)
July 2013	1.00	.85
August 2013	1.05	.78
September 2013	1.22	.90
October 2013	1.50	1.00
November 2013	1.58	1.15
December 2013	1.70	1.18
January 2014	1.85	1.26
February 2014	2.00	1.33
March 2014	2.05	1.40
April 2014	2.10	1.56
May 2014	2.15	1.66
June 2014	2.25	1.70

Heavy metal stock solutions were prepared for toxicity test on zooplankton as industrial effluent contains other organic and inorganic material which changed chemical nature of metals. In present study, Cu and Pb were used for toxicity test on *Daphnia magna*. Daphnia magna is widely used as a test organism in a variety of ecological studies. D. magna is relatively easy to maintain in the laboratory, has a short life cycle and can be maintained at high population densities in relatively small volumes (Martins, 2007). It was chosen because it is a very good indicator of heavy metal pollution (Altmda, 2008). According to Michels *et al.,* (2000) *Daphnia magna* is also known to be sensitive to many chemicals that are commonly found in the aquatic environment, and can respond to these substances with a variety of physiological and behavioural characteristics.

Initially the range finding test was performed on the basis of literature survey. Cu was tested in the range of

.05 to .150 mg/l. Experiment with Pb was conducted at concentrations ranging from .110 to .300 mg/l.

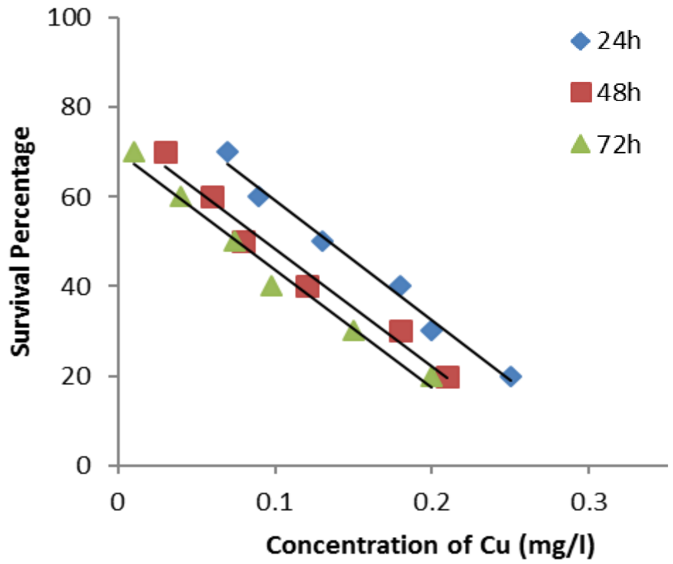
The median lethal concentration (LC_{50}) of Cu to *Daphnia magna* for 24, 48 and 72 h were 0.130, 0.080 and 0.074 mg/l respectively. According to Kungolos et al. (2002) and Gholami *et al.,* (2013) the LC_{50} value for 24 h were .049 mg/l and .030 mg/l respectively. Khangarot *et al.,* (1987) found the 48h LC_{50} value for copper sulphate as 0.1 mg/l for *Daphnia magna.*

In case of Pb lethal concentration (LC_{50}) for 24, 48 and 72 h were 0.250, 0.235 and 0.200 mg/l respectively. Altmda *et al.,* (2008) found the 24 h LC_{50} for lead nitrate as .441 mg/l for *Daphnia magna*. All values were different from each other so it makes difficult to interpret. According to Ghazy *et al.,* (2003) metal toxicity even to the same organism can vary with changes in the water quality because of the influence of water chemistry and metal speciation on metal bioavailability.

The presented order of toxicity for D. Magna was Cu > Pb. Similar results were made by Milan *et al.,* (1995) during studies on the toxicity of heavy metals (Cu, Zn and Pb) on *Daphnia magna.* The order of toxicity is as follows: Cu > Zn > Pb. According to Khangarot *et al.,* (1987) the order of toxicity of heavy metals for *Daphnia magna* was: Hg > Ag > Cu > Zn > Cd > Co > Cr > Pb > Ni > Sn.

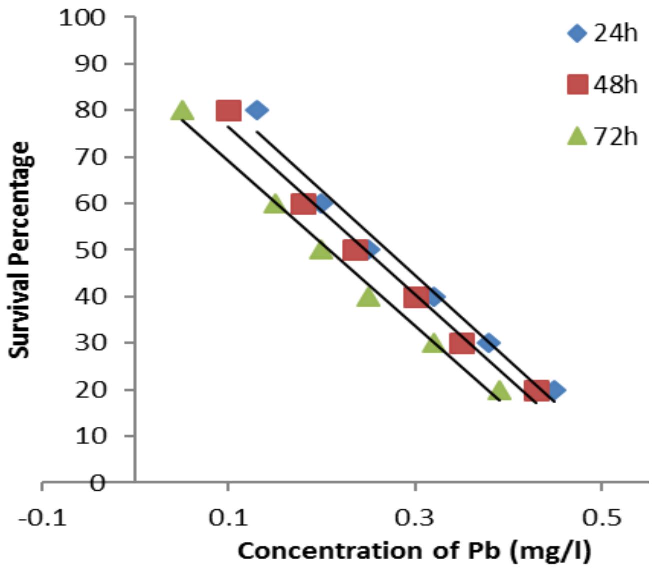
Cu salt cause a slight bluish greenish trace to the body of D. magna. Due to toxic effect of heavy metals they depicted restless movements, antennal movement retarded completely. Similar results were found by Verma *et al.,* (2013) and Altmda *et al.,* (2008) that studied on the toxicity of Cu on Ceriodaphnids.

Fig-1. Relationship between the survival percentage and Copper Sulphate concentrations along with the regression lines and equations for *Daphnia magna*



Population growth and movement decreased with increasing concentration of heavy metals. Unterstein *et al.,* (2003) found alterations in the swimming patterns after exposing D. Magna to 10 µg/l Cu. Ghazy *et al.,* (2003) have studied that the filter feeding cladocerans are more sensitive to toxic materials than predatory cyclopoid copepods. This is due to differences in mechanisms of food collection as well as in the intensity of contact with water having heavy metal in ionic form.

Fig-2: Relationship between the survival percentage and Lead Nitrate concentrations along with the regression lines and equations for *Daphnia magna*



Regression values were 0.986, 0.976 and 0.981 with respect to 24, 48 and 72h for Cu and 0.978, 0.986 and 0.992 with respect to 24, 48 and 72h for Pb respectively indication good correlation between the metal concentrations and survival percentage (Fig. 1 and 2). The slope values and regression values are depicted in Table-2.

Table-2. LC_{50}, Slope Function and Regression Values

Metal	Exposure Period	Parameters	Values
Copper	24h	LC_{50}	0.130 mg/l
		Slope function	y = 86.34 - 269.6x
		R^2	0.986
	48h	LC_{50}	0.080 mg/l
		Slope function	y = 74.78-262.8x
		R^2	0.976
	72h	LC_{50}	0.074 mg/l
		Slope function	y = 70.11 - 263.4x
		R^2	0.981
Lead	24h	LC_{50}	0.250 mg/l
		Slope function	y = 98.79 - 180.7x
		R^2	0.978
	48h	LC_{50}	0.235 mg/l
		Slope function	y = 94.51-179.9x
		R^2	0.986
	72h	LC_{50}	0.200 mg/l
		Slope function	y = 86.83 -

	177.2x
R^2	0.992

CONCLUSION

Toxicity bioassay test used for monitoring the quality of the aquatic environment are an important and useful tool. From the results obtained it can be inferred that the heavy metals are toxic to the zooplankton in general. *Daphnia magna* were more sensitive to Cu than Pb. Further studies will be needed to evaluate the chronic effect of Cu and Pb on the *D. magna*.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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