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RESEARCH ARTICLE

Acute toxicity of copper sulphate and potassium chromate to "tailless freshwater flea", *Simocephalus vetulus* (Crustacea-Cladocera).

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Abstract

The objective of this study was to evaluate the acute toxicity (LC_{50}) and behavioural alterations by heavy metals, copper sulphate and potassium chromate to "tailless freshwater flea", *Simocephalus vetulus* (Crustacea-Cladocera). *S. vetulus* were subjected to static bioassay tests to ascertain the LC_{50} values and their 95% confidence limits of copper sulphate and potassium chromate which were calculated according to trimmed Spearman-Karber method. The LC_{50} values of 24, 48, 72 and 96hr exposure for copper sulphate and potassium chromate to *S. vetulus* were found to be 0.87, 0.58, 0.45, 0.37 mgL^{-1} and 0.70, 0.33, 0.23, 0.16 mgL^{-1} respectively. Behavioural alterations like hyperactivity, fast appendage movements and avoidance were noticed at initial stage and at later stage erratic swimming and spinning, reduced activity were common in both metals while darkening of cuticle in copper and cuticular lightening in chromium were peculiar alterations. LC_{50} values indicated that *S. vetulus* were more sensitive to potassium chromate than copper sulphate. The behavioural response and changes of *S. vetulus* showed the most sensitive indication of potential toxic effects. Hence LC_{50} values of present study showed to be an important predictive and sensitive biomarker in aquatic monitoring and pollution management.

Introduction:-

Industrial development manifested due to setting up of new industries or expansion of existing industrial establishments has resulted in the generation of industrial effluents, spatially small scale cottage industries which discharge untreated effluents and ultimately cause air, water, soil and soil solid waste pollution (Nasrullah *et al.*, 2006). Most industries discharge their effluents into water bodies such as rivers, streams, lakes, etc. The major concern about these effluents is that if it discharged untreated, they may exhibit acute or chronic toxic effects on organisms in the receiving water bodies and result in ecological damage (Adewuyi *et al.*, 2010).

The main substances involved in chemical pollution through industrial effluent are heavy metals, organic compounds, especially organo-halogenated substances, detergents-"surfactants", pesticides, polycyclic Aromatic Hydrocarbons (PAHs) and Poly Chloro Biphenyls (PCBs), nitrates, phosphates, and drug residues (Kümmerer, 2001; Zimmo *et al*., 2004; Emmanuel *et al*., 2004; Lacour *et al*., 2006).

Heavy metals after entering into environment finally find their way into aquatic ecosystem and affects aquatic flora and fauna as well as human beings. Copper, though an necessary micronutrient, when present in excess causes different toxic effects on aquatic flora and fauna by changing their behaviour, physiology and histoarchitecture (Singh, 2014).

Among various metals, Copper a “grey listed metal” (Mason, 1996) naturally occurs in rocks, soil, water, sediments, air as well as are widely used in agriculture and various industries. After taking entry in aquatic system copper becomes highly toxic to flora and fauna. Cu and its compounds have been designated as priority pollutants by (EPA, 2000). Cu is one of the heavy metals, that is necessary at small concentrations but at higher levels it becomes toxic to zooplankton (Bossuyt and Janssen, 2004). Cu salts are widely used as an algaecide for controlling phytoplankton in fish ponds and lakes as well as an herbicide, used in aquatic weed control for which proper standardization of doses is lacking. The unplanned excessive doses can alter the physiology, histology, survival rate, growth and reproductive potential of fishes. The toxic effects of copper on fishes and other aquatic organisms are not very well studied in comparison to other metals (Witeska, 2005; Lodhi *et al*., 2006; Sharma and Shukla, 2006; Verma *et al*., 2010; Reddey *et al*., 2016).

Another metal Chromium is considered non essential for plants, but an essential trace element for animals required for basal metabolism (APHA, 1998). The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters as 100µg/L. The U. S. EPA primary drinking water standard MCL (maximum contamination level) is 0.l mg/L for total chromium (APHA, 1998). Cr is a heavy metal with two oxidation stages: Cr (III) and Cr (VI); the first one is a micronutrient required for some organisms, whereas the Cr (VI) is a carcinogenic toxic substance that is discharged into the environment as pollutant from industrial activities (Martınez-Jeronimo *et al*., 2006). Cr is used in the textile, leather, galvanizing and metallurgic industries, and their discharges contain additionally important amounts of salts. This is a stress producing factor for freshwater aquatic organisms (Gomez-Dıaz and Martınez-Jeronimo, 2009). Effect of chromium on reproduction, mortality and total growth rate have been recorded on various organisms including *Daphnia.* (Sharma, *et al*., 2008; Gopi *et al.,* 2012).

Crustaceans specially, prawn, shrimps, lobsters and crabs are on top of foreign exchange earners among aquaculture products have important place in aquatic food web and are more sensitive to the toxicants than aquatic vertebrates therefore wildly used in toxicity testing and biomonitering (Shukla and Sharma, 1992; Sharma and Shukla, 2006; Verma *et al*., 2010).

The freshwater crustaceans specially Cladocerans are commonly used in measuring pollution level, drinking water quality and in testing of human body fluids, due to their macroscopic size, easy culture methods, short life cycle and parthenogenetic mode of reproduction (Smirnov, 2014). The aquatic creatures like “tailless freshwater flea”, *Simocephalus vetulus* (Crustacea-Cladocera) being the sensitive crustaceans can serve as important biological indicator for aquatic pollution and also plays an important role in food webs of aquatic ecosystems. It occupies a key position, thus allows the transfer of energy and matters from lower trophic levels to higher trophic levels in fresh water aquatic ecosystem (Hakima *et al*., 2013).

Keeping this in view, the present study was under taken to evaluate the LC_{50} values of copper sulphate and potassium chromate to the “tailless freshwater flea” *S. vetulus* and to study its behavioural alterations due to the heavy metal toxicity.

Materials and Methods:-

Collection and culture of *S. vetulus:-*

S. vetulus (Crustacea-Cladocera) were collected from the freshwater pond located near Ikkar railway station at Haridwar and identified (Michael and Sharma, 1988). The samples were brought to laboratory and their culture was prepared. Five gm. of dried animal compost and 25 gm. of soil garden were mixed in one liter of pond water in a trough. The mixture was filtered through 0.15 mm pores membrane and kept at laboratory temperature for 2 days. For the preparation of the medium, one volume of filtered liquid was mixed with 6 to 8 volumes of pond water following the method of (Davis and Ford, 1992). The stock culture of *S. vetulus* was done in a five liter glass jar, which can be optimized further for growth depending upon the observations (US-EPA, 2000).
S. vetulus were fed once in 24 hours (or as per experimental need) with mixture of dried prawn powder in liquid form.

Preparation of toxicant and analysis:-

Stock solution of Copper sulphate ($CuSO_4.5H_2O$: AR: Merck Specialities Private Limited, Mumbai, India) and Potassium chromate (K_2CrO_4: AR: Merck Specialities Private Limited, Mumbai, India) was prepared by dissolving weighed amount of salt in double distilled water. Two to three drops of glacial acetic acid was added to stock solution of copper sulphate so as to prevent the precipitation. The experimental medium was analyzed for its physico-chemical parameters (Temperature, pH, Dissolved oxygen and Total hardness) as per standard methods of (APHA *et al*., 2012).

Experimental set up:-

All toxicity tests were carried out on neonates (age < 24 hr) obtained by isolating the adults from the stock culture. A series of five concentrations of test medium were prepared on the basis of toxic ranges determined by exploratory tests, in 100ml glass beaker containing 50ml of dilution water (DW).10 neonates of 24hr old *S. vetulus* were carefully introduced in each beaker containing test medium water. 50ml of DW in a beaker, served as control. All tests were carried out up to 96hr following standard procedure (APHA *et al*., 2012). Percent mortality (PM) was recorded for all the concentration of $CuSO_4.5H_2O$ and K_2CrO_4 at 24, 48, 72 and 96hr. The animals failing to response were considered as dead. Behavioral responses were observed for each concentration comparing with control (DW). Experiments were replicated thrice.

Statistical analysis:-

The LC_{50} values and their 95% confidence limits were calculated according to trimmed Spearman-Karber method (Hamilton *et al*., 1977) with software on P.C.

Results and Discussion:-

The physico-chemical parameters (Temperature, pH, Dissolved oxygen and Total hardness) of medium water, LC_{50} values and their 95% confidence limits with $CuSO_4.5H_2O$ and K_2CrO_4 are given in Tables 1 and 2 respectively, and LC_{50} values of copper and chromium for different species of crustaceans studied by other researchers compared with present study is appended in Table 3.

In the present study, LC_{50} values with $CuSO_4.5H_2O$ and K_2CrO_4 toxicity at 24, 48, 72 and 96hr for *S. vetulus* were recorded to be 0.87, 0.58, 0.45, 0.37 and 0.70, 0.33, 0.23, 0.16mgL^{-1} respectively at pH-7.3±0.2; DO- 6.5±0.2mgL^{-1}; Total hardness-290±2.4mgL^{-1} and Temperature.- 23±2°C. A clear cut inverse relationship between exposure duration and LC_{50} values were noticed in present study. During the present study, it was observed that 96hr LC_{50} values of *S. vetulus* due to Cr toxicity were lower than Cu toxicity, showing higher toxicity of chromium than copper. This might be due to essentiality of Cu towards crustaceans.

Static bioassay tests have been widely used to assess the toxic potential of various toxicants as well as sensitivity of various organisms at different trophic levels in ecosystem (APHA *et al*., 1998). Present findings are in confirmity with the findings of Guilhermino *et al*., (2000) who recorded 0.399 mgL^{-1} for 24hr LC_{50} on *D. magna*, Nandini *et al*., (2007) who recorded 710 μgL^{-1} for 24hr on *Moina macrocopa*, while some authors reported lower values viz. Mano *et al*., (2011) reported LC_{50} of 16.4 and 10.4 μgL^{-1} at 24 and 48hr respectively on *Diaphanosoma brachyurum* and Tellioğlu, (2014) reported LC_{50} (18.7 and 12.7 μgL^{-1}) due to Cu toxicity at 24 and 48-hr respectively on *Ceriodaphnia reticulata*. Whereas the concentration of LC_{50} (0.16 mgL^{-1}) in the present study due to K_2CrO_4 at 24hr was reported higher by Guilhermino *et al*., 2000 reported LC_{50} value of chromous chloride 40.501 mgL^{-1} on *D. magna* at 24hr, Rajaretnam and Stanley, 2015 reported LC_{50} value 3.5 mgL^{-1} on *D. magna* at 24hr with $K_2Cr_2O_4$, while some authors reported lower LC_{50} values for chromium toxicity Martınez-Jeronimo *et al*., 2006 reported 0.2076 mgL^{-1} on *D. magna* at 48hr, Diamantino *et al*., 2000 reported LC_{50} Value of sodium dichromate 0.29 mgL^{-1} on *D. magna* Straus at 48hr. The differences may be due to variation in physico-chemical parameters of diluents water and as well as in lab condition.

Present study shows that chromium is more toxic than copper as also reported for different crustaceans (Guilhermino *et al*., 2000; Rodgher and Espíndola, 2008; Rajaretnam and Stanley, 2015).

Heavy metals copper and chromium induced marked behavioural alterations in *S. vetulus* during present study as initial hyperactivity, mucus secretion, fast appendage movements and avoidance at initial stage while of both the metal exposures, while at later stage erratic swimming, spinning, reduced activity darkening of carapace in copper exposure while rough and light carapace in chromium exposure were peculiar features.

Various behavioural alterations observed in present study due to copper and chromium toxicity have also been reported by various workers in crustaceans (Thiel and Baeza, 2001; Meerhoff *et al*., 2006; Sharma *et al*., 2008;

Loureiro *et al*., 2013) and other aquatic animals (Ezeonyejiaku *et al*., 2013; Kamble and Kamble , 2014; Mariappan and Karuppasamy, 2014; Kumar *et al*., 2015).

Changes in animal behaviour are the earliest indication to the environmental stress (Clotfelter *et al*., 2004). Hyperactivity, fast appendage movement observed in present study may be due to avoidance and irritation caused by toxicants as also observed by other workers (Lodhi *et al*., 2006; Sharma and Shukla, 2006). Mucous secretion may be a protective device against the toxicants as it binds heavy metals to check their further entry (Plonk and Neft, 1969; Lock and Overbreak, 1981; Srinivas and Balaparameswara, 1996). Loss of balance, sluggishness at later stages may be due to alteration in CNS (Devi and Fingerman, 1995) or may be due to alteration in muscle fibres (Schultz and Kennedy, 1977).

Table 1. Physico-chemical parameters of medium water.

S.N.	Parameter	
1	Temperature (°C)	23±2
2	pH	7.3±0.2
3	Dissolved oxygen (mgL^{-1})	6.5±0.2
4	Hardness (mgL^{-1})	290± 2.4[1]

Table 2. LC_{50} values of copper sulphate and potassium chromate to tailless freshwater flea, *S. vetulus*.

Time (hr)	Copper sulphate			Potassium chromate		
	LC_{50} (mgL^{-1})	95% confidence limits (mgL^{-1})		LC_{50}(mgL^{-1})	95% confidence limits(mgL^{-1})	
24	0.87	0.63	1.19	0.70	0.55	0.90
48	0.58	0.45	0.75	0.33	0.24	0.45
72	0.45	0.32	0.64	0.23	0.16	0.33
96	0.37	0.28	0.48	0.16	0.11	0.24

Table 3. LC_{50} values of copper sulphate and potassium chromate to tailless freshwater flea, *S. vetulus* and comparison with different species of crustaceans.

S.N.	Species	LC_{50}	Metals	Duration (hr)	Reference
1.	*D. magna*	0.399 mgL^{-1}	Copper	24	Guilhermino *et al*., 2000
2.	*Moina macrocopa*	710 μgL^{-1}	$CuCl_2$	24	Nandini *et al*., 2007
3.	*Diaphanosoma brachyurum*	16.4 and 10.4 μgL^{-1}	$CuSO_4$	24 and 48	Mano *et al*., 2011
4.	*Ceriodaphnia reticulata*	18.7 and 12.7 μgL^{-1}	Copper	24 and 48	Tellioğlu, 2014
5.	*S. vetulus*	0.87 and 0.58mgL^{-1}	$CuSO_4$	24 and 48	Present study
6.	*S. vetulus*	0.45 and 0.37mgL^{-1}	$CuSO_4$	72 and 96	Present study
7.	*D. magna*	40.501 mgL^{-1}	Chromous chloride	24	Guilhermino *et al*., 2000
8.	*D. magna*	3.5 mgL^{-1}	$K_2Cr_2O_4$	24	Rajaretnam and Stanley, 2015
9.	*S. vetulus*	0.70 mgL^{-1}	K_2CrO_4	24	Present study
10.	*D. magna*	0.2076±0.0164 mgL^{-1}	Cr(VI) 20°C	48	Martınez-Jeronimo *et al*., 2006
11.	*D. magna*	0.1544±0.0175 mgL^{-1}	Cr(VI) 25°C	48	Martınez-Jeronimo *et al*., 2006
12.	*D. magna* Straus	0.29 mgL^{-1}	Sodium dichromate	48	Diamantino *et al*., 2000
13.	*S. vetulus*	0.33 mgL^{-1}	K_2CrO_4	48	Present study
14.	*S. vetulus*	0.23 mgL^{-1}	K_2CrO_4	72	Present study
15.	*S. vetulus*	0.16 mgL^{-1}	K_2CrO_4	96	Present study

Conclusion:-

It is evident from present study that both heavy metals copper and chromium are toxic to "tailless freshwater flea", *S. vetulus*. Various behavioural alterations after proper standardizations can serve as biomarkers in reference to metal toxicity and *S. vetulus* can serve as better bioindicator for environmental pollution and can be easily used in biomonitoring.

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