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 (LC₅₀) 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 LC₅₀ values and their 95% confidence limits of copper sulphate and potassium chromate which were calculated according to trimmed Spearman-Karber method. The LC₅₀ 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. LC₅₀ 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 LC₅₀ 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 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 algaeicide 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 0.1 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-Diaz and Martinez-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 biomonitoring (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$_{2}$O; AR: Merck Specialities Private Limited, Mumbai, India) and Potassium chromate (K$_{2}$CrO$_{4}$; 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$_2$O and K$_2$CrO$_4$ at 24, 48, 72 and 96hr. The animals failing to respond 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$_2$O and K$_2$CrO$_4$ 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$_2$O and K$_2$CrO$_4$ 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 conformity 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 48hr respectively on Ceriodaphnia reticulata. Whereas the concentration of LC$_{50}$ (0.16 mgL$^{-1}$) in the present study due to K$_2$CrO$_4$ 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$_2$CrO$_4$, while some authors reported lower LC$_{50}$ values for chromium toxicity Martinez-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 Strauss 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               | Value           |
|------|-------------------------|-----------------|
| 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        |

**Table 2.** LC₅₀ values of copper sulphate and potassium chromate to tailless freshwater flea, *S. vetulus*.

| Time (hr) | Copper sulphate | 95% confidence limits (mgL⁻¹) | Potassium chromate | 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.** LC₅₀ values of copper sulphate and potassium chromate to tailless freshwater flea, *S. vetulus* and comparison with different species of crustaceans.

| S.N. | Species               | LC₅₀ | Metals       | Duration (hr) | Reference                      |
|------|-----------------------|------|--------------|---------------|-------------------------------|
| 1.   | *D. magna*            | 0.399 mgL⁻¹ | Copper      | 24            | Guilhermino *et al.*, 2000    |
| 2.   | *Moina macrocopa*     | 710 µgL⁻¹ | CuCl₂       | 24            | Nandini *et al.*, 2007        |
| 3.   | *Diaphanosoma brachyurum* | 16.4 and 10.4 µgL⁻¹ | CuSO₄ | 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 | CuSO₄ | 24 and 48 | Present study                 |
| 6.   | *S. vetulus*          | 0.45 and 0.37mgL⁻¹ | CuSO₄ | 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 | K₂Cr₂O₇ | 24 | Rajaretanam and Stanley, 2015 |
| 9.   | *S. vetulus*          | 0.70 mgL⁻¹ | K₂CrO₇ | 24 | Present study                 |
| 10.  | *D. magna*            | 0.2076±0.0164 mgL⁻¹ | Cr(VI) 20°C | 48 | Martinez-Jeronimo *et al.*, 2006 |
| 11.  | *D. magna*            | 0.1544±0.0175 mgL⁻¹ | Cr(VI) 25°C | 48 | Martinez-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 | K₂CrO₇ | 48 | Present study                 |
| 14.  | *S. vetulus*          | 0.23 mgL⁻¹ | K₂CrO₇ | 72 | Present study                 |
| 15.  | *S. vetulus*          | 0.16 mgL⁻¹ | K₂CrO₇ | 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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