ABSTRACT
The cladoceran “tailless fresh water flea” *Simocephalus vetulus* (Crustacea-Cladocera) is well established as a model for eco-toxicology and being the sensitive crustacean can serve as better bio-indicator of metallic pollution. The myogenic heart of the *S. vetulus* is regulated by inhibitory cholinergic neurons that modulate cardiac function, including heart rate and susceptibility to cardio-active drugs known to affect the human heart. Present study deals with the effect of copper sulphate (96hr LC$_{50}$, 25%, and, 10% of 96hr LC$_{50}$ values, 0.037, 0.092, and 0.037mg/l respectively) on physiology of heart beat rate of *S. vetulus* for 96hr, 7, 14 and 21 day exposure. The mean heart beat rate of acute (96hr LC$_{50}$) was 297.8 and for control 418.6 beats/min., sub-acute (25% 96hr LC$_{50}$) 429.6, 392.6 and 345.4 beats/min. for 7, 14 and 21 days respectively and for control 460.4, 450.0, and 465.2 beats/min. for 7, 14 and 21 days respectively, and chronic (10% of 96hr LC$_{50}$) was 415, 398.8 and 385.6 beats/min. for 7, 14 and 21 days respectively, comparing with control being 460.4, 450.0 and 465.2 beats/min. for 7, 14 and 21 days respectively. The mechanisms of copper toxicity in reference to heart beat rate of *S. vetulus* have been discussed which can serve as better biomarker in reference to metal toxicity.

Keywords: Copper Sulphate, Heartbeat, Simocephalus vetulus, Tailless Fresh Water Flea

INTRODUCTION
Rapid industrialization, intensive chemical use in agriculture and anthropogenic activities had inverse effect on aquatic ecosystem there by adversely affecting flora and fauna (Tiwari *et al.*, 2008). Heavy metals like copper are common contaminants from agricultural and industries of minerals, petrochemicals, paints, where copper is often used as an algaeicides, it may eventually reach water bodies as run offs (Nandini *et al.*, 2007; Blake *et al.*, 2014). Cu an essential element assists with metabolic activities and acts as a catalyst for cellular respiration. Excess copper can result in oxidative stress during displacement of zinc in proteins and disruption of internal ion balance in gills of aquatic organisms (Gheorghiu *et al.*, 2010).

In general, invertebrates are more sensitive than fish to copper toxicity (USEPA, 2007). Cu is one of the heavy metals, that is required at low concentrations but at higher levels it becomes toxic to zooplankton (Gama-Flores *et al.*, 2007).

Cladocerans are important aquatic organisms because they transfer energy from primary producers to consumers of higher trophic level, such as fish. At the same time, they 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 span and parthenogenetic mode of reproduction (Hanazato, 2001; Smirnov, 2014).

The heart, in cladocera is reduced as a bulbous chamber in first true segment (Wagler, 1927), in Branchiura, heart is triangular located in last thoracic segment (Martin, 1932), in Decapoda, heart is triangular sac, situated in pericardial sinus, above the hepatopancreas (Lodhi *et al.*, 2009). Cladocerans possess an open circulatory system and a myogenic heart (Maynard, 1960; Yamagishi *et al.*, 2000; McMahon, 2001; Smirnov, 2014). Heart beats and the flow of hemolymph can be easily observed, as cladocera are translucent (Maynard, 1960; Smirnov, 2014). Toxic effect of heavy metals on cardiac...
Physiology has been studied in fishes (Al-Yousuf and El-Shahawi, 1999; Ashraj, 2005) and crustaceans (Tiwari et al., 2008; More, 2011; Rajaretnam and Stanley, 2015). Toxicity of copper on physiology of heart beat has been investigated in crustaceans (Ketpadung and Tangkroch-olan, 2006; More, 2011; Singh, 2014; Rajaretnam and Stanley, 2015) and other aquatic animals (Scott and Major, 1972; Brown et al., 2004). But less in reference physiology of heart beat to metal toxicity in cladocerans (Fernandez-Gonzalez et al., 2011; Rajaretnam and Stanley, 2015).

*S. vetulus* are small crustaceans that are inexpensive, easy to care and translucent colors. The latter property allows to easily observing the beating heart without dissecting and sacrificing the animals. Keeping this in view, the present study was under taken to evaluate the effect of copper sulphate on the physiology of heart beat rate of the “tailless freshwater flea” *S. vetulus* due to the heavy metal toxicity.

**MATERIALS AND METHODS**

**Culture and Maintenance**

*S. vetulus* (Crustacea-Cladocera) were collected from the freshwater pond located near Ikkar railway station at Haridwar (Uttarakhand), India. The samples were brought to laboratory and their culture was prepared by method of Davis and Ford (1992).

**Preparation of Toxicant:** Stock solution of Copper sulphate (CuSO₄·5H₂O: 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 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 (APHA et al., 2012).

**Experimental Set up and Recording of Heart Beat:** Acute, sub-acute and chronic effect of copper sulphate on physiology of heart beat rate was carried out on adults and neonates (age < 24 hr) of *S. vetulus* obtained by isolating from the stock culture. The heart beat recording procedure was based on that of Baylor (1942). A small amount of cotton was placed into a 1.5 ml glass well. A *S. vetulus* was added with enough aged medium water to immerge the animal. The cotton prevented the *S. vetulus* from swimming but still allowed some movement. Heart beat rate was recorded by a Panasonic DMC-LZ8 camera. Heart beat rate was determined by playing a 06-second portion of the recording in slow motion with the help of movie maker software and counting the beats/min.

**Statistical Analysis:** All the experiments were replicated thrice and results were analyzed with Student’s t-test and one-way ANOVA using Minitab software. All error bars represent the standard errors of the means.

**RESULTS AND DISCUSSION**

**Results**

*S. vetulus* possessed a myogenic heart. Like some arthropods, its heart was located dorsally, posterior to the brain, and was slightly flattened, elongated as translucent body and heart beat was easily observed without dissecting the animals (Plate: 1).

In the present study, exposure of *S. vetulus* to copper sulphate showed marked alterations in heart beat rate during acute, sub-acute and chronic test. The experimental medium was analyzed for its physico-chemical parameters (Temperature- 23±2°C, pH- 7.3±0.2, Dissolved oxygen- 6.5±0.2 mgL⁻¹ and Total hardness- 290± 2.4 mgL⁻¹) as per standard methods of (APHA et al., 2012). Acute effects of copper sulphate on physiology of heart beat of *S. vetulus* are summarized in Table 1, Figure 3, sub-acute Table 2, Figure 4 and chronic exposure are summarized in Table 2, Figure 5.

A significant decrease in heart beat rate was observed in response to 96hr LC₅₀ (0.37mg/L) of copper sulphate, the decrease in heart beat rate being 297.8±4.17bpm in comparison to control being 418.6±5.30bpm.

The differences in heart beat rate of exposed and control of *S. vetulus* were moderately significant (t= 4.00, p<0.01) at 24h; highly significant (t= 10.92, p<0.001; t= 13.00, p<0.001 and t= 17.89, p<0.001) at...
48, 72 and 96 hr. The overall fluctuations in heart beat rate from 24-96h were insignificant (F=1.22, p>0.05) in control and moderately significant (F=32.71, p<0.01) in exposed animals. In sub-acute (25% of 96h LC₅₀) exposure the heart beat rate was also lower in experimental animals than controls throughout the experiments (Table 2 and Figure 4). The differences in heart beat rate in control and experimental animals were moderately significant at 7 day (t=4.25, p<0.01) and highly significant 14 day (t=9.05, p<0.001) and 21 day exposure (t=27.93, p<0.001). The overall fluctuations were found to be significant in control (F=2.99, p<0.05) and moderately significant in exposed (F=105.85, p<0.01) from 7 to 21 days of exposure.

In chronic (10% of 96h) exposure the heart beat rate was also lower in experimental animals than controls throughout the experiments (Table 2 and Figure 5). The differences in heart beat rate in control and experimental animals were highly significant at 7 day (t= 5.49, p<0.001), 14 day (t= 9.69, p<0.001) and 21 day exposure (t= 19.42, p<0.001). The overall fluctuations were found to be significant in control (F=2.99, p<0.05) and moderately significant in exposed (F=12.37, p<0.01) from 7 to 21 days of exposure. S. vetulus exhibited a significant decrease in heart rate immediately following the 25% and 10% of the 96hr LC₅₀ values the heart rate.

Table 1: Effects of Copper Sulphate on Heart Beat/Min. of S. Vetulus after Acute Exposure

<table>
<thead>
<tr>
<th>Duration of Exposure (in Hours)</th>
<th>Heart Beat/min. (Mean ±SE)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>409.8±5.88</td>
<td>369.8±8.08**</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>405.2±4.63</td>
<td>344.8±3.02***</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>412.6±4.30</td>
<td>334.8±4.15***</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>418.6±5.30</td>
<td>297.8±4.17***</td>
<td></td>
</tr>
</tbody>
</table>

Note- ** denote differences in mean to be moderately significant at p<0.01
*** denote differences in mean to be highly significant at p<0.001

Figure 1: Photograph of Tailless Water Flea, Simocephalus Vetulus
Figure 2: Photomicrograph of Anterior Region of S. Vetulus
ANT=Antennae, CRA=Carapace, EYE=Eye, HD=Head, HT=Heart, INT=Intestine, OC=Ocellus, SP=Sensory Papillae
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Figure 3: Effects of Copper Sulphate on Heart Beat/Min. of *S. vetulus* after Acute Exposure

Figure 4: Effects of Copper Sulphate on Heart Beat/Min. of *S. vetulus* after Sub-Acute Exposure (25% of 96hr LC₅₀)

Table 2: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Sub-Acute and Chronic Exposure

<table>
<thead>
<tr>
<th>Duration of Exposure (Days)</th>
<th>Heart Beat/min. (Mean ±SE)</th>
<th>Experimental 25% of 96h LC₅₀</th>
<th>Experimental 10% of 96h LC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25% of 96h LC₅₀</td>
<td>10% of 96h LC₅₀</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>460.4±6.59</td>
<td>429.6±2.99**</td>
<td>415.0±5.00***</td>
</tr>
<tr>
<td>14</td>
<td>450.0±2.77</td>
<td>392.6±5.70***</td>
<td>398.8±4.49***</td>
</tr>
<tr>
<td>21</td>
<td>465.2±3.07</td>
<td>345.4±2.99***</td>
<td>385.6±2.71***</td>
</tr>
</tbody>
</table>

Note- ** denote differences in mean to be moderately significant at p<0.01
*** denote differences in mean to be highly significant at p<0.001
Figure 5: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Chronic Exposure (10% of 96hr LC$_{50}$)

**Discussion**

The results revealed that copper induced marked decrease in heart beat of *S. vetulus* during acute, sub acute and chronic exposures. Heart beat activity in cladocerans and crustaceans have been studied and mentioned to the wide range of stimuli *i.e.* tactile, chemical, pesticides, external electric field, temperature and hypoxic rate of breathing structures (Mac Mohan and Wilkens, 1975 (*Homarus americanus*); Usanov et al., 1999 (*D. magna Straus*); Usanov et al., 2001b (*D. magna*); Ketpadung and Tangkrock-olan, 2006 (*Portunus pelagicus*); Kaas et al., 2009 (*D. magna*); Chung et al., 2012 (*Procambarus clarkii* and *Macrobrachium rosenbergii*). The rate of heart beat is faster in smaller animals of a species and slower in larger species *Barytelphusa guerini* (More, 2011).

The heart beat rate is also affected and influenced by various environmental conditions and toxicant present in aquatic environment and can be used as sensitive bio-monitoring techniques for aquatic bodies (Ketpadung and Tangkrock-olan, 2006 ; Kaas et al., 2009; Medesani et al., 2011).

Various heavy metals also have direct effect on heart beat rate, which is very well known (Tiwari et al., 2008; Kozak et al., 2010). Copper sulphate toxicity showed significantly decreased heart rate throughout the experimental period, during acute (96hr LC$_{50}$), sub-acute (25% 96hr LC$_{50}$) and chronic (10% 96hr LC$_{50}$) exposure periods. High concentration of copper sulphate, the thermoresistance of the cardiac muscle of *D. magna* is significantly decreased (Pashkova et al., 1998).

This possibly result in decreased impulses in the cardiac nerves reduction the hemolymph flow so that the heart beat rate decrease under acute, sub-acute and chronic conditions to the toxicant. Decrease in heart beat rate observed in the present study is similar to observations of the other crustaceans *Barytelphusa guerini* (More, 2011). Heart beat frequency has been studied in many crustacean species in response to environmental stressors and chemicals (Tiwari et al., 2008; Singh, 2014). Decrease in heart beat in later stage of experiment may be due to altered carbohydrate metabolism (Radhakrishnaiah and Bussapa, 1986). Crustacean’s heart beat rate differs under different environmental conditions and rhythm varies in temperature, pH, size, sex, presence and absence of embryo (Seidl et al., 2002; More, 2011). Changes in heart rate of other aquatic animals are well known indicators of some maladjustment in the environment (Williams et al., 1991; Brown et al., 2004; Kozak et al., 2010).

**Conclusion**

It is evident from present investigation that heavy metal copper, despite being a micronutrient. Significantly lower the heart beat of Tailless water flea, *S. vetulus* in acute (*t*=17.89, *p*<0.001), sub-acute...
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(t=27.93, p<0.001) and chronic exposure (t=19.42, p<0.001). Due to their translucent colors heart beat can be counted without dissecting and sacrificing the animals. S. vetulus can serve as better bioindicator and heart beat rate can serve as biomarkers in reference to metal toxicity.

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