

Effect of copper on crawling speed in the freshwater snails *Pila globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp.

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Abstract

A laboratory study was carried out to observe the changes in crawling speed in four freshwater gastropod species *Pila globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp. due to copper (Cu) exposure. The movement of the snails were observed manually and recorded for 15 min period. A Kruskal-Wallis Test revealed that there were significant difference in crawling speed in *Pila globosa*, *Lymnaea* sp., and *Bellamya* sp. among control, 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu exposed concentration. A Kruskal-Wallis test revealed that there were no significant difference in crawling speed in *Planorbis* sp. among control, 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu exposed concentration. The result showed that Cu in the media had less effect in the changes in crawling speed of *Planorbis* sp. which explained that this species is more resistant to 0.01 mgL⁻¹ Cu, and 0.1mgL⁻¹ Cu concentrations compared to *Pila globosa*, *Lymnaea* sp., and *Bellamya* sp. The gastropods showed response against the Cu concentration in the experimental media by withdrawing the foot, tentacles and closing the operculum. This behavior might be to escape the toxicity of the metal concentration in the media. All the four molluscs studied showed no mucus secretions during the exposure in 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu test media.

Keywords: Copper, gastropod, mollusc, media, toxicity

Introduction

Deepor Beel is a floodplain wetland and a Ramsar Site (Ramsar site no. 1207) declared in 2002 is a large natural freshwater wetland that holds significant biological and ecological value (Deka and Goswami, 1992; NWA, 2013) [1, 19]. Deepor Beel is situated at the south western fringe of Guwahati, the state capital of Assam - a North Eastern state of India (MoEF, 2018) [18]. Vertebrate diversity in Deepor Beel includes 68 species of fishes, 11 species of amphibians, 33 species of reptiles, 234 known species of birds, 24 species of mammals and supports 18 threatened species of vertebrates. Diversed invertebrates found in Deepor Beel include 65 species of diatoms, 171 species of zooplankton, 5 species of bryozoans, 15 species of molluscs, 55 species of aquatic insects, 3 species of prawns and 2 species of crabs. There is a report of 58 species of aquatic macrophytes distributed all over the Deepor Beel (Barman & Saikia, 1995; Saikia & Saikia, 2011; Goswami and Kalita, 2012; Saikia, 2019; Battacharjya *et al.* 2021) [1, 14, 25]. *Bellamya bengalensis* was the dominant molluscan species in the wetland, followed by *Indoplanorbissexustus*, *Pila globosa*, and *Lymnaea acuminata*. The species *Corbicula assamensis* was recorded in a few numbers (Saikia, 2019) [24]. Sharma and Sharma (2013) [28] reported 13 molluscan species from Deepor Beel, namely *B. bengalensis*, *B. crassa*, *B. dissimilis*, *Pila globosa*, *P. theobaldi*, *Brotia costula*, *Paludomus blanfordiana*, *P. conica*, *P. reticulate*, *Lymnaea acuminata*, *L. luteola*, *Gyraulus convexiusculus*, *Indoplanorbis bisexustus*.

Aquatic organisms are known as the bioindicators of early warning systems of toxicity in aquatic systems early (Kramer and Botterweg, 1991) [16]. The extensive use of copper sulphate (CuSO₄), which is known as a toxic metal without any known important biological role has created environmental contamination worldwide. The presence of

CuSO₄ in aquatic system can lead to the production of Reactive Oxygen Species (ROS) such as superoxide anion radical (O²⁻), hydroxyl radical (OH⁻), and non-radical hydrogen peroxide (H₂O₂) in aquatic invertebrates including mussels causing oxidative stress. Aquatic molluscs have been used as a suitable organism in both active and passive bio-monitoring (Goldberg, 1986; Salanki, 1989; Borcherdig and Volpers, 1994) [4, 13, 26]. In active monitoring response of the artificial or modified populations, behavioral patterns, movement, feeding, respiration, reproduction, neural regulation, cellular and subcellular functions under toxic conditions (Salanki *et al.* 2000; Saikia, 2019) [24, 27]. Various anthropogenic activities, fertilizers and pesticides used in adjacent agriculture areas, solid waste and heavy metal waste dumpings from the Guwahati City cause threat to the ecosystem and biodiversity of the Deepor Beel (RIS, 2002; Roy and Kalita, 2011; Choudhury and Gupta, 2017) [8, 23]. Presence of heavy metal Cu during post monsoon 336.15 mg/kg and pre monsoon 33.69 mg/kg of sediment in the Deepor Beel is due to anthropogenic activities and the concentration increases after the post monsoon (Dash *et al.*, 2021) [10].

Any work on behavioral toxicology is based on the knowledge about the basic behavioral characteristics of the particular test species, which reflects the "integration of exposure conditions and represent an acute cumulative effect". An earlier study had investigated the toxic effects of a short term exposure of CuSO₄ in *Corbicula* sp. (Brahma & Gupta, 2020) [6]. In this research work it has been tried to observe the differences in the behavior of *Pila globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp. by observing on changes in crawling speed in copper.

Materials and Methods

Procurement of copper (Cu)

The heavy metal used in the experiment was Copper sulphate pentahydrate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, manufactured by Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India.

Sampling of gastropods

Gastropods were collected from Deepor Beel from two locations-

Location 1: Near view tower and Deepor Beel Wildlife Sanctuary Office (presence of macrophyte vegetation)

Latitude- $26^\circ 6' 46''$ N
Longitude- $91^\circ 39' 22''$

Location 2: Near the bridge (presence of mud, small pebbles and macrophytes)

Latitude- $26^\circ 6' 47''$ N
Longitude- $91^\circ 39' 18''$ E

Sampling

Mature gastropods *Pila globosa* (length 4.2 ± 0.4 cm, width 2.5 ± 0.3 cm); *Lymnaea* sp. (length 3.2 ± 0.3 cm, width 0.8 ± 0.2 cm); *Planorbis* sp. (length 0.6 ± 0.1 cm, width 0.5 ± 0.1 cm); and *Bellamya* sp. (length 1.4 ± 0.2 cm, width 1.1 ± 0.1 cm) were sampled from the Deepor Beel, Assam, India, from Location 1 and 2 by direct handpicked from the base of stream which is a mix of sand, small pebbles and mud, at around 7:00-8:00 a.m. The collected live snails were brought in a plastic bucket with some stream water in it. They were kept carefully in the polythene boxes and brought to the laboratory. The sampled molluscs were identified by following the Key to the various Taxa provided in the book "Handbook of Freshwater Molluscs of India" by Subba Rao (1989) [29].

Acclimatization

Before running the experiment collected snails were acclimatized in the laboratory in a glass aquarium (50 x 30x 30 cm) of 40 L capacity for 7 days. Aeration was provided continuously and no food was supplied. Aquarium was covered with nylon net so that any snail could not crawl out of it. Any dead snail was removed from the aquarium.

Crawling rate (Speed)

Following the protocol of Byzitter *et al.* (2012) [7], crawling rates were measured for gastropods at 0 h and 12 h in control (without Cu concentration) or in test water containing Cu at concentrations 0.01 mgL^{-1} and 0.1 mgL^{-1} . A large size petri

dish of 14 cm diameter by 2 cm depth was filled with 200 ml of stream water or Cu test media, giving a depth of 15 mm in petri dish which was sufficient to submerge the snail. In a graph paper $2 \text{ cm} \times 2 \text{ cm}$ grids were made and placed below the petri dish. The movement of the snail was observed and recorded for 15 min period. The observations were repeated for 5 times for each test group. The travelling speed of a snail was calculated as, $\text{Speed} = \text{distance}/\text{time}$.

The distance was converted into an average crawling rate of mm s^{-1} for analysis.

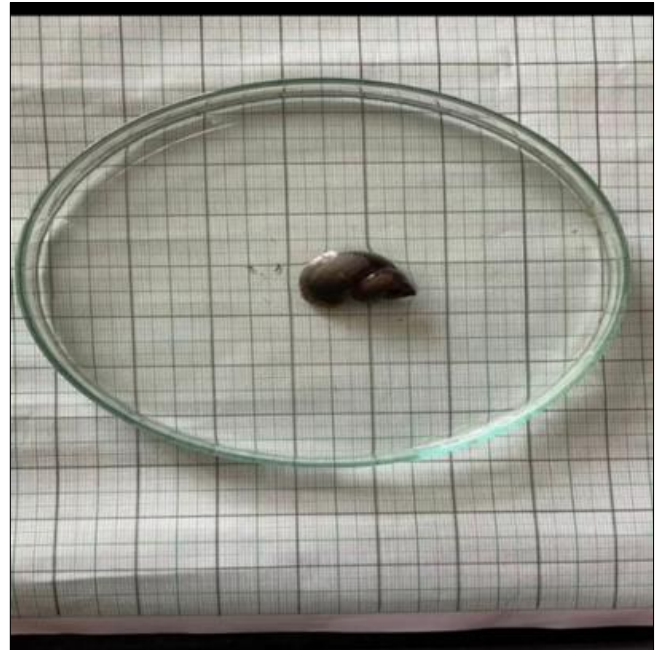


Fig 1: Crawling movement of *Bellamya* sp.

Statistical analysis

Normality of the data was checked with one-sample Kolmogorov-Smirnov Test and data without normal distribution was analysed with non-parametric Kruskal-Wallis and Mann-Whitney. Microsoft Excel 2007 and Statistical package for Social Sciences (IBM SPSS Statistics 20) for Windows were used for presentation.

Results

Changes in crawling speed in molluscs species

Average crawling speed of *P. globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp. in control and 0.01 mgL^{-1} Cu and 0.1 mgL^{-1} Cu exposed groups are tabulated in Table 1.

Table 1: Correlation of changes in crawling speed in *Pila globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp. Molluscs species in control, in 0.01 and 0.1 mgL^{-1} Cu test media

Species	Average distance travelled (In control) (Cm)	Average distance travelled (0.01 mgL^{-1} Cu) (Cm)	Average distance travelled (0.1 mgL^{-1} Cu) (Cm)
<i>Pila globosa</i>	34 _a	12 _a	1.33 _c
<i>Lymnaea</i> sp.	51.3 _a	21.3 _b	9.3 _c
<i>Planorbis</i> sp.	7.3 _a	9.3 _a	6 _a
<i>Bellamya</i> sp.	24.6 _a	12.6 _b	3.33 _c

Different subscript denote significant ($p < 0.05$)

Pila globosa

A Kruskal-Wallis Test revealed that there were significant difference in crawling speed among control, 0.01 mgL^{-1} Cu and 0.1 mgL^{-1} Cu exposed concentration ($p < 0.001$) (Table 1).

Mann-Whitney Test revealed that crawling speed of *Pila globosa* in control was significantly higher from that in 0.01 mgL^{-1} Cu and 0.1 mgL^{-1} Cu exposed groups ($U = 6.501$, $p < 0.001$). There was significant difference in crawling speed between 0.01 mgL^{-1} Cu and 0.1 mgL^{-1} Cu exposed groups ($U = 5.001$, $p < 0.031$).

***Lymnaea* sp.**

A Kruskal-Wallis test revealed that there were significant difference in crawling speed among control 0.01mgL⁻¹ Cu, and 0.1 mg L⁻¹ Cu exposed concentration ($p < 0.001$) (Table 1). Mann-Whitney Test revealed that crawling speed of *Lymnaea* sp. in control was significantly higher from that in 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu exposed groups ($U = 7.000$, $p < 0.001$). There was significant difference in crawling speed between 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu exposed groups ($U = 4.022$, $p < 0.001$)

***Planorbis* sp.**

A Kruskal-Wallis test revealed that there were no significant difference in crawling speed among control, 0.01mgL⁻¹ Cu and 0.1mgL⁻¹ Cu exposed concentration ($p < 0.145$) (Table 1).

***Bellamya* sp.**

A Kruskal-Wallis Test revealed that there were significant differences in crawling speed among control, 0.01 mgL⁻¹ Cu, and 0.1 mgL⁻¹ Cu exposed concentrations ($p < 0.001$) (Table 1). Mann-Whitney Test revealed that crawling speed of *Bellamya* sp. in control was significantly higher from that in 0.01 mgL⁻¹ Cu, and 0.1mgL⁻¹ Cu exposed groups ($U = 6.000$, $p < 0.001$). There was significant differences in crawling speed between 0.01mgL⁻¹ Cu, and 0.1mgL⁻¹ Cu exposed groups ($U = 5.002$, $p < 0.001$).

Discussion

Movement by muscular foot is a very important physiology not only for locomotion in environment but also for their various important life activities such as finding food, protection from predators, and reproduction. To determine whether there would be any effect on gastropod molluscs due to chemical contamination, the toxic effect of copper sulphate was done on selected mollusc species *Pila globosa*, *Lymnaea* sp., *Planorbis* sp., and *Bellamya* sp. The study on changes in crawling speed was done manually by direct observation and camera record. The observation in changes in crawling speed was found to be effected with significant changes in crawling speed of the gastropods. The concentrations of 0.01 mgL⁻¹ Cu, and 0.1mgL⁻¹ Cu exposed groups were selected by referring to Central Water Commission, Govt. of India report, as there was record of 0.011 mgL⁻¹ of copper concentration recorded in Pandu sampling site (CWC, 2018)^[9]. The present study revealed that copper could change the speed of the movement of the gastropods *Pila globosa*, *Lymnaea* sp., and *Bellamya* sp. more significantly, and movement of *Planorbis* sp. was found to be less effected. The result showed that Cu in the media had less effect in the changes in crawling speed of *Planorbis* sp. which explained that this species is more resistant to 0.01 mgL⁻¹ Cu, and 0.1mgL⁻¹ Cu concentrations compared to *Pila globosa*, *Lymnaea* sp., and *Bellamya* sp. The gastropods showed response against the Cu concentration in the experimental media by withdrawing the foot, tentacles and closing the valve. This behaviour might be to escape the toxicity of the metal concentration in the media. Behavioural changes like reduced movement and withdrawal of foot were also reported in *Corbicula fluminea*, *Lamellidens marginalis*, and *Lamellidens jenkinsianus obesa* due to presence of metal contamination in the water (Fournier *et al.*, 2004; Kumar *et al.*, 2012; Brahma and Gupta, 2020)^[6, 12, 17]. Kamble and Kamble (2014)^[15] also reported toxicity of copper sulphate in *Bellamya bengalensis*. All the four molluscs studied showed no mucus secretions during the

experiment. *Lamellidens corrianus*, *Melania hainesiana* and *Physa acuta* also did not release any mucus substance during exposure to low concentrations of Cu (Otludil and Ayaz, 2020; Brahma 2023)^[5, 21]. Molluscs could be a bioindicator of the toxicity in the water (Oeshlmann and Oeshlmann, 2003; Borchherding, 1992; Borchherding and Volpers, 1994)^[3, 4, 20].

Conclusion

The present study reveals that copper in the water could affect the movement of the benthic organism the studied mollusc species and thereby can reduce the crawling speed in harmful manner. Further study would give a clear scientific knowledge of the mechanism of the decrease in crawling speed of the molluscs due to harmful toxins. Determination on biochemical activities could provide an important research breakthrough about the harmful effects of heavy metals on aquatic organisms and to remediate the harmful chemicals. This would be very important step towards the conservation of biodiversity.

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