Journal of Biological Sciences and Medicine
Available online at www.jbscim.com

ISSN: 2455-5266

Research Article **Open Access**

Evaluation of Lethal concentration of Ammonium Sulphate to freshwater fish *Mystus Vitatus* (BLOCH)

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ARTICLE INFO

Article History:
Received
08 July 2016

Accepted
10 August 2016

Available online
30 September 2016

Key words:
Ammonium Sulphate; LC_{50}; *Mystus vittatus*; Safe level; Toxicity

ABSTRACT

The present study was undertaken to evaluate the lethal toxicity and stress levels of commercial formulations of Ammonium Sulphate toward freshwater *Mystus vittatus*. The 96 h LC_{50} values, determined in a semi-static system by probit analysis was 500 mg/L for Ammonium Sulphate, indicating that the fish were more sensitive to Ammonium Sulphate. There were large variations in the safe levels estimated by different methods for the metal. In addition to dose and dose-time dependent increase in mortality rate, stress signs in the form of behavioral changes were observed in response to the test salt.

Introduction

The quality of human life is directly or indirectly dependent on the health, vitality and vigour of the environment around us. Abiotic and biotic components, including air, water, soil, food, plants and animals constitute our environment, and hence life is sustained through complex interactions. Deterioration in the health of any of these components can have serious knock-on effect on the continuity of life. The aquatic environment, which covers two-third of the planet, is inhibited by the majority of species in different ecological niches; moreover, many of them are important source of human food. It has been estimated that approximately 70% of the human population resides within 60 km of coastal regions and this percentage is increasing (Gray 1991). In addition, a significant proportion of the world's largest cities are connected, either directly or indirectly to the marine environment (Jha and Jirong 2004).

The aquatic environment therefore, plays a vital role for ecosystem functioning, human health and civilization. Ammonium Sulphate is a chemical of considerable attention mainly due to

its tendency to elevate the ammonia load in the aquatic bodies following its agricultural application (Banerjee and Paul 1996; Zwaan et al. 2001; UNEP 2003). The toxicity of ammonium solutions does not usually cause problems for human and other mammals because a special mechanism prevents ammonia to build up in blood stream. Ammonia (NH3) is converted to Carbonyl phosphate by the enzyme Carbonyl phosphate synthase and then enters the urea cycle to be either incorporated in to amino acid or excreted in the urine. However, fishes, amphibians and most aquatic invertebrates lack this mechanism; they can usually eliminate ammonia from their bodies by direct excretion (Campbell and Reece 2002; Tilak et al. 2002).

The present study is thus aimed at examining the toxicity and effects of Ammonium Sulphate on fresh water fish *Mystus vittatus* by determining the LC_{50} values and analyzing behavioral changes due to toxic stress. The study further focused on estimation of the safe level as well as strengthened the base line data that could be used to estimate comparative sensitivity to these salts.

Material and Methods

Experimental fish specimen and chemicals

The specimen (10 ± 2.0g; 5.5 ± 1.0cm) were collected from local lentic habitats in the vicinity of Kanpur, India and brought to the laboratory in wide mouthed plastic containers containing natural water without any stress and mechanical injuries. The fish were subjected to repeated washings with tap water and various dip treatments viz., in formalin (0.4%) for 15 min., in 0.1% $KMnO_4$ solution for 30 min., in Benzylkonium Chloride (1-4 ppm) for 1 hour to remove external infections like, bacteria, protozoa, monogenetic trematods and arthropods, etc. The fishes were then acclimatized for two weeks under laboratory conditions in semi static systems. They were fed boiled eggs, minced goat liver and poultry waste materials during acclimatization. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water.

Determination of sub-lethal concentrations

Acute toxicity assay to determine the 96 h LC_{50} value of Ammonium Sulphate conducted with definitive test in semi-static system in laboratory as per standard methods (APHA, AWWA, WPCE, 2005). The range finding test was carried out prior to the definitive test to determine the concentration of the test solution. The experiment was conducted in glass aquaria containing 20 L of dechlorinated and gentle aerated water. A set of 10 fish specimen were randomly exposed to each of the 0.1, 0.2, 0.3, 0.4, 0.5 mg/L concentrations. Another set of 10 fish were also simultaneously maintained in tap water (0.00 mg/L) as the control. The experiment was set in triplicate and fish were not fed during the experimentation as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). Exposure time was 96 h after which mean mortality from a particular dose and its replicate was calculated. A fish was considered dead when it did not respond after gentle prodding with a glass rod; dead fish were removed from the tank immediately. Fish behavior was observed during the exposure. The median lethal concentration (LC_{50}) of the test salt was calculated from the data obtained in acute toxicity bioassays following the probit analysis method as described by Finney (1971). The 95% confidence limits of the LC_{50} values obtained by Finney (1971) method were calculated with the formula of Mohapatra and Rengarajan (1995).

Data analysis

The data obtained were statistically analyzed by statistical package SPSS (version 16). The data were subjected to one-way ANOVA and Duncan's multiple range tests was used to determine the significance difference at 5 % probability level.

Results

Physicochemical characteristics of the test water

The physicochemical characteristics of the test water were temperature from 25 to 28 ºC, pH values fall between 7.4 to 7.8, while salinity ranged from 0.07 to 0.15 mg/L. The dissolved oxygen concentration ranged from 6.5 to 7.5 mg/L, conductivity values varied from 250 to 275µM/cm, while total hardness varied from 210 to 240 mg/L $CaCO_3$ during the experimental period.

Median lethal concentration and application factor

The mortality or any visible changes in behavior of the exposed specimens were not observed in the control group. In treated groups, the fish mortality increased with increase in the test concentrations of the chemical (Table 1) and the specimens swarm to surface more often than did the controls. The median lethal concentration (96-h LC_{50} value) of test chemical was estimated using acute toxicity bioassay as 500 mg/L for *Mystus vittatus*. A dose dependent increase and time dependent decrease were observed in mortality rate as the exposure time increased from 24 to 96 h; the median concentration was reduced.

Table 1. Mortality in *Mystus vittatus* (BLOCH**)** at 96 h due to Ammonium sulphate exposure.

Exposed concentration (mg/L)	**Number of Fish Exposed**	**Mortality (%)**
Control	**20**	**0**
100	20	10
200	20	30
300	20	50
400	20	65
500	20	75
700	20	85
800	20	100

Discussion

Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Sunderam et al. 1994). The results of the LC_{50} (median lethal concentration) of the present study at 96 h was 500 mg/L for Ammonium sulfate. The results showed that Ammonium sulfate was toxic to the fish at different concentrations and times of exposure. The test result of the 96 h LC_{50} of *Mystus vittatus* exposed to ammonium sulfate obtained in the present study was slightly higher than the 96 h LC_{50} value of 33.9 mg/L for $Ca(OH)_2$ estimated by Ufodike and Onusiriuka (2008) of composite fertilizers for African catfish (*Clarias gariepinus*). This is because that the environmental factor and metabolism mechanism may be different.

The results of acute testing demonstrate that nitrogenous fertilizers exhibit acute toxicity to rainbow trout. In another study, 96-h LC_{50} values of 28:0:0 and 10:34:0 for the rainbow trout were found to be 0.585 g/L and 1.342 g/L, respectively (Mac Kinlay and Buday 1997). Toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species (Abdul-Farah et al. 2004). Physiological parameters like quality, temperature, pH, dissolved oxygen and turbidity of water, amount and kind of aquatic vegetation, concentration and formulation of chemical and its exposure also greatly influence such studies (Gupta et al. 1981).

The difference might be related to fertilizer composition, fish and physicochemical characteristics of the test water (Saha et al. 2002; Palanivelu et al. 2005; Kushwaha et al. 2012). Fertilizer might positively or negatively affect the ecosystem quality to the benefit or detriment of live aquatic organisms including fish (Yaro et al. 2005). In the present study, all the deaths were only 10.0% after transferring 96 h toxicity test survivors to flow through tanks, which is similar with previous reports (Xu and Oldham 1997; Little et al. 2002). Changing the Behavioral as a result of stress is the indication of potential toxic effects. The various behavioral changes like restlessness, abnormal swimming behavior, and vigorous jerks of body, loss of balance, myotonia and anorexia observed in *Mystus vittatus* are similar to the observations of Chandra (2008) in fishes exposed to various pesticides.

Conclusion

The result of the present study showed that Ammonium sulphate was more toxic for *Mystus vittatus*. Acute toxicity studies have been documented as the very first step in determining the water quality requirements of fish and expose toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. However, chemical determination of any insistent toxicant concentration in water as well in sediment may not provide information on the severity of contamination, especially in the case of sublethal levels.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

The authors are thankful to the director of the National Bureau of Fish Genetic Resources (Lucknow, India) for providing support materials and the laboratory facility for conducting this study.

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