Short Communication

Response of Dipteran larvae exposed to acute doses of different heavy metals and its impact on aquatic environment.

ABSTRACT

Industrial effluents contain heavy metals, which are the main source of water pollution due to their bioaccumulation and magnification in different trophic level. The aim of this work to estimate LC_{50} of Pb, Cd and Hg in *Chironomus striatapennis* which is a primary consumer of aquatic ecosystem.Fourth instars were collected from breeding aquarium reared under laboratory conditions and exposed for 96 hours to different doses of Pb, Cd and Hg for static bioassay of LC_{50}. Ten fourth instar larvae were placed in 100 ml beaker with 50 ml of each test solution. Each concentration consists of five trials. A control was also maintained wherein organisms were exposed to distilled water. Larvae were not fed during the toxicity tests. All beakers were free from tube forming materials. Data were subjected to probit analysis. Result showed that sensitivity of larvae was Hg> Cd >Pb and Chi square for Heterogeneity were also found significant in all three metals. *C. striatapennis* showed noticeable response in LC_{50} study and sensitive to low doses of heavy metals. Several secondary consumers have preferred this larva as their food. So unplanned industrialization may increase the level of heavy metals in the aquatic ecosystem which will accumulate slowly but definitely in different trophic level and at the same time unusual death of these larvae may indirectly change the equilibrium of the aquatic ecosystem.

Key Words:LC_{50}, Mercury, Lead, Cadmium, *Chironomus.*

INTRODUCTION

Industrialization means a change in the technology to produce goods and service as well as an engine of growth in developing countries [1,2]. It is known that there exist darkness under the lamp, industrialisation also leaving behind serious environmental issues like water pollution[3]. Both fresh and marine water are polluting everyday by untreated or improperly treated industrial wastewater. Over 80% of the world's wastewater and over 95% in some least developed countries is released to the environment without treatment [4]. It is estimated that in India 13,500 million litres per day industrial wastewater is generated from urban cities and discharging nearby aquatic bodies with or without treating the waste water [5]. Industrial wastes from different industries, such as mining operations, metal plating, radiator manufacturing, tanneries, smelting and alloy industries, storage battery industries are the significant source of heavy metals [6]. Among the heavy metals, Cd, Pb and Hg, are considered as most hazardous water pollutants [7,8]. Due to their high solubility in water, heavy metals could be absorbed by living organisms once they enter the aquatic food chain [9]. Benthic primary consumer like chironomid larvae (Order Diptera, Family Chironomidae) are continuously exposed to such environment, and may contribute to the accumulation and bio transfer of these heavy metals to upper trophic level and considered as good biological indicator of aquatic environment degradation [10,11,12].*Chironomus striatapennis* was found highly sensitive when exposed to different doses of Arsenic salt [13]. LC_{50} is a statistical parameter which illustrates a complete picture of mortality in a population and also organism's tolerance to a particular xenobiotic [14]. The objective of the study is to determine the LC_{50} of Pb, Cd and Hg in *Chironomus striatapennis* to find how this macroorganism is responding to these heavy metals. This in turn provide information regarding the level of these metals in the industrial effluents which will not vulnerable for this primary consumer.

MATERIAL AND METHODS

Collection of Chironomid larvae

Fourth instar larvae of *Chironomous striatapennis* were collected from fresh water pond locating at Kanchrapara (22_56018.664800N, 88_28010.034400E) district North Twenty four Parganas, West Bengal, India and placed in aerated plastic bags and transported to the laboratory. Larvae of chironomid reared under laboratory conditions by using the breeding aquarium which was filled to a depth of approximately 20 cm with pond water and given fish flakes for food [15].This was the source of all test organisms. Atomic absorption spectrophotometry was done to confirm that larvae were not contaminated with the Lead (Pb), Mercury (Hg) and Cadmium(Cd).

Toxicity Test of Heavy metals

For contamination, stock of 1mg l^{-1} concentration was prepared initially with Cadmium acetate (SRL, 99% purity), Lead acetate (SRL, 99% purity) and Mercuric chloride (SRL, 98% purity) in double distilled water and kept for twenty four hours. Test solution of different concentrations were prepared from that stock, through a series of dilution. Initially a series of tests were conducted in concentrations ranged between 0.0005 mgl^{-1} and 1 mg l^{-1}, where test organisms been exposed for 96 hours. Finally for Cd, concentrations of 0.001mg l^{-1}(d1), 0.003mg l^{-1}(d2), 0.007mg l^{-1}(d3), 0.015 mg l^{-1}(d4), 0.03mg l^{-1}(d5) and 0.062 mg l^{-1}(d6); for Hg,0.0005mg l^{-1}(d1), 0.001mgl^{-1}(d2), 0.003mg l^{-1}(d3), 0.007 mgl^{-1}(d4), 0.015mg l^{-1}(d5) and 0.031 mgl^{-1}(d6), and for Pb,0.003mgl^{-1}(d1) 0.007mg l^{-1}(d2), 0.015mg l^{-1}(d3), 0.031 mgl^{-1}(d4), 0.062mg l^{-1}(d5) and 0.125 mg l^{-1}(d6) were considered for the experiment. Tenfourth instar larvae were placed in 100 ml beaker with 50 ml of each test solution. Each concentration consists of five trials. A control was also maintained wherein organisms were exposed to distilled water. Larvae were not fed during the toxicity test. All beakers were free from tube forming materials. The criterion for death is immobility and/or lack of reaction to mechanical stimulus. After 96 hours, recorded data were subjected to probit analyses[16] by using Probit Programme Version 1.5.

RESULTS

LC_{50} and LC_{90}values and 95% confidence limit for Hg, Cd and Pb in the fourth instar of *Chironomus striatapennis* are presented in Table 1. The result revealed that sensitivity of larvae was Hg>Cd>Pb. Chi-square for Heterogeneity were also found significant in all three metals in compare to tabulated value of Chi-square (7.815, P<0.05). Percentage of mortality of larvae exposed to three heavy metals is presented in Fig.1.

Table 1: LC_{50} Confidence Limit of *C. striatapennis*

	Mercury	**Cadmium**	**Lead**
Exposer Period	96 Hour	96 Hour	96 Hour
LC_{50} mg l^{-1}	0.001	0.003	0.007
LC_{90} mg l^{-1}	0.010	0.012	0.104
95% Confidence Limit for LC_{50}	**Lower Limit:** 0.000	**Lower Limit:** 0.000	**Lower Limit:** 0.000
	Upper Limit: 0.003	**Upper Limit:** 0.005	**Upper Limit:** 0.019

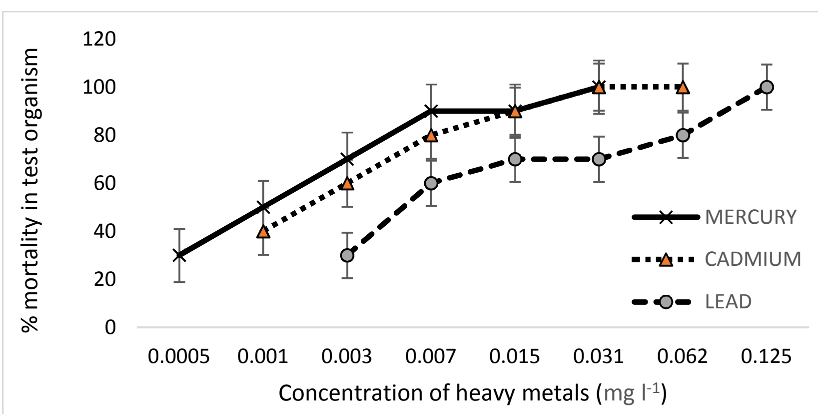

Fig. 1: Concentration of Hg, Cd, Pb and percentage mortality in *C striatapennis*

DISCUSSION

Mercury, a prevalent toxicant isavailable in the environment due to anthropogenic activity as well as natural sources. Present study revealed that Chironomids were more susceptible to Hg than other two heavy metals and observed LC_{50} exposed to Hg was 0.001 mgl^{-1}, which was same as human permissible limit (0.001mgl^{-1})of BIS[17]and less than acceptable limit in industrial effluent(0.01mgl^{-1}) [18], but in industrial effluent this metal may available in more high concentration[19].Mercury reduce the growth and decrease the locomotion activity which lead to increase the probability of mortality rate of the larvae [20]. Moreover, increase in Hg concentration decreases the survival rate of this larva as was observed in *Eriocheir sinensis*[21].

The study revealed that *C. striatapennis* was more susceptible to Cd than Pb and LC_{50} of these three heavy metals showed that lead is least toxic for this insect. Toxic effect of cadmium reduced the uptake of essential metals, specifically Calcium (Ca) ion channel due to their similarity of size and charge which can disrupt the normal physiological actions of Ca ion. Though *Chironomus* contains Metallothionein (MT), a metal binding protein (MBP), having high cysteine content with numerous thiol groups, which may help cellular tolerance to Cd through high affinity sequestration of the toxic metal by MT [22]. In spite of that, sever amount of cadmium may increase the lethality of this organism. Pb was found less toxic but it also have similar kind of effect to prevent or imitate the action of Ca ion of Calcium dependent or allied process [23]. Moreover, Pb accumulation by this larvae is higher than other heavy metal due to the presence of MBP which may cause bio- magnification of this heavy metal in the food chain[24].

Our study revealed that the concentration of these metals (LC_{50}) used in this experiment, though below the human permissible limit but not suitable for the survival of the larvae of this insect. The maximum acceptable limit for Pb and Cd in industrial effluents are 0.1 mg/l and 0.01 mg/l respectively [25]. LC_{50} was found less than such acceptable limit. Whereas, LC_{90} for this insect was recorded for Pb (0.10 mgl^{-1}) and Cd (0.012 mgl^{-1}) which were similar to the acceptable limit in the industrial effluents.

Chironomus have antioxidant defense system, contains antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase. Level of these enzymes decreased with the increase in stress situation like increasing concentration of heavy metals in aquatic environment and larvae died due to toxicity of the metals[26]. Chironomids are macrobenthos and are the primary consumer of the aquatic food chain[27]. Due to unscrupulous industrial development in the developing countries, there are increased in the industrial effluents containing heavy metals like Hg, Cd and Pb which ultimately reach the fresh water sources of those areas. The bioaccumulation of these metals in aquatic organisms is dangerous not only for their own survival and biology, but also for humans because of the possible passage of contaminant through the food chain [28].

Though there is no noticeable changes are found in higher vertebrates like fish in those aquatic ecosystem, but our LC_{50} results indicated that *C. striatapennis* was highly sensitive to low doses of heavy metals. Several secondary consumers consider chironomids as there food. So heavy metal pollution may indirectly distorting the equilibrium of the aquatic ecosystem. This study provides information for industries to release effluents after proper treatment so that level of these heavy metals should below the effective level. That is essential for the sustainable development and to stop the biodiversity loss of the ecosystem.

CONCLUSION

LC_{50} assay revealed that larvae of *Chironomus striatapennis* was more sensitive to Hg than Cd and Pb respectively. It was also observed that LC_{50} values were less than standard permissible limit of these heavy metals. As this larvae is preferred by different secondary consumers, so unplanned industrialization may increase the level of heavy metals in the aquatic ecosystem which will accumulate slowly but definitely in different trophic levels and at the same time unusual death of these larvae may indirectly change the equilibrium of the aquatic ecosystem.

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