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Behavioural toxicology of midge larvae *Chironomous sp.* after acute in situ exposure of different industrial effluents and drinking water

Debika Bhunia[1], Subhodeep Sarkar[2], Kushal Banerjee[3], Abantika Nandy[3], Soumendra Nath Talapatra[3,*]

[1]Department of Zoology, Maulana Azad College, Rafi Ahmed Kidwai Road, Kolkata – 700013, India

[2]Department of Environmental Science, Asutosh College, S. P. Mukherjee Road, Kolkata – 700026, India

[3]Department of Environmental Science, University of Calcutta, 51/1 & 2 Hazra Road, Kolkata – 700019, India
*Phone: 91-33-2461-5445

*E-mail address: ecologylive@yahoo.co.in

ABSTRACT

Behavioural activities in relation to toxicological aspects involve behavioural changes of aquatic organisms under the exposure of a contaminant. The present study aims to know behavioural activities of midge larvae *Chironomus sp.* at the in-situ acute exposure of different chemicals containing wastewater viz. lead-acid battery industrial effluent, mixed industrial effluent and fresh tap water (chlorinated) as drinking water in comparison to control (dechlorinated) water sample (aged tap water). The Chironomus larvae were kept in three different experimental chambers (perforated wall test vessels) with the exposure of different water samples. These samples were made with no dilution, 50 % dilution, 2.5 % dilution and control water sample. The behavioral activities for larvae of *Chironomus sp.* were measured at 0h, 2h, 24h and 48 h in in-situ condition. The behavioral activities viz. crawling, looping, ventilation, paralyses and subsequently death of the larvae were recorded in the field condition. A significant differences ($P < 0.05$, $P < 0.01$ and $P < 0.001$) were observed with increasing time of exposure while in few cases the data were increased without significance level. It was recorded that after exhibiting behavioural activities viz. crawling, looping, ventilation and paralyses finally all species were died 100 % of the population in lead acid battery effluent following both 24h and 48hr exposure. In addition, death of larvae were 70 % in mixed industrial effluent and 50 % in fresh tap water (chlorinated) after 48h exposure as compared to control sample water. In conclusion, the present results indicate that the larvae of *Chironomous sp.* are suitable indicators in the evaluation of the effluent quality in the studied stream, potential to know by behavioural toxicological study for heavy metals and organic pollution. Although it is a preliminary observation by assessing behavioural toxicology but future study in relation to biochemical and genetic damage of Chironomous larvae with the exposure of toxic water samples will provide bigger view.

***Keywords*:** Behavioural toxicology; Water pollution; Midge larvae; *Chironomous sp.*; Industrial effluent

1. INTRODUCTION

Behavioral toxicology study of animals in relation to toxicity by different pollutants has already been established (Warner, 1967, Beitinger, 1990; Little, 1990; ASTM 2000; Dell'Omo, 2002). Behavioural analysis involves the monitoring of aquatic organisms behaviour to indicate exposure to a contaminant (Solnik, 2012).

Chironomids, 'non-biting midges' (Diptera: Chironomidae) are the most widely distributed and frequently the most abundant insect in freshwater (Halpern et al., 2001). Midges are holometabolous, that is they go though complete metamorphosis consisting of egg, larva, pupa, and adult stages. *C. riparius* mates in aerial swarms. After mating, the female deposits the eggs on the water surface. Larvae undergo four instars; the first (L1) is planktonic and the second to fourth larval stages (L2, L3, L4) are in direct contact with sediment. Larvae L2 to L4 are collector gatherers, feeding mainly on detritus and its associated bacteria and fungi. This species represents an important food source for fish, making it a useful species for documenting the bioaccumulation (Armitage et al., 1995).

According to OECD (2004), *Chironomus riparius*, is found in sediment and has established for toxicity testing. Chironomids are recognized as useful tools in studies of sediment toxicity (Ankley et al. 1994). Chironomids species can tolerate and develop in polluted waters such as waste stabilization ponds where they become a dominant macroinvertebrate (Broza et al., 2000). In heavily metal polluted streams, they were found to compose up to 80 % of the total fauna, whereas they constituted less than 10 % in an unpolluted section of the same stream (Winner et al., 1980). In an aquatic environment, most anthopogenic chemicals eventually accumulate in the sediment (Ingersoll 1995). Sediment provides a habitat for many aquatic organisms, such as the larval stages of midges, which are exposed to sediment-associated chemicals both directly and via food intake. These chemicals may be directly toxic to benthic animals or bioaccumulate in the food chain.

Behavioral responses in toxicological study by individual or combinations of chemical(s) stress are among the first and most sensitive parameters (Walshe,1951; Warner, 1967), which integrate both biochemical alterations at the sub-organism level and ecologically relevant changes at the whole-organism level (Beitinger, 1990; Scherer, 1992). It was reported that exposure of heavy metal and/or organic compounds polluted water chironomus larvae have showed various behavior such as locomotion (crawling/swimming and looping), respiration (ventilation) movements (Brackenbury 2000; Pereira, 2010; Solnik, 2012).

Many studies have already been carried out on determination of sediment toxicity and heavy metal exposure by using *Chironomus sp*. globally (Warner, 1967; Winner et al., 1980; Beitinger, 1990; Giesy et al., 1990; Scherer, 1992; Hoke et al. 1993; Pellinen and Soimasuo 1993; Ankley et al. 1994; Harkey et al. 1994; Armitage et al., 1995; Ingersoll 1995; Brown et al. 1996; Harrahy and Clements 1997; Bleeker et al. 1998; Halpern et al.2001; Stuijfzand et al. 1998; Broza et al., 2000; Pereira, 2010; Solnik, 2012) as well as in India (Majumder and Gupta 2010; Midya et al., 2013) but no one has attempted in relation to in-situ exposure of industrial effluents and drinking water on behavioural activities as behavioural toxicology study in Indian context.

The present study aims to detect behavioral activities of *Chironomus sp.* at the in-situ acute exposure of different chemicals containing wastewater such as mixed industrial effluent, lead-acid battery industrial effluent as well as fresh tap water as drinking water (Photo 1 & 2).

Photo 1. Grew character (*Chironomus sp*.).

Photo 2. Larval character (*Chironomus sp*.).

2. MATERIALS AND METHODS

The wastewater sampling site were selected at Nature Park (latitude = 22°30′ N and longitude = 88°15′ E) for mixed industrial effluent, Sonarpur (latitude = 22° 26′ N and longitude = 88° 25′ E) for Pb acid battery effluent and Maheshtala (latitude = 22° 30′ N and longitude = 88° 15′ E) for fresh tap water (chlorinated) as drinking water sample and aged tap water (dechlorinated) as control sample, all the sites are about 8m above the sea level. All the experimental samples were compared with control sample dechlorinated water (aged tap water). The present experiment was conducted on an in-situ habitat of *Chironomus sp.* and these species were found near the drainage system, where the kitchen wastewater (grey water) is mixed.

The Chironomus larvae were kept them in three different experimental chambers (perforated wall test vessels) with the exposure of fresh tap water (chlorinated), mixed industrial effluent and lead-acid battery industrial effluent and one chamber for control sample water. These samples were used as with no dilution, 50 % dilution, 2.5 % dilution and control water sample was used without dilution.

The behavioral activities for larvae of *Chironomus sp.* were measured at 0h, 2h, 24h and 48 h in in-situ condition. The larvae were randomly selected from just beside habitat. Each study was done on 20 larvae kept in test chamber filled with 100 ml sample water and a few detritus particle from their stream. The behavioral activities viz. crawling, looping, ventilation movements of the larvae were recorded for 0h, 2h, 24h, 48h respectively in the field condition. The set up was done in two replicas.

The identification of the larvae was done by the help of book, internet and visual identification. Behavioral study after exposure to toxicants was done by visual observation, image and video capture. All the activities of the larvae during the exposure of above mentioned wastewater were recorded and tabulated in the data table.

All the mean values of data were analyzed to determine statistically significant differences between experimental and control groups by using Student's 't' test at 0.05 level of significance.

3. RESULTS

In the present study, the average percentage values of behavioral responses of *Chironomous sp.* were observed in different industrial effluents as well as fresh tap water (drinking water) compared to control sample as aged (dechlorinated) tap water. The behavioral activities for the larvae of *Chironomus sp.* were measured at 0h, 2h, 24h and 48h in in-situ condition. The behavioral activities viz. crawling, looping, ventilation of the larvae were recorded in the field condition. A significant differences ($P < 0.05$, $P < 0.01$ and $P < 0.001$) were observed (Table 1).

In case of crawling behavior, it was observed in 0h and 2h, a significant differences ($P < 0.01$) in the exposure of lead-acid battery industrial effluent (0.90 ±0.9, 0.90 ±0.9) but all species were died in 24h and 48h exposure and fresh tap water (chlorinated) as drinking water 0h and 2h (0.70 ±0.5 and 0.70 ±0.5) also showed significant ($P < 0.01$) increased values while in 24h exposure, the data (0.80 ±0.8) was higher at significance level of $P < 0.05$ but the increased data (0.40 ±0.4) for 48h was not showed significant differences while the value (0.80 ±0.8) was higher with significant ($P < 0.05$) differences only in 24h exposure for mixed industrial effluent, other values for 0h, 2h (0.30 ±0.5, 0.30 ±0.5) were not changed and

48h (0.40 ±0.4) was not significantly increased when compared to control sample as aged (decholorinated) tap water (0.30 ±0.5, 0.30 ±0.5, 0.40 ±0.4, 0.40 ±0.4) for 0h, 2h, 24h and 48h exposure.

Table 1. Behavioural activities in *Chironomous sp.* exposed to different contaminants containing water samples compared to control sample
(*P < 0.001; **P < 0.01; ***P < 0.05).

Sl. No.	Contaminant	Dilutions (%)	Behavioural responses (%)													
			0h			2h			24h				48h			
			Crawling	Looping	Others	Crawling	Looping	Others	Crawling	Looping	Others	Died	Crawling	Looping	Others	Died
1.	Control	0	0.30 ± 0.5	0.10 ± 0.3	0.30 ± 0.5	0.30 ± 0.5	0.20 ± 0.4	0.20 ± 0.4	0.40 ± 0.4	0.20 ± 0.4	0.30 ± 0.5	0	0.40 ± 0.4	0.10 ± 0.3	0.40 ± 0.4	0
2.	Drinking water	0	0.70 ± 0.5**	0.20 ± 0.4	0.20 ± 0.4	0.70 ± 0.5**	0.30 ± 0.5	0.20 ± 0.4	0.80 ± 0.8***	0.30 ± 0.5	0.20 ± 0.4	0	0.40 ± 0.5	0.20 ± 0.4	0.30 ± 0.5	50
3.	Mixed industrial effluent	50	0.30 ± 0.5	0.70 ± 0.5*	0.10 ± 0.3	0.40 ± 0.4	0.80 ± 0.8*	0.30 ± 0.5	0.80 ± 0.8***	0.30 ± 0.5	0.40 ± 0.5	0	0.40 ± 0.4	0.20 ± 0.4	0.30 ± 0.5	70
4.	Lead acid battery effluent	2.5	0.90 ± 0.9**	0.30 ± 0.5	0.20 ± 0.4	0.90 ± 0.9**	0.60 ± 0.5**	0.40 ± 0.5	-	-	-	100	-	-	-	100

In case of looping behavior, it was observed in 2h exposure the data (0.60 ±0.5) with a significant differences (P < 0.01) for lead-acid battery industrial effluent while in 0h exposure the value (0.30 ±0.5) was increased without significant difference but all species were died in 24h and 48h exposure and in fresh tap water (chlorinated) as drinking water the values (0.20 ±0.4, 0.30 ±0.5, 0.30 ±0.5, 0.20 ±0.4) were increased without any significant differences while in 0h and 2h exposure for mixed industrial effluent the values (0.70 ±0.5 and 0.80 ±0.8) were significantly higher (P < 0.01 and P < 0.05) but for 24h and 48h the values (0.30 ±0.5, 0.20 ±0.4) were increased without any significant differences when compared to control

sample as aged (decholorinated) tap water (0.10 ±0.3, 0.20 ±0.4, 0.20 ±0.4, 0.10 ±0.3) for 0h, 2h, 24h and 48h exposure.

In case of other behavior like ventilation and finally paralyses, it was observed for lead-acid battery industrial effluent, the data (0.40 ±0.5) was increased without significant level for 2h exposure while decreased value (0.20 ±0.4) was found in 0h exposure but all species were died in 24h and 48h exposure. The data (0.20 ±0.4, 0.20 ±0.4 and 0.30 ±0.5) were decreased 0h, 24h and 48h while increased in 2h exposure without significant differences for fresh tap water (chlorinated) as drinking water sample while the values (0.10 ±0.3 and 0.30 ±0.5) were decreased in 0h and 48h but values (0.30 ±0.5 and 0.40 ±0.5) were increased without any significant differences for mixed industrial effluent when compared to control sample as aged (decholorinated) tap water (0.30 ±0.5, 0.20 ±0.4, 0.30 ±0.5, 0.40 ±0.4) for 0h, 2h, 24h and 48h exposure.

4. DISCUSSION AND CONCLUSION

The present study was documented on behavioral toxicology of midge larvae *Chironomous sp.* exposed to different industrial effluents as well as fresh tap water used as drinking water compared to control (dechlorinated) sample aged tap water. Our work indicate that there were significantly ($P < 0.05$, 0.01 and 0.001) variable changes of behavioral activities after in-situ toxicants exposure. Sardo and Soares (2010) have identified locomotion as an early warning parameter as chonic toxicity in aquatic oligochaete, *Lumbriculus variegatus*. The same pattern of inhibition of growth of *Chironomus spp.* has already been observed following exposure to a number of other environmental contaminants and stressors, including uranium (Muscatello and Liber, 2009), mercury (Chibunda, 2009; Azevedo-Pereira and Soares, 2010), and reduced food availability (Liber et al., 1996).

It was already reported in behavioural toxicology study of how drugs, chemicals, or other environmental contaminants (generally referred to as “toxicants”) change the way in which organisms behave (Beitinger, 1990; Vuori, 1994; Gerhardt, 1995; Solnik, 2012). The recent research work is based on the behavioral activities viz. crawling, looping, ventilation and paralyses of the larvae at different time interval, 0h, 2h, 24h, 48h respectively in the field condition exposed to different industrial effluents and drinking water. Similar results were observed in in-situ condition by Gerhardt, 1995, Gerhardt and de Bisthoven, 1995 that populations of *Chironomus* gr. *thummi* larvae from two differently polluted lowland streams (Dommei, high cadmium and zinc; Ijse, medium copper and organic xenobiotics) for behavioural and morphological responses to pollution. Behaviours such as locomotion (swimming and looping), respiration movements (ventilation) and inactivity were quantified with impedance conversion technique. Chironomids from the Dommel were more active than larvae from Ijse. In Ijse, deformed larvae showed less emergence, less locomotion and more ventilation than non-deformed larvae. In Dommel, deformed and normal larvae were equally fit (behaviour, emergence). According to Ding et al (2000), they have focused in their similar study on the effects of run-off contaminants on non-target species, observed thinner and elongated bodies accompanied with inhibition of swimming, crawling, body reversal, and eventually full paralyses in aquatic oligochate *Lumbriculus variegatus* under ivermectin concentrations of 0.3 to 300nM. The same study was identified behavioural endpoints as being much more sensitive than other endpoints such as survival.

The LC_{50} at 72h was 560 nM, while their IC_{50}s at 3h for swimming, reversal, crawling speed and frequency were 1.1, 16, 51, and 91 nM respectively. But they have not studied on *Chironomus spp.* exposed to drinking water sample while drinking water after post cholorination and freshly supplied tap water may contain some chemicals that led to abnormal behavioural activities. In the present study, which is supported by other studies have also observed immobility and reduced locomotion behaviour in *L. variegates* by Solnik (2012). It was established that chlorine and its compounds are largely used for the production of pathogen-free drinking water. World Health Organization Drinking Water Guidelines (Health canda, 1993) has already been provided an appropriate context for the subject matter that disinfection is unquestionably the most important step in the treatment of water for public supply for the destruction of microbiological pathogens, an essential phenomenon and invariably involved the use of chlorine that already established powerful biocides. Also it is capable of reacting with other water constituents to form new compounds with potentially long-term health impacts.

The present results (Table 1) were clearly indicated different behavioural activities such as crawling, looping ventilation, paralysis and eventually death. The crawling behavior as locomotion was observed only at 0h and 2h with a significant differences ($P < 0.01$) in the exposure of lead-acid battery industrial effluent but 100 % of the population were died before paralyses at 24h and 48h exposure. For fresh tap water (chlorinated) sample as drinking water, it was also observed significantly increased rate of behavioural activities at 0h, 2h ($P < 0.01$) and 24h ($P < 0.05$) exposure but 48h showed no significant differences because of paralyses followed by death of 50 % population. For mixed industrial effluent, the value was higher with significant ($P < 0.05$) differences only at 24h exposure while the data for 0h and 2h exposure were unchanged and 48h exposure showed increasing activities without significant differences and it was found paralyses followed by death of 70 % population. All the differences were compared to control sample as aged (decholorinated) tap water at 0h, 2h, 24h and 48h exposure and no paralyses followed by death were observed in the test species.

In case of other locomotion behavior as looping behavior, it was observed for lead-acid battery industrial effluent a significant differences ($P < 0.01$) at 2h exposure while 0h exposure the value was increased without significant difference and, 100 % of the population were died before paralyses in 24h and 48h exposure and in fresh tap water (chlorinated) as drinking water the values were increased without any significant differences because of paralyses followed by death of 50 % population while at 0h and 2h exposure for mixed industrial effluent the values were significantly higher ($P < 0.01$ and $P < 0.05$) while at 24h and 48h the values increased without any significant differences and paralyses followed by death of 70 % population. All the differences were compared to control sample as aged (decholorinated) tap water at 0h, 2h, 24h and 48h exposure and no paralyses followed by death were observed in the test species.

In case of other behavior like ventilation and subsequently paralyses, the data show increased activities for lead-acid battery industrial effluent, without significant level for 2h exposure while decreased value was found at 0h exposure while all species were died in 24h and 48h exposure. The values were decreased 0h, 24h and 48h while increased at 2h exposure without significant differences and 50 % population were died for fresh tap water (chlorinated) as drinking water sample while the values were decreased at 0h and 48h but values were increased without any significant differences for mixed industrial effluent where 50 % population were died. All the differences were compared to control sample as aged (decholorinated) tap water for 0h, 2h, 24h and 48h exposure and no paralyses followed by death were observed in the test species.

The present behavioural activities by toxicants in effluent water and drinking water are evidenced with previous research works (Bat and Raffaelli, 1998; Brackenbury 2000; Chibunda, 2009; Azevedo-Pereira and Soares, 2010; Pereira, 2010; Majumdar and Gupta, 2012; Solnik, 2012; Midya et al., 2013). It was reported that exposure of heavy metal and/or organic compounds polluted water Chironomus larvae showed various behavior such as locomotion (crawling/swimming and looping), respiration (ventilation) movements and eventually paralyses. As reported earlier that the behaviour of Chironomus varied between the different treatments. The avoidance of sediment increased with increasing Zn, Cu and Pb concentrations and were significantly different ($P < 0.05$) from the control, indicating that the contaminants had a sublethal effect. Even on the first day, concentrations of 30 μg Cu g^{-1}, 15 μg Zn g^{-1} and 20 μg Pb g^{-1} induced marked changes in burrowing behaviour. The avoidance by Chironomus of contaminated sediment may reduce exposure to metal in the sediment, but, once emerged, individuals become vulnerable to predation by birds, fish and epibenthic crustaceans. This makes the ability of surviving animals to rebury in clean sediments, which are ecologically relevant sublethal test end point.

It was observed from the present results that lead acid battery effluent is more toxic followed by mixed industrial effluent and fresh tap water (chlorinated) used as drinking water compared with control (dechlorinated) water sample (aged tap water). The toxicity by heavy metals and organic compounds in the sample water enhances the behavioural activities in the midge larvae, *Chironomous sp.* All the samples were continuously mixed with grey water, where the species has inhabited and the grey water from kitchen having potent organic compounds (Leal et al., 2012). The toxicity may increase with the grey water because there was a continuous flow of kitchen wastewater within the perforated test vessels and existing test species were showed more behavioural activities. In support Chironomidae larvae are tolerant indicator species as observed by Bat and Akbulut, 2001 and they exhibit potent detoxification mechanism (Wiseman et al., 2013).

In conclusion, the data indicate that the larvae of *Chironomous sp.* are suitable indicator in monitoring the effluent quality in the studied stream, therefore confirming the potential of this taxonomic group as indicator of heavy metals and organic pollution (Solnik, 2012; Midya et al., 2013; Wiseman et al., 2013). This is a preliminary observation to know toxicity of industrial effluents as well as drinking water when exposed to midge larvae by assessing behavioural toxicology. Further study might be relevant in relation to biochemical and genetic damage with the exposure of different chemical containing effluents and drinking water.

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