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Relative tolerance of some tropical freshwater microcrustaceans to acidification

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Abstract: The present work reports on the effects of low pH values on the mortality of 3 freshwater zooplankton available in the freshwater ponds of Midnapore district. Acute bioassay tests on *Daphnia lumholtzi*, *Moina brachiata*, and *Heliodiaptomus viduus* to varying acidic pH levels of water were performed in the laboratory using serial grades of hydrochloric acid solution. Among the 3 genera, *M. brachiata* was found to respond sharply to acid stress in a 1-h assay, since 100% mortality was achieved at pH 5.79 (2.347 mM HCl), as compared to *H. viduus*, which showed 100% death in 1 h only below pH 4.9 (2.978 mM HCl). In fact, it appears that *H. viduus* is more tolerant to short-term acidification than *M. brachiata* after a 1-h exposure. This study determined that adult *M. brachiata* had the lowest Lc_{50} values and *H. viduus* had the highest Lc_{50} values at both 1 h and 24 h. The survival pattern of *D. lumholtzi* closely resembled that of *H. viduus* rather than *M. brachiata*. Higher sensitivity of *M. brachiata*, even to the slightest alteration of pH as revealed by chi-square tests (significant differences with others), has prompted us to consider this species as a sensitive indicator of aquatic pollution, particularly acidification.

Key words: Freshwater, acidification, zooplankton, pH, bioassay

1. Introduction

The variability of freshwater aquatic systems is very pronounced in a tropical country such as India. This delicate ecosystem is highly threatened by multifarious factors such as habitat alteration, dumping of wastes leading to eutrophication, and loading of industrial effluents (Payne, 1986). The impact of most of these factors has been seen in the lowering of pH values, dissolved oxygen (DO) levels, and species diversity, and also in the increasing of biochemical oxygen demand (BOD), chemical oxygen demand, total dissolved solids, microbial load, etc. (Giri et al., 2008). The acidification of an aquatic system has a profound negative effect on the fish population and leads to a loss of biodiversity in the planktonic and benthic fauna (Wærvågen and Nilssen, 2003). Surveys have demonstrated that some zooplankton species are sensitive to acidity, while others are more tolerant (Havens, 1993). Lakes having a higher (alkaline) pH are found to be richer in zooplankton species. It was observed that 2–3 times the number of genera are found in waters with a pH greater than 5 as compared to those with a more acidic pH (Havens, 1993). Oscillations of pH alongside oxygen levels occur in small bodies of water (such as ponds) due to the respiration of aquatic flora and fauna (Hofmann, 1977). The natural cause of low pH levels in freshwater bodies is the accumulation of rain (pH ≤ 5.6). Besides this, the acidification of freshwater in an area is dependent on the quantity of calcium carbonate (limestone) in the soil (Wetzel, 1992). Much of the damage to aquatic life in sensitive areas with little buffering capacity is a result of a sudden runoff of large amounts of highly acidic water and certain ions into lakes, or unusually heavy rains. pH may also alter the toxicity of other substances to organisms, especially by releasing heavy metals (Park et al., 2009). Since the soil of the Midnapore district of the state of West Bengal is largely lateritic, the freshwater sources tend to naturally be slightly acidic.

The most economical and effective method of gauging chemical toxicity is to perform a sensitivity test that uses aquatic organisms as indicators of toxicity (Slooff et al., 1983). Microcrustacean plankton are often critical components of ecosystems due to their trophodynamic role (Fischer, 2001). They are sensitive to most toxic material, which is exhibited through their altered abundance, arrested growth and biological activity, the disappearance of some species altogether, and a change in population number (Sprules, 1975). Cladocera and Copepoda represent common zooplanktonic taxa in any tropical freshwater ecosystem and form the lower trophic level in the intricate aquatic food web. They display

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early reproduction, rapid development, high population growth rates, and a higher tendency to produce resting eggs. Different zooplankton showed different habitat preferences through different seasons and years (Halder et al., 2008). Major cladoceran species belonging to the genus *Moina* usually exhibit a preference for semiperennial or temporary bodies of water, or become especially abundant, which provides them with suitable conditions for only a brief period.

Effects of innumerable abiotic and biotic factors on *Moina* spp. have been studied by authors like Rojas et al. (2000), Deng and Xie (2003), Martínez-Jerónimo et al. (2007), and Golder et al. (2007).

Earlier, ecotoxicological studies were executed upon some freshwater micrometazoans like *Moina brachiata* using the single pesticide DDT (Sharma and Dattagupta, 1983). Acute and chronic effects of several chemical substances (Slooff et al., 1983) and toxic organic substances like lindane, methoprene, pyrene, bleach effluents, dinoseb, and ivermectin on the biology of an individual genus or freshwater zooplankton community have been assessed by several authors (Peithera et al., 1996; Chu et al., 1997; Nikkilä et al., 1999; Pintar et al., 2004; Chèvre et al., 2005; Garric et al., 2007). The toxicity of heavy metals such as chromium, copper, nickel, and zinc on the survival of *Moina macrocopa* was studied by Wong (1992). The exposure time-dependent cadmium toxicity on the same cladoceran species was assessed by Gama-Flores et al. (2007). Similarly, Wang et al. (2008) studied the acute and chronic toxicity of cadmium on saltwater *Moina mongolica* Daday. Heinlaan et al. (2008) assessed the toxicity of ZnO, CuO, and TiO_2 on the crustaceans *Daphnia* sp. and *Thamnocephalus* sp. Acute toxicities of boron, titanium dioxide and aluminum nanoparticles on *Daphnia magna* were observed by Strigul et al. (2009). Zhu et al. (2010) explained the mode of toxicity and bioaccumulation of nanoparticle aggregates of TiO_2 in *Daphnia magna*, whereas Bodar et al. (1989) assessed the toxicity of heavy metals in the early life stages of *Daphnia magna*.

Numerous studies have documented the responses of zooplankton species to a variety of environmental variables, including pH (O'Brien and deNoyelles, 1972; Kring and O'Brien, 1976; Locke, 1992). Cladocerans have been considered to be vital indicators of lake acidification for a long time (Krause-Dellin and Steinberg, 1986). Effects of lake acidification on crustacean zooplankton populations and community structure were observed by Schartau et al. (2001), Walseng et al. (2008), and Havens et al. (1993). The latter of these ranked acid sensitivities of 6 common crustacean zooplankton taxa, determined from a multilake field survey and from laboratory bioassays. An experiment conducted by Fischer et al. (2001) highlighted the cumulative effects of both sensitivity and species interactions on compensatory dynamics in zooplankton responses to acidification.

Out of the many categories of freshwater bodies, small ponds are unpredictable habitats with large temporal and spatial variability in abiotic factors (Koivisto, 1995). The present research has attempted to blend field-based information with experimental results to record the tolerance of microcrustacean zooplankton to changing acidic conditions with an aim to devise tools for the ecological monitoring of freshwater ecosystems.

2. Materials and methods

2.1. Method of collection

The zooplankton were collected during the early morning with a standard ring Nylobolt net (mouth diameter of 30.48 cm, 52-µm mesh size) from the subsurface water area.

2.2. Selection of test organisms

Organisms were studied under a Zeiss (1000 1098) microscope and identified following the standard literature sources (Battish, 1992; Michael and Sharma, 1988). Initially, 3 different species of microcrustaceans commonly found in considerable abundance from particular freshwater ponds of the Midnapore (West) district of West Bengal were selected. *Moina brachiata* (Jurine, 1820) (eudominant) and *Heliodiaptomus viduus* (Gurney, 1916) were collected from a pond in the village of Khoirulachak (Site 1) located about 3 km away from the town of Midnapore (22.25°N, 87.65°E). *Daphnia lumholtzi* (Sars, 1885) (eudominant) with *Heliodiaptomus viduus* (dominant) were collected from a pond in the village of Gangadaschak (Site 2; 22.27°N, 87.55°E) within the Pingla block of the Midnapore (West) district. The entire experiment was focused on the sensitivity of crustacean species and was conducted during the period of January–April 2010, which corresponded more or less with the population peaks of the selected zooplankton (Figure 1).

2.3. Test medium

Subsurface pond water was collected from each pond, and physicochemical parameters were recorded using a water quality checker (TOWA 22 A, Japan) and by following standard methods (APHA, 2005). To carry out this bioassay, pond water itself was used as the test medium for the population collected naturally.

2.4. Test chemicals

Hydrochloric acid (E. Merck, India) was used in this bioassay experiment as a common agent that controls the pH of the test medium and renders a controlled acidic environment in the laboratory. Alternatively, pure NaCl (E. Merck) of equivalent molarity was used to conduct parallel bioassay experiments.

2.5. Standardization of doses

Deionized and distilled water was used to produce diluted solutions from concentrated HCl. The concentrations

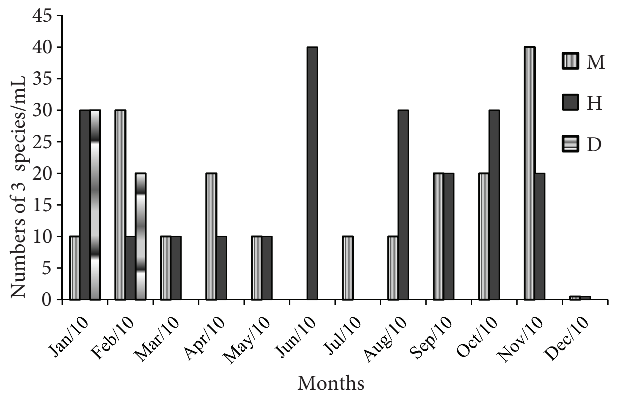

Figure 1. Annual occurrence of 3 species (numbers per milliliter) in freshwater wetlands of Midnapore during 2010 (**M** = *Moina* sp., **H** = *Heliodiaptomus* sp., and **D** = *Daphnia* sp.).

that were chosen for the experiment were based on pilot tests of acute toxicity that ranged from 0.12 M to 0.35 M HCl. Studies were pursued with a freshly prepared stock solution of 0.235 M HCl. The working solution (1.073–3.189 mM) responsible for the required pH variations was prepared by mixing 10 gradual amounts of HCl stock solution into each 10 mL of test water. Water parameters were also measured for both the control and test water at 1 h and 24 h after adding the working concentrations of acid.

2.6. Design of experimental protocol

Before each experimental setup, the individuals of a particular population were acclimatized in glass aquaria. The healthy and less-stressed test species were then screened for further bioassay. Gravid females were not selected for this experiment in order to avoid confusion in animal counting.

The experiments were maintained at 25 ± 1 °C and conducted in small beakers containing 10 mL of pond water. The design of the experiment (summarized in Table 1) included 3 replicates for 10 sets of trials and a control, each containing 10 fully active microcrustaceans, exposed to HCl test concentrations corresponding to different pH values (Figure 2). Not depicted in Table 1 is the alternative bioassay with the same ranges of NaCl to verify if the Cl^- influenced the mortality of the specimen. Static acute bioassay was performed to assess the relative response of the 3 test species by observing their rate of mortality for short durations, i.e. just after 1 h and later at 24 h, in each case. For this, nonresponsive individuals without heart beats were considered dead after examination under the microscope. The number of immobile, moribund, or dead specimens were recorded manually for 1-h and 24-h acute tests and analyzed. LC_{50} values were assessed by fitting the dose–response curves following the logistic model using SPSS 17. The chi-square test was used to compute the significant differences in the responses of organisms in each case.

3. Results

All 3 selected species (Figure 1) showed peak population together during January and February 2010. The physicochemical parameters of freshly collected filtered pond water during the specified period were recorded as in Table 2. Comparing the parameters of the 2 study sites, temperature and pH were found to differ least whereas turbidity differed the most.

Upon the addition of specified HCl working solutions to the test trials, certain changes were noted in other physicochemical parameters of the natural water sample associated with descending pH and are represented in Figure 2. Conductivity and total dissolved solids (TDS) were found to be inversely proportional to pH and HCl concentrations. The H_3O^+ so formed in test media after being spiked with HCl was responsible for the changes in pH (Table 1). Alternatively, spiking with equivalent strengths of NaCl (1.073–3.189 mM and control) in another set of experiments with the same microcrustaceans neither brought about any change in the pH nor resulted in mortality at all (and therefore is not depicted in the graphs). At the same time, higher death was found in

Table 1. Summary of experimental setup for static acute bioassay ($_n$ = neonate, $_a$ = adult).

Experiment	Range of HCl concentration	H_3O^+ measured in test water	Obtained pH	No. of replicates for each concentration	Species tested
10 grades with HCl	1.073–3.189 mM	2.82×10^{-7} to 7.94×10^{-6}	6.55–5.1	3	D
				3	M_n, M_a
				3	H
1 control	No HCl added	1.58×10^{-8}	7.08	3	D
				3	M_n, M_a
				3	H

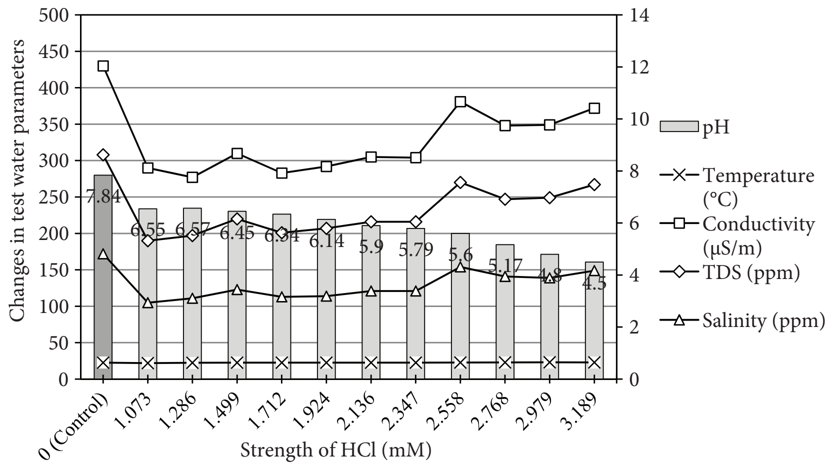

Figure 2. Gradual changes in physicochemical parameters of pond water with decreasing pH upon addition of HCl.

bioassays performed with the same concentrations of HCl but variable media, viz. tap water, distilled water, and pond water.

Variability in mortality rate among replicate samples of the same species was low and no significant difference in survivorship was found in controls. Toxic concentrations of H_3O^+ for organisms at 24 h were mostly lower than those at 1 h (Figure 3–5). The relative survival patterns of selected species to the lowering of pH at 1 h and 24 h are shown in Figures 4 and 5, respectively. Table 3 indicates the lowest LC_{50} value for adult *M. brachiata* and the highest LC_{50} value for *H. viduus* at both the 1-h and 24-h bioassay. Therefore, it is suggested that *M. brachiata* is most sensitive to acidification whereas *H. viduus* is the most tolerant species. Neonatal *M. brachiata* seemed to be less sensitive to acidification than adult *M. brachiata* (Table 1) in both 1-h and 24-h acute tests (Figure 3). pH < 5.9 was found to be lethal for *M. brachiata* (Table 3); pH < 5.17 and pH < 4.9 were lethal for *D. lumholtzi* and *H. viduus*, respectively.

Chi-square test results from the comparison of sensitivities of the 3 test organisms at both 1-h and 24-h assay (Table 4) showed no significant differences between *H. viduus* and *D. lumholtzi* (χ^2 = 2.072 and χ^2 = 1.606 at df 9, respectively*)*. The chi-square test at 1 h indicates that significant differences exist between the responses of *M. brachiata* and *H. viduus* (9.502) and between the responses of *M. brachiata* and *D. lumholtzi* (8.752). Similar highly significant chi-square results were obtained for *M. brachiata* with each of the remaining species at 24-h assay (9.62 and 7.741, respectively). Optimum and lethal values of pH for *M. brachiata* exposed to short-term acid contamination (Table 4) illustrated the narrowest range among the 3 test microcrustaceans.

4. Discussion

Predicting the effects of environmental change on ecosystems is a major challenge in ecology. In accordance with Krause-Dellin and Steinberg (1986), the present

Table 2. Physicochemical parameters of collected sample water during January–April 2010.

Water parameters	Site 1 (Mean ± SE)	Site 2 (Mean ± SE)
Temperature (°C)	24.20 ± 3.50	25.98 ± 2.44
pH	7.08 ± 0.28	6.71 ± 0.75
DO (mg/L)	4.87 ± 0.91	5.25 ± 1.19
BOD (mg/L)	1.55 ± 1.63	1.26 ± 1.01
Turbidity (NTU)	52.00 ± 26.91	8.33 ± 5.32
TDS (ppm)	250.80 ± 76.88	277.67 ± 110.51
Conductivity (μS/m)	354.40 ± 113.05	391.5 ± 155.22
Salinity (ppt)	1.41 ± 0.45	1.57 ± 0.61

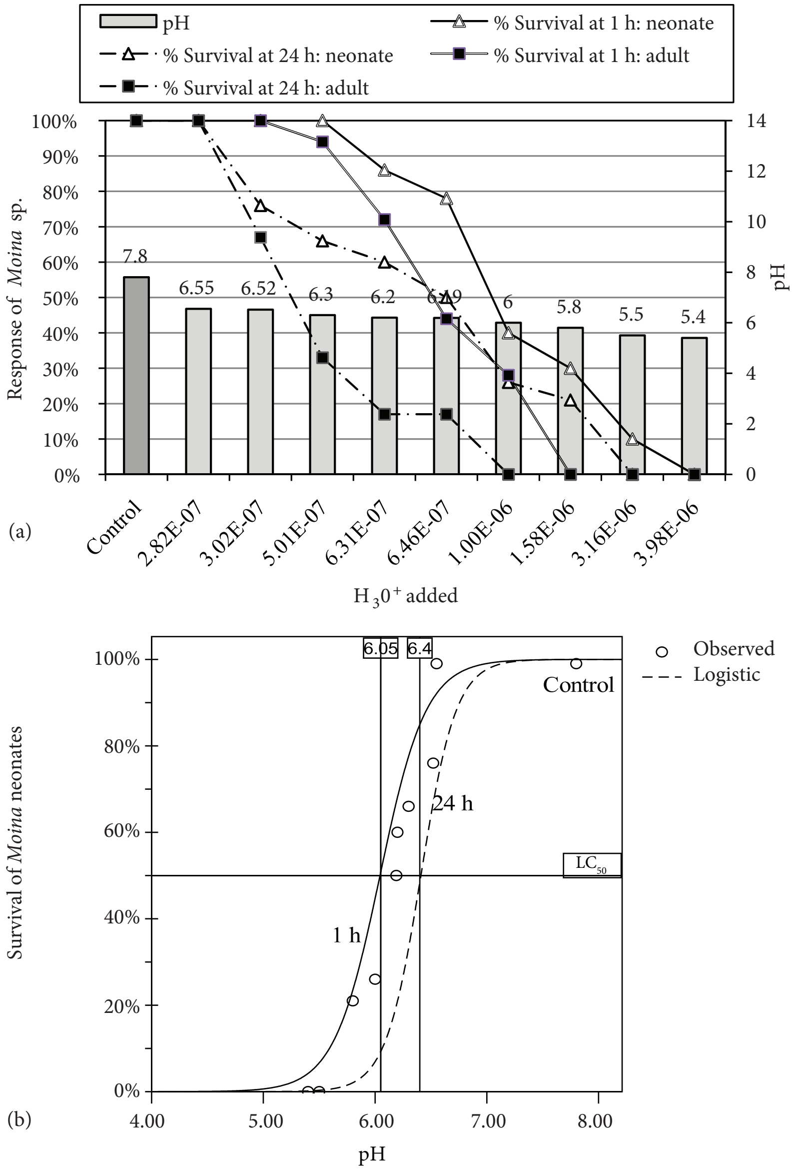

Figure 3. (a) Relative survival in neonate and adult *M. brachiata* exposed to H_3O^+ additions after 1 h and 24 h; (b) dose–response curve of neonate M. *brachiata* against pH at 1 h and 24 h.

study also employed a calanoid copepod in addition to cladocerans as indicators of acidification. *Moina brachiata*, *Daphnia lumholtzi*, and *Heliodiaptomus viduus* were specifically selected for acute bioassays because they were dominant in their respective habitats and could ensure sufficient population size for the present study based on field-collected specimens. Moreover, the study period was restricted to winter 2010 since all 3 selected species (Figure 1) showed cooccurring abundance from January to February.

M. brachiata was most sensitive to small changes in pollutant concentration, which, in this case, were represented by a decrease in pH values (Figures 4 and 5). Therefore, it is a better indicator of aquatic pollution than the other community members, particularly *H. viduus* and *D. lumholtzi*. Supported by findings of Bodar et al. (1989), neonates of *M. brachiata* had a higher resistance to acidic water than fully grown adults (Figure 3), but the reason for this has yet to be explored. Although LC_{50} data have been determined in the present work (Table 3), dose–response approaches to estimating the lethal effects of toxicants on organisms have been criticized for lacking real ecological meaning. However, LC_{50} values may be starting points for more ecologically relevant studies (Newman et al., 1996).

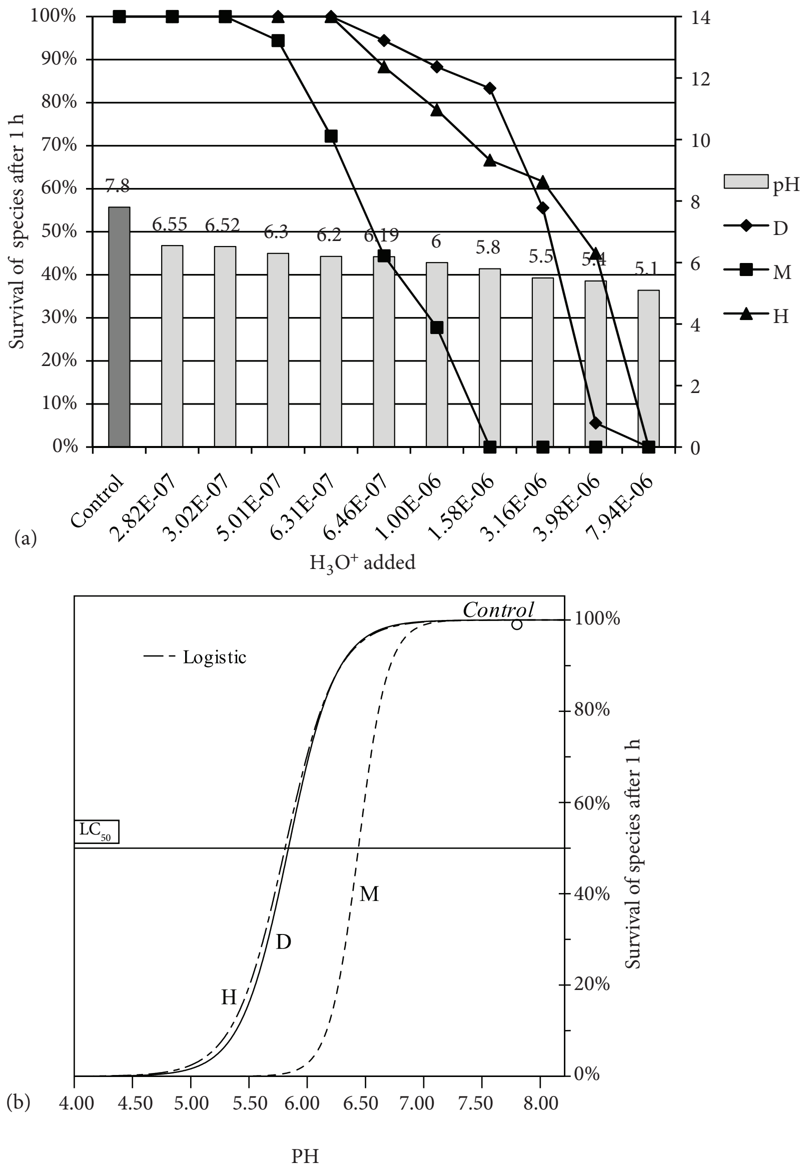

Figure 4. (a) Relative survival in 3 test species exposed to H_3O^+ additions after 1 h; (b) dose–response curve of species against pH at 1 h (D = *D. lumholtzi*, M = adult *M. brachiata*, H = *H. viduus*).

The reason for selecting pond water as the test medium was the ability to assess the effect of lowered pH on the natural population. Another reason behind pond water being ideal as a medium was that since the test specimens were not cultured in a laboratory, pond water provided them with their native environment. In an experimental study by Chen et al. (2004), it was found that stress mediated by low food supply alone in conjunction with pH 5.5 led to the diminished survival of cladoceran *Simocephalus vetulus*. The usage of both tap water and distilled water as the media in 1 of our experiments resulted in the considerable mortality of all 3 species, revealing the fact that food scarcity appears to become a major stress factor for their survival. Kooijman et al. (2003) indicated that the toxicity of chemicals that directly affect survival is aggravated by food limitation. Typically, food is not provided during acute tests (Versteeg et al., 1997). Therefore, no additional food was supplied during the toxicity tests.

HCl is used for various purposes. Such acidic contaminants may easily enter bodies of water and hamper aquatic life, thus necessitating the study of the effects of resulting acidification on aquatic biota. Zuo et al. (2006) used various oxidants of chlorine to evaluate the removal

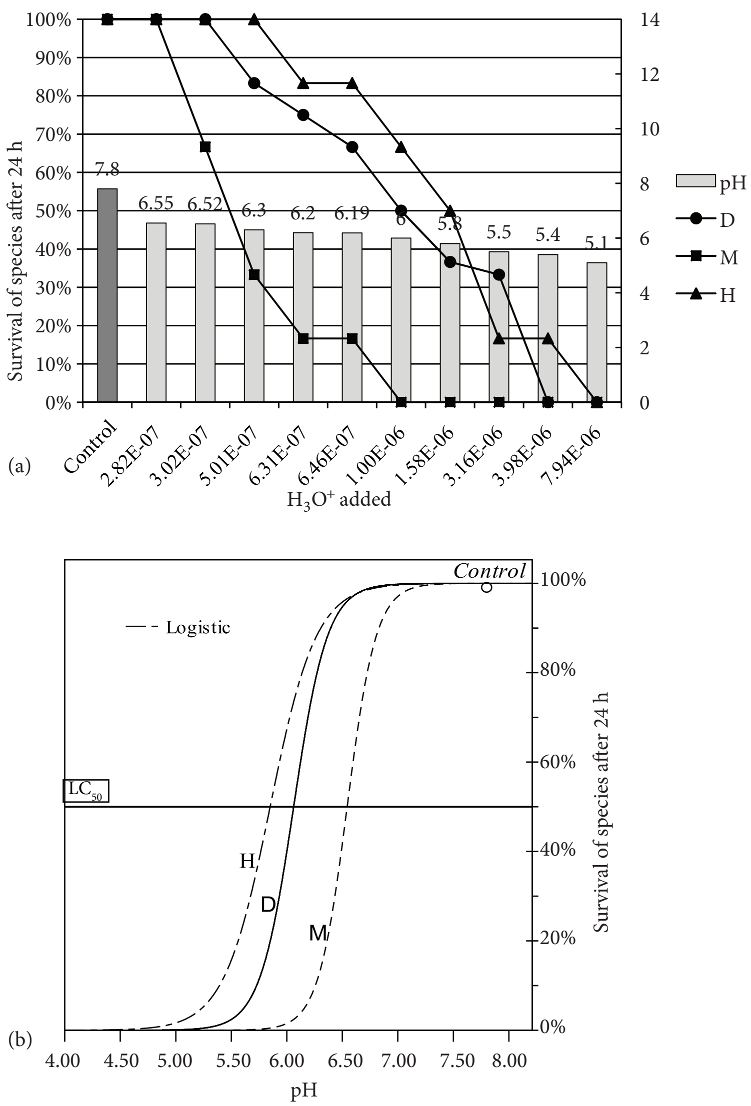

Figure 5. (a) Relative survival of 3 test species exposed to H_3O^+ additions after 24 h; (b) dose–response curve of species against pH at 24 h.

effect on *Mesocyclops leukarti*. This is in tune with the findings of Bernot et al. (2005), who stated that chlorine was highly toxic in acute (48-h) bioassay with *Daphnia magna* and who negatively accounted toxicity in their experiments to chloride and other anions. The present investigation used NaCl in a parallel assay with HCl to nullify the effect of chloride on the mortality of organisms when provided in low concentrations. Only an increase in salinity is attributed to the release of chloride ions in the sample water from HCl or NaCl. The H_3O^+ so formed in test media, after spiking with HCl, was actually responsible for the changes in pH and was the primary factor in affecting the mortality of organisms.

The present study selected a pH range of 5.0–7.8, whereas Fischer et al. (2001) detected a detrimental effect on *Daphnia dubia* using pH levels of 6.1, 5.6, 5.3, and 4.7. While assessing the distribution of plankton species in lakes with pH levels of 3.5–7.6, Blouin (1989) noted that plankton never occurred at low pH values (pH < 4.6), which is similar to our findings (Table 3). It was also observed by Beklioglu and Moss (1995) that increasing pH values led to an increase in the numbers of *Daphnia hyalina*, particularly at pH 10, but population density declined at pH 11. Higher pH may cause poor plankton growth (Boyd, 2000), yet an elevation of pH was not analyzed in the present studies, wherein zooplankton showed a narrower tolerance range (the lower limit being pH 5), perhaps due to an acclimatization problem in the laboratory. Optimum hatching conditions observed by Rojas et al. (2000) for *Moina* sp. were pH 5–9. On the other

Table 3. LC_{50} and 100% mortality values for HCl additions as well as optimum and lethal acidic pH ranges for 3 species at 1-h and 24-h acute toxicity bioassays (all values are the mean of 3 replicates of responses).

	LC_{50} (mM)		100% (mM)		Optimum acidic pH		Lethal range of acidic pH	
Test species	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
D	2.18	1.92	3.6	3	>6.2	>6.51	5.1	5.3
M_n	1.93	1.52	3.2	2.7	>6.31	>6.53	≤5.38	≤5.47
M_a	1.4	1.38	2.4	2.2	>6.45	>6.56	≤5.79	≤6
H	2.2	2.15	3.8	3.6	>6.2	>6.34	≤4.9	5

hand, optimum reproductive conditions for *M. brachiata* in the present study were pH > 5.9.

pH not only determines the availability of certain chemical elements in the water, but it also enhances the toxicity of some metals (Park et al., 2009). Several authors like Locke (1992), Havens et al. (1993), and Fischer et al. (2001) emphasized that pH controls zooplankton community structure and food webs in acidified lakes. Out of different acidification variables tested by Walseng et al. (2008), pH showed the strongest correlation with microcrustacean composition. Thus, the present paper also gives sufficient importance to the solitary effect of pH on microcrustacean species.

Computer simulations and laboratory studies have revealed that sublethal toxicant concentrations have the potential to reduce a population's vital rates and ultimately lead to their extinction (Hallam et al., 1993; Bernot et al., 2005). Therefore, isolated bioassay endpoints may not provide sufficient information to accurately predict population level effects.

Toxicity values from numerous tests with *Daphnia* and *Ceriodaphnia* were compared using a 2-tailed chi-square test to determine if 1 species was more or less sensitive than the other, based on pure chance (Versteeg et al., 1997). It can be inferred from the present chi-square test results that the survival pattern of *M. brachiata* significantly differed from that of *D. lumholtzi* and *H. viduus* in both 1-h and 24-h bioassay experiments. Based on taxonomic closeness, sensitivities of both cladoceran species were expected to be highly similar, yet the response of *D. lumholtzi* closely resembled that of *H. viduus* (Table 4) in spite of belonging to a separate order (i.e. Cladocera and Copepoda, respectively). Comparison of the results with data obtained from complex outdoor systems in the future is required for the practicability and sensitivity of indoor studies and also emphasizes the importance of long-term testing for the prediction of environmental effects.

Despite the diversity of test species available, the only or preferred invertebrate species recommended for acute or chronic toxicity testing is the cladoceran *Daphnia magna* (Grandy, 1995; Koivisto, 1995). Acid-sensitive species, such as large *Daphnia* sp., were reduced in numbers or extirpated from acidified lakes (Locke, 1992) and only thrived in fishless habitats (Koivisto, 1995). The present

Table 4. Chi-square test values for survival responses of each pair of 3 test organisms at specified time periods (* = significant results; the *M. brachiata* considered here are adults).

χ^2	*M. brachiata*		*D. lumholtzi*		*H. viduus*	
	1 h	24 h	1 h	24 h	1 h	24 h
M. brachiata	-	-		7.741* df 8		9.62* df 9
D. lumholtzi	8.752* df 9		-	-		1.606 df 9
H. viduus	9.502* df 9		2.072 df 9		-	-

df 9	df 8
P(0.5) = 8.34	P(0.5) = 7.34
P(0.1) = 14.68	P(0.1) = 13.36

extensive work shows that *D. lumholtzi* is only occasionally found in Midnapore district and within a limited season, and so bioassay data on artificially cultured *Daphnia* spp. cannot be extrapolated to other natural cladoceran communities. Interestingly, the influence of lateritic soil may be considered for future studies as a root cause of *Daphnia*'s disappearance in this district.

Overall, the current study tried to provide a preliminary report on the toxicity of anthropogenic chemicals that can induce changes in the aquatic ecosystems by altering the physicochemical parameters of water and ultimately posing a threat to aquatic organisms. It is well established that food webs in small water sources display very complex interactions such that clear-cut interpretations of any kind of manipulation of a single factor are difficult. It is suggested that a combination of laboratory and field investigations, coupled with monospecies and community assessments, may provide the best route for understanding the effects of toxicants for regulatory purposes. Additional information is necessary to obtain a better understanding of the responses of the zooplankton community to man-induced modifications of water quality.

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