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Effect of Sub-Lethal Concentration of Iron on Growth and Survival of *Daphnia* (Müller, 1785)

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Abstract

In the present study the effect of iron on growth and survival of *Daphnia* (Müller, 1785) has been studied. Neonates of *Daphnia* were exposed to different water-borne iron concentrations. Although iron is toxic at high concentrations, they are also essential elements required for many biological processes. Iron is a vital metal for *Daphnia* for normal development and reproduction. Mass culture of *Daphnia* has been done in plastic tubs. During experiment, the effects of iron on *Daphnia* were studied at an exposure equivalent to the 48 h LC_{50}. Based on the LC_{50}, seven Fe^{2+} levels (prepared through serial dilution from a stock solution of 5 mgL^{-1}) for the chronic toxicity tests were selected. These were 0, 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 mg $L^{-1}$of Fe^{2+}. The population density of *Daphnia* increased with increase of sub-lethal dosages of iron and decreased significantly at higher toxic dosages, indicating that iron is required as micronutrients for stimulating growth in terms of population density.

Keywords: *Daphnia,* micronutrients, population density, reproduction

Introduction

Zooplankton may contribute to the transfer of trace metals to higher tropic levels and have been chosen as one of the recommended groups for the base line studies of metals in both marine and fresh water environments. It is well known that exposure to mercury and cadmium can generally render inhibitory effects. However, the effects of sub-lethal exposure of cladocerans to metals, which are essential for life at trace levels, are not yet well known. As one of the major metal contaminants in freshwater ecosystems, iron is of eco-toxicological interest. The effects of sub-lethal iron exposure, especially at low concentrations on growth and survival of cladocerans are poorly understood. Therefore, the need of the hour is to standardize the sub-lethal dosages of iron required for optimum growth and survival of *Daphnia*.

Population growth studies are helpful to quantify sub-lethal effects of toxicants including heavy metals to zooplankton because small changes in survivorship and fecundity are eventually summed up in peak abundances and growth rates (Halbach et al., 1983). For this, single species test are appropriate because they yield information both rapidly and quantitatively to evaluate the direct effects of toxicants which could be extrapolated to natural conditions with some precision (Koivistio, 1995).

Daphnia a planktonic crustacean of the order cladocera, having size 0.2 and 0.5 mm in length is commonly called water fleas due to its salutatory swimming style. They are mainly fresh water farms and are found abundantly in most lakes and ponds. They move in the open water of lakes, or either gets attached to vegetation or near the bottom of the water body. The present study is designed to quantify sublethel concentration of Iron and its effect on growth and survival of *Daphnia.*

Material and Methods

Daphnia, obtained from the natural ponds (seven bungalows, Andheri-west locality) were brought to the wet lab of Central Institute of Fisheries Education, Mumbai, India and kept in tanks. *Daphnia* was collected from tank and brought to the laboratory and sample was examined under a

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microscope to pick up *Daphnia* with the help of a fine dropper and inoculated @ 10 no. / 100 ml water in beakers. A nutrient medium (slurry) was prepared by adding 2.5 kg of raw cow dung (RCD), 0.5 kg of groundnut oil cake (GNOC), 0.25 kg single super phosphate (SSP) with constant aeration. It was kept for 2-3 days for mineralization of slurry in order to allow the obnoxious gases like ammonia, hydrogen sulphide to escape. The slurry was added @ 4 ml L^{-1} for 2-3 days to 1000 L water. On third day, *Daphnia* was inoculated @ 40-50 individuals/L. Slurry application was continued @ 2 ml L^{-1} at an interval of every 2 days with constant and mild aeration.

Based on a preliminary range finding test, six final concentrations (4, 8, 16, 32, 64 and 128 $mgL^{-1}$of $FeSO_4.7H_2O$) were chosen to determine the LC_{50} of iron. Based on the LC_{50}, seven each of Fe^{2+} levels (prepared through serial dilution from a stock solution of 5 mgL^{-1}) for the chronic toxicity tests were selected. These were control, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg $L^{-1}$of Fe^{2+}. Experiments were conducted in 100 ml glass beakers containing 50 ml of the water medium containing Fe^{2+} in triplicates. In each test vessels, 20 individuals (mixed age group) were introduced. Following initiation of population growth experiments, the number of living *Daphnia* alive in each replicate were counted and transferred to a fresh medium with appropriate levels of Chlorella and Fe^{2+}. The experiments were terminated after a declining trend was observed in most of the test populations, lasted for 21-35 days.

The population growth rate was calculated following the exponential growth equation (Krebs, 1985)

$$r = (\ln N_t - \ln N_0)/t$$

where, N_0 and N_t are the initial and final population densities and 't' is time in days. The 'r' and the peak population densities were analyzed for statistical significance (One-way ANOVA) following Sokal & Rohlf (2000).

Results and Discussion

Growth curve of *Daphnia* in 35 days at different iron concentration is shown in Fig. 1-7. The population density is 20 numbers mL^{-1} at 0 day, where the peak concentration and declining level were indicated at different concentration of iron (Fig. 1-7). Rate of population increase (r) of *Daphnia* under different concentrations of iron are given in Fig. 8. The value of 'r' was positive up to 4.0 $mgL^{-1}$of iron and become negative from 0.8 $mgL^{-1}$of iron. This shows that population density increased up to 4.0 mg $L^{-1}$of iron and then decreased from 8.0 mgL^{-1}.

Mortality (%) of *Daphnia* exposed to different concentrations of iron in 24 and 48 h are given in Fig. 9 and Fig. 10. The mortality of *Daphnia* increased with increase in iron concentrations and time of exposure. The mortality (%) was more at 48 h than 24 h at similar iron concentrations. The LC_{50} values (mgL^{-1}) of iron for *Daphnia* in 24 and 48 h and their 95% confidence limits (indicated in parentheses) were recorded as 2.04 (1.75-2.33) and 1.17 (1.02-1.32) respectively.

Generally in toxicity test, death is a decisive criterion because it is easy to determine and have obvious biological and ecological significance. Median lethal concentration is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms in a particular length of exposure. In the present study, the 24 and 48 h LC_{50} values of Iron was 2.04 and 1.17 mgL^{-1} respectively. These values are similar to those reported in *Moina macrocopa* (Wong, 1992). The 24 h and 48 h LC_{50} values and 95% CL was in the same range as earlier reported for *Daphnia* (Biesinger & Christensen, 1972; Winner & Farrell, 1976; Khangarot & Ray, 1987).

It is clear from the present study that population growth of *Daphnia* was affected by the iron. Iron was stimulatory effect on the population growth of *Daphnia* up to certain sub- lethal dosages below their respective LC_{50} values. The population density increased significantly than control (Fig. 1) at 0.25 mgL^{-1}, 0.50 mgL^{-1}, 1.0, 2.0 and 4.0 mg L^{-1} of iron concentrations (Fig. 2, 3, 4, 5 and 6). The population density reached its highest peak of 270 numbers / mL in 21 days at 1.0 mg L^{-1} iron. Thereafter the population density decreased and reached a peak of 100 numbers /mL in 11 days at 8.0 mgL^{-1} of iron and declined up to 45 numbers in 19 days (Fig. 7). The rate of population increase (r) was positive in the control, 0.25, 0.50, 1.0, 2.0 and 4.0 mgL^{-1} of iron and negative in 8.0 mgL^{-1}.

At higher test concentration of iron (8.0 mgL^{-1}) *Daphnia* showed a sign of hyperactivity and increased locomotive activity during the early hour of exposure followed by a decrease of swimming activity prior to death. At lower test concentrations, animals showed normal movements for a longer

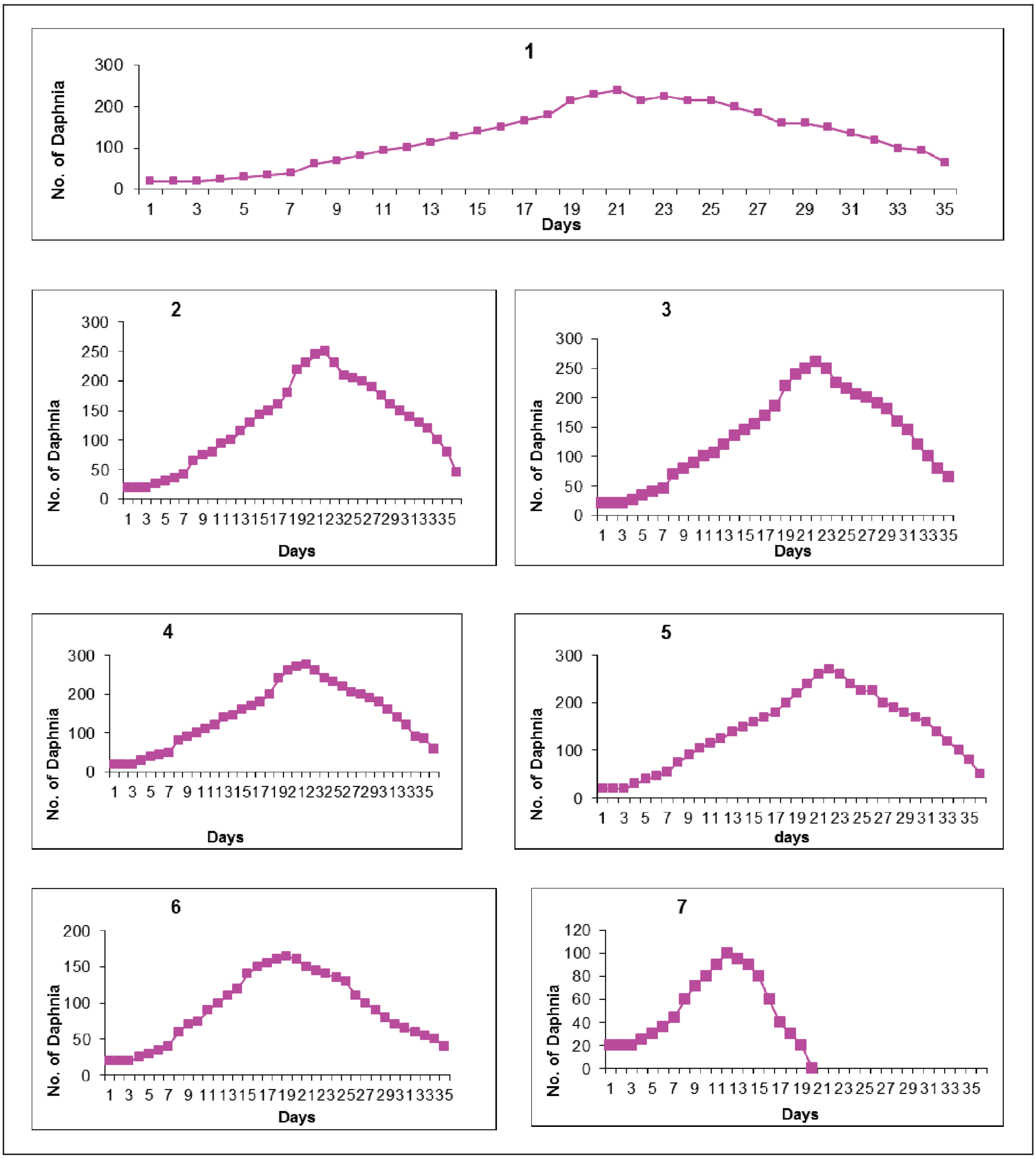

Fig. 1-7. Growth curve of *Daphnia* in 35 days at different iron concentration. The initial population density is 20 numbers / ml at 0 day

1. Reaches peak of 240 numbers / ml in 20 days and declines to 45 numbers / ml in 35 days at 0.0 **iron mgL**$^{-1}$
2. Reaches peak of 250 numbers / ml in 21 days and declines to 45 numbers / ml in 35 days at 0.25 **iron mgL**$^{-1}$.
3. Reaches peak of 260 numbers / ml in 21 days and declines to 40 numbers / ml in 35 days at 0.50 **iron mgL**$^{-1}$
4. Reaches peak of 275 numbers / ml in 21 days and declines to 60 numbers / ml in 35 days at 1.0 **iron mgL**$^{-1}$
5. Reaches peak of 270 numbers / ml in 21 days and declines to 50 numbers / ml in 35 days at 2.0 **iron mgL**$^{-1}$
6. Reaches peak of 165 numbers / ml in 18 days and declines to 30 numbers / ml in 35 days 4.0 **iron mgL**$^{-1}$
7. Reaches peak of 100 numbers / ml in 11 days and declines to 45 numbers / ml in 19 days at 8.0 **iron mgL**$^{-1}$

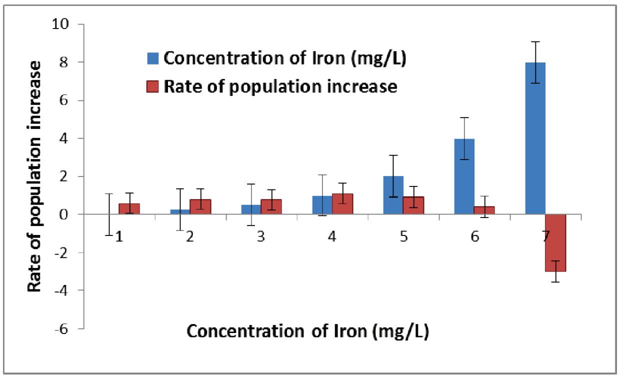

Fig. 8. Rate of population increase (r) under different concentrations of iron

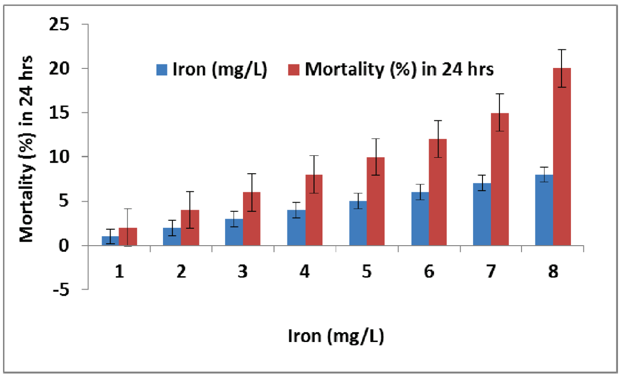

Fig. 9. Mortality of *Daphnia* in 24 h under different concentrations of iron

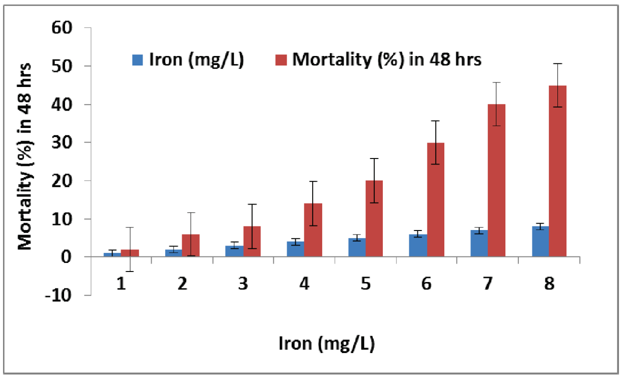

Fig.10. Mortality of *Daphnia* in 48 h under different concentrations of iron

period of exposure. In most of the cases, before immobilization, *Daphnia* remained on the surface of water and then settled down at the bottom and showed uncoordinated body movement and finally became immobilized. Similar behavior was observed in *Daphnia* by using copper (Khangarot & Rathore, 2002). The effect of different concentrations of Cd or Zn on the population growth of *Brachionus havanaens* showed decreased population abundance with increasing levels of the metal concentration in the medium (Juarez-Franco et al., 2007).

There may be positive effects of sub-lethal exposure of cladocerans to metals such iron, which are essential for life at trace level. In case of iron, population, growth varies positively from 0.586 - 1.097 at lower dosages and become negative at higher dosages (Fig. 8). This shows that iron at sub-lethal dosages increases the population density but at higher dosages becomes toxic to the animals. Statistically 'r' is adversely influenced by the heavy metal concentration in the medium. Similar values and conclusions were derived by using Cu and Zn (Nandini et al., 2007) and Cd and Zn (Juarez-Franco et al., 2007).

Statistically, there was a significant impact of iron on the peak population density of *Daphnia* ($p<0.001$). Post-hoc analysis showed that the time taken to reach the peak population density of *Daphnia* was significantly affected by the different metal concentrations ($p<0.05$, Tukey's test). An α- posteriori analysis of the population growth rate of *Daphnia* showed that at the highest metal concentration (4.00-8.00 mgL^{-1} in Iron), there was a significant decrease in population density as compared to the controls or to the lowest metal concentration ($p<0.05$, Tukey's test).

Cladoceran *Daphnia* population was significantly affected by the sub lethal exposure to different concentrations of zinc and also was directly related to the time taken by the population to reach to the peak. Iron is an essential trace metals for the population growth of *Daphnia* up to certain sub lethal dosage and will negatively affect the population growth near toxic dosages. *Daphnia* can be considered as a model organism for toxicity studies of various pollutants.

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