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Original Research Paper

# Evaluation of Nickel Toxicity in Freshwater Snail, *Pila globosa* (Swainson) in Relation to Body Size

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## ABSTRACT

Small (20±2 g) and large (40±2 g) size groups of the freshwater snail *Pila globosa* were exposed to different concentrations of nickel (mg/L), and 96 hr $LC_{50}$ through percent and probit mortality of the animals and also by Dragstead and Behren's method were determined. There was a linear relationship between the percent or probit mortality and the nickel concentration of both the size groups of snails. Thus, the percent and/or probit mortality increased with the increase in concentration of nickel. The percent mortality plotted against log concentration of nickel gave sigmoid curves, whereas the probit mortality plotted against log concentration gave straight lines in both the groups of snails. The 96 hr $LC_{50}$ obtained for small and large size groups of snails are 117.6 mg/L and 206.3 mg/L respectively. These values obviously indicated a significant ($P<0.001$) increase in the $LC_{50}$ of nickel with the increase in the size of the snail. The results of the present study indicate that the small size groups of snails are more sensitive to nickel than the large size groups.

## INTRODUCTION

The level of toxicity of a toxicant can be measured in terms of its concentration or dose which kills a known population of specific species within a fixed period of time. It, however, is influenced by various extrinsic and intrinsic factors such as nutritional state, humidity, light and size (Venkata Reddy et al. 2007), temperature (Min & Kim 2006), pH and salinity (Sreenivasa Reddy et al. 2008). Acute toxicity tests are generally used to determine the concentration of a toxicant that produces adverse effects on a specific percentage of test organisms in the short span of time.

The investigations on effects of heavy metals on aquatic organisms have mostly involved first with the determination of $LC_{50}$ (Anandhan & Hemalatha 2009). Heavy metals including nickel are being released into the aquatic bodies over a long period of time through various anthropogenic activities. Burning of fossil fuels and residual oils, coal mine spoils, sewage sludge and production of Ni-Cd batteries are the primary source of nickel that enters into freshwater ecosystem and exhibit toxic effect on its biota (E1-Enany & Issa 2000). Average yearly worldwide emission of Ni was $55{,}650 \times 10^3$ kg in atmosphere, $113 \times 10^6$ kg in aquatic ecosystem and $416.5 \times 10^6$ kg in soil (Shukla et al. 2009). There are reports that nickel concentrations of 0.03-0.05 mg/L reduce fecundity, reproduction and respiration in *Daphnia magana* and sea urchin, *Lytchnius pictus* (Timourian & Watchmaker 1972). Exposure of molluscs to sublethal/chronic levels of nickel generally results in a reduction in growth. Calabrese et al. (1977) reported that the growth of oysters *Crassostrea virginica* was not influenced at the $LC_5$ value but was markedly reduced at the $LC_{50}$. Nickel sulphate induced sex linked recessive lethal mutations in *Drosophila melanogaster* (Rodriguez-Arnaiz & Ramos 1986). The information on $LC_{50}$ studies in freshwater molluscs is rather scanty. Hence, the present study is taken up to determine the toxicity levels of nickel for the small and large size groups of freshwater snail, *Pila globosa* in order to understand the impact of size on nickel toxicity.

## MATERIALS AND METHODS

The snails were collected from local irrigation canals and ponds in and around Anantapur. The small and large size groups of snails having the total body weight 20 ± 2g and 40 ± 2g respectively were used in the present investigation. They were maintained in the laboratory in a large aquarium and the water was renewed once a day to provide freshwater rich in oxygen. The snails were fed *ad libitum* with pieces of an aquatic plant *Hydrilla* for ten days before using them for experimentation. The water used has pH range of 7.6 ± 0.2 and total hardness 100 ± 5 mg/L as $CaCO_3$. Temperature, chlorinity and dissolved oxygen content of water were checked periodically during the course of investigation and were maintained at 28°C ± 0.5°C, 0.08 ± 0.003% and 8.79 ± 0.4 mg/L respectively. Water was aerated once a day to prevent influence of hypoxia.

Pure nickel chloride was used as a source of metal with the molecular weight 273.71 to study the effect of nickel on the freshwater snails. The percent mortality of small and large size groups of snails in different concentrations was determined immediately after 96 hours of exposure. For this, the

two sizes of experimental animals were divided into batches of twenty four each and exposed to different concentrations of nickel, each batch to one concentration, ranging from 75 to 200 mg/L for small size and 150 to 275 mg/L for large size groups of animals. These ranges were obtained on the trial and error basis. Mortality rate was observed in each concentration of nickel immediately after 96 hours exposure. A batch of animal in each size group maintained along side in freshwater with 0.1 mL of hydrochloric acid per litre to nullify chloride effect served as control. The mortality at each concentration derived from the mean of three repetitions was converted into percent mortality values. From this, the probit mortality values were obtained (Finney 1971). As the evaluation of toxicity of metals to an aquatic organism is by the determination of its $LC_{50}$, the percent mortality and probit mortality values of the two groups were plotted separately against nickel concentration and $LC_{50}$ values were derived from the two curves. For subsequent verification of the $LC_{50}$ values obtained by graphical methods, Dragstedt and Behren's method as given by Carpenter (1975) was employed. As per this method the small and large sized animals were exposed to log 2 concentrations of nickel, 50, 100, 200, 400 mg/L for 96 hours.

The percent mortality values were calculated from the cumulative mortality and with those values $LC_{50}$ values were obtained by adopting the following formula.

$$\text{Log LC}_{50} = \text{Log.A} + \frac{50 - a}{b - a} \times \text{Log.2}$$

A = Concentration of the metal which has a percent mortality immediately below 50%.
a = Percent mortality observed immediately below 50%
b = Percent mortality observed immediately above 50%

Thus, the mean $LC_{50}$/96 hr for each size group of snails was obtained through percent and probit mortality curves and Dragstedt and Behren's method as described earlier (Koppar et al. 1993).

## RESULTS AND DISCUSSION

Studies using toxicity tests for determination of the effects of heavy metals on aquatic organisms have traditionally been carried out under controlled conditions. The results thus obtained are of considerable value in establishing criteria for limiting the discharge of these contaminants into aquatic bodies.

In the present study, $LC_{50}$ values obtained for nickel at 96hr are 117.6 and 206.3 mg/L for small and large size snails respectively (Tables 1, 2 and 3, Figs. 1 and 2). It is clear from the data that there was a linear relationship between the percent or probit mortality and the nickel concentration in both the size groups of snails. The values reflect a greater tolerance capacity of these animals to nickel concentration. Eisler & Hennekey (1978) reported that molluscs are tolerant to nickel with 96 hr $LC_{50}$ values ranging from 72 to 350 mg/L. For a freshwater mussel *Lamilledence marginalis*, the $LC_{50}$ value of nickel is reported as 14.53 mg/L at 96 hrs of exposure (Sreedevi et al. 1992). In the present study, high tolerance of snails to nickel could be due to their sedentary

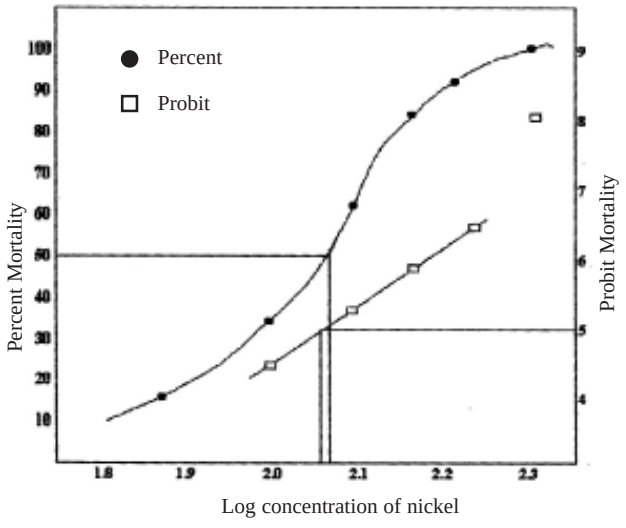


Fig.1: 96 hrs percent and probit mortality of the small size *Pila globosa* in different log concentrations of nickel. Each point is mean of three replicates. $LC_{50}$ values are indicated on both percent and probit mortality curves.

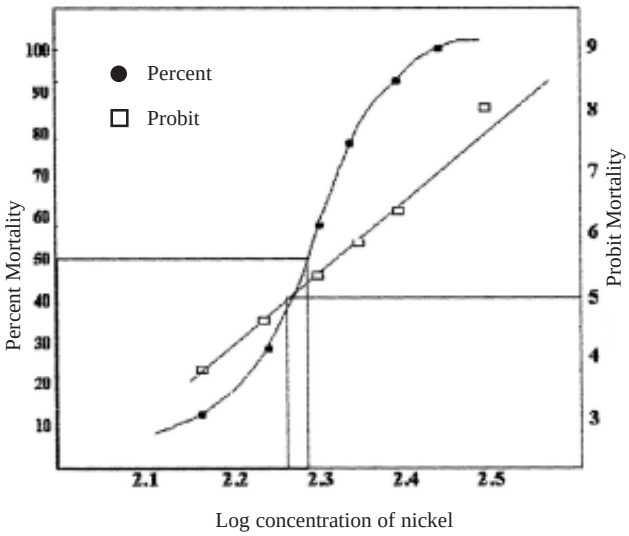


Fig. 2: 96hrs percent and probit mortality of the large size *Pila globosa* in different log concentrations of nickel. Each point is mean of three replicates. $LC_{50}$s are indicated on both percent and probit mortality curves.

Table 1: 96 hrs percent and probity mortality values of the small and large size *Pila globosa* in different concentrations of nickel. Each value is a mean of three replicates.

| S.No | Concentration mg/L | Log concentration | No. of snails exposed | No. of snails dead | No. of snails alive | Percent mortality | Probit mortality |
|---|---|---|---|---|---|---|---|
| **Small Animals** | | | | | | | |
| 1 | 75 | 1.8751 | 24 | 4 | 20 | 16.7 | 4.01 |
| 2 | 100 | 2.0000 | 24 | 8 | 16 | 33.3 | 4.56 |
| 3 | 125 | 2.0969 | 24 | 15 | 9 | 62.5 | 5.31 |
| 4 | 150 | 2.1761 | 24 | 20 | 4 | 83.3 | 5.95 |
| 5 | 175 | 2.2430 | 24 | 22 | 2 | 91.7 | 6.34 |
| 6 | 200 | 2.3010 | 24 | 24 | 0 | 100 | 8.09 |
| **Large Animals** | | | | | | | |
| 1 | 150 | 2.1761 | 24 | 3 | 21 | 12.5 | 3.82 |
| 2 | 175 | 2.2430 | 24 | 7 | 17 | 29.2 | 4.45 |
| 3 | 200 | 2.3010 | 24 | 14 | 10 | 58.3 | 5.20 |
| 4 | 225 | 2.3522 | 24 | 19 | 6 | 79.2 | 5.81 |
| 5 | 250 | 2.3979 | 24 | 22 | 2 | 91.7 | 6.34 |
| 6 | 275 | 2.4393 | 24 | 24 | 0 | 100 | 8.09 |

Table 2: 96 hrs percent mortality values of small and large size animals of *Pila globosa* in log.2 concentrations of nickel. Dragestedt and Behren's method as described by Carpenter, 1975). Percent mortality is calculated from cumulative mortality. Each value is a mean of three replicates.

| S.No. | Concentration | Log concentration | No. of animals exposed | No. of animals alive | No. of animals dead | Cumulative | | Percent mortality |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Alive | Dead | |
| **Small Animals** | | | | | | | | |
| 1 | 50 | 1.6990 | 24 | 24 | 0 | 38 | 0 | - |
| 2 | 100 | 2.0000 | 24 | 14 | 10 | 14 | 10 | 41.66 |
| 3 | 200 | 2.3010 | 24 | 0 | 24 | 0 | 34 | 100 |
| 4 | 400 | 2.6021 | 24 | 0 | 24 | 0 | 58 | - |
| **Large Animals** | | | | | | | | |
| 1 | 50 | 1.6990 | 24 | 24 | 0 | 58 | 0 | - |
| 2 | 100 | 2.0000 | 24 | 24 | 0 | 34 | 0 | - |
| 3 | 200 | 2.3010 | 24 | 10 | 14 | 10 | 14 | 58.33 |
| 4 | 400 | 2.6021 | 24 | 0 | 24 | 0 | 38 | 100 |

nature or greater adaptability to toxicants by the capacity of speedy elimination of the metal and/or the activation of detoxification mechanisms. Further, the operculum closing mechanism of these animals could restrict the flow of polluted water over the soft parts and hence, could elevate the toxicity range. James et al. (2003) also gave similar explanation for freshwater fish exposed to copper toxicity. The marine mussel *Mytilus edulis* on exposure to mercury, copper, cadmium and zinc conferred increased tolerance to the toxicity of inorganic mercury and this acquired tolerance was attributed to the apparent induction of metallothionein in which presumably initiated cellular compensatory responses (Roesijadi & Fellingham 1987).

Increase in $LC_{50}$ of nickel with the increase in size indicates the requirement of higher concentration of nickel for the mortality of the large sized snails than the small ones. Probably, higher metabolic rate of small snails than the larger ones could be one of the reasons for more sensitivity of former size animals than the latter. With the increase in metabolism in small animals there is every possibility for rapid incorporation of metal in soft tissues. Moore & Ramamurthy (1984) have stated that sensitivity of invertebrates to nickel is inversely related to the age/size of the animal. This type of relation was also observed in freshwater fish *Mystus vittatus* when exposed to copper (Subathra et al. 2007).

Various symptoms of poisoning can also be observed from studies involving the determination of $LC_{50}$. In the present study a few symptoms of nickel poisoning were observed in both the size groups of snails. Closing of operculum of snails immediately on their transfer to the toxic medium, followed by a slow progression of the body and extrusion of tentacles and pulps were observed in both, lethal and below lethal concentration of nickel. On further stay in lethal concentration, a sheet of mucus was seen near the opening of the shell valve. Slowly, the animals became inactive and finally the operculum got detached form the foot, which led to their death. A copious quantity of mucus was found exuding out of the foot region. The snails became very le-

Table 3: 96 hrs $LC_{50}$ values of nickel to the small and large animals of *Pila globosa*. The values are derived from the percent and probit mortality curves as well as the value obtained through Dragestedt and Behren's methods.

| S.No | Method | $LC_{50}$/96hrs (mg/L) Small animals | Large animals |
|---|---|---|---|
| 1 | Percent mortality | 112.2 | 195.0 |
| 2 | Probit mortality | 114.8 | 190.5 |
| 3 | Dragestedt & Behren's method | 126.0 | 223.5 |
| | Mean | 117.6 | 206.3 |
| | SD± | 6.0 | 19.3 |

The difference in $LC_{50}$ values between the two size groups is statistically significant ($P<0.01$). SD: Standard Deviation

thargic and the responses of the foot to mechanical stimuli like pricking with a needle, was very feeble. When the snails were touched before their eventual death, the retraction of foot became slow and sluggish, compared to the immediate withdrawal of animals maintained in normal water. The symptoms of poisoning in the lethal concentration were more effective in small size animals than in those of larger ones. Balaparameswara Rao & Jayasree (1987) reported that juvenile snails are more susceptible than adult snails, *Bellamya dissimilis* while exposed to acute concentrations of copper and zinc. The results of the present study also support their statements, as it is observed that the small size groups of snails are more sensitive to nickel than the large size group in the lethal and near lethal concentration. On the whole, with the knowledge of toxicity evaluation it could be possible to establish limits and levels of susceptibility of the freshwater snail *Pila globosa* to nickel in its normal habitat at different stages of its growth.

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