SUMMARY AND CONCLUSIONS
Human population explosion, unplanned urbanization deforestation, profit
oriented capitalism and technological advancement, has inadequately
introduced pollution into the aquatic environment. Out of all the pollutants
cause pollution of aquatic environment, heavy metals are predominant.
Some of these metals are essential in limited quantities to normal growth of
organisms. Even essential metals are toxic if the concentration exceeds the
normal requirement. Nickel is a silvery white group VIII metal plays dual role,
as an essential trace metal and as a potential toxic element. The physical
properties of nickel such as corrosion, resistance, high strength, and
durability over a wide range of temperature, pleasing appearance and good
electrical conductivity are chief advantages of its use in 3000 different alloys.
Nickel enters into groundwater and surface waters by leaching of rocks and
soils, from biological cycles, from industrial waste disposal, and causing
imbalances in aquatic fauna. Molluscs represent one of the largest
populations of aquatic life and serve as proteins food supplement for South
Indian poor people. Protection of these animals from the deleterious effects
of metals is possible only by analysing the response patterns of them to
various concentrations at different periods of exposure. The present study
has been taken up with this view, in which some of the biochemical and
histological responses in the selected organs like the mantle,
hepatopancreas and foot of an edible freshwater snail, *Pila globosa* exposed
to lethal and sublethal concentration of nickel were analysed. As the body
size is one of the important intrinsic factors which exhibits a profound
influence on the toxicity of any metal, the present investigation has been
carried out in two size groups, small and large, of the snails in order to
assess the impact of size on the response patterns of these animals to nickel
at two different growth stages.
2. As LC₅₀s are highly useful in establishing the tolerance limits and safe levels of toxic agents for the biota of aquatic environment, and also in evaluating the lethal and sublethal concentrations, the present study was commenced with the determination of LC₅₀s of nickel to both the size groups of snails by dose–response curves. They have been subsequently verified by Dragestedt and Behern's method. As the period of exposure is an important factor, 96 hours was preferred with the view that the effects of metal become consistent within this period. The 96 hours LC₅₀s obtained for the small and large size groups of snails were 117.6 mg/l and 206.3 mg/l respectively. These values showed a high tolerance capacity of freshwater snails to nickel, and a significant increase of it with the increase in size of the snail. High tolerance could be due to the sluggish nature of the animal; and the increase of it with size could be due to the decrease in their metabolic rate. Secretion of mucus and swelling of foot were two important symptoms of poisoning observed at the lethal and sublethal concentrations; the intensity, however, was concentration and size–dependent.

3. Lethal concentrations of nickel alone are not sufficient in assessing various response patterns of the snails to the toxicant. These studies have serious limitations like the possibility of ignoring the occurrence of adaptation of the test animal to the metal. Hence, further studies in this investigation were carried out in the small and large size groups of snails exposed to a lethal and a sublethal concentration of nickel. As two different LC₅₀s obtained for the small and large snails, the average of two, approximately 150 mg/l, was taken as the lethal concentration for both the size groups; and one tenth of this, i.e., 15 mg/l, was taken as the sublethal concentration in order to compare the influence of body size over the toxicity of nickel. All the further
studies were made in the mantle, hepatopancreas and foot of snails at different time intervals of exposure i.e., 12, 24, 36 and 48 hours in the lethal concentration and 5, 10, 20 and 30 days in sublethal concentration; with a view that the results obtained at these exposure periods could indicate some specific events of responses of snails to acute and chronic toxicity of nickel on short–term and long–term exposures.

4. Molluscs, among invertebrates, accumulate many metals in their tissues to several orders of magnitude above their ambient water concentrations. Relative to controls, significant amount of nickel had accumulated in the mantle, hepatopancreas and foot of both size groups of snails exposed to the lethal and sublethal concentrations. But, the amount of accumulation was more in both size groups exposed to the lethal concentration than to sublethal; and the rate of accumulation was directly proportional to the time of exposure in the lethal concentration where as inversely proportional in the sublethal concentration. Among the organs, the concentration of nickel accumulated in the both lethal and sublethal concentrations were in the order: hepatopancreas>mantle>foot. The data revealed that the accumulation of nickel in the organs is not only concentration dependent but also organ–and size dependent. As hepatopancreas is the primary site of detoxification, this organ received more amounts of nickel, may for its storage, and/or for detoxification and disposal of the metal. Mantle is a secretary and secondary respiratory structure; hence, more accumulation of nickel in this organ is also possible. Foot being a muscular nature slow diffusion of nickel into through haemolymph could be one of the possible reasons for less accumulation in it. Significant accumulation of nickel in the organs of both size groups on exposure to both the lethal and sublethal
concentrations indicated high potentiality of this metal for bioaccumulation and persistence. Increase in the rate of accumulation over time of exposure in the lethal concentration could indicate the domination of metal concentration in the organs over its elimination/excretion. It could lead suppression of metal sequestration and detoxification capacities. On the other hand, significant decrease in the rate of accumulation in the sublethal concentration could indicate the domination of metal elimination/excretion over its uptake; as well as slower rate of absorption of metal ions due to their less availability. Between the two size groups, rate of accumulation of nickel was greater in the organs of small animals than those of large ones at lethal concentration. More accumulation in small snails could be related to their greater cell surface area and high metabolic activity when compare to large animals. In the sublethal exposure, less accumulation of nickel at 20 and 30 days in small animals than large ones indicated their greater efficiency in elimination or depuration of the metal; such capacity decreased with the increase in size of the animal on long-term exposure to chronic toxicity.

5. The rates of oxygen consumption and SDH activity in the lethal concentration decreased in the organs of both size groups at all exposure periods, the magnitude of which was in the order: 12<24<36<48 hours. Among the organs the decrease was more in hepatopancreas and less in foot; and in between the size groups it was in the order: small>large. In the sublethal concentration, though a decrease was observed at days 5 and 10, it regressed at days 20 and 30 in the organs small sized snails. In the organs of large size group the decrease observed was in the order: 5<10>20<30 days. The suppression of oxidative metabolism in the organs of snails exposed to lethal concentration induced a shift to anaerobic glycolysis by
stimulating the LDH activity in the organs in order to meet the energy requirements under high toxic stress. The gradual decrease in SDH and elevation in LDH activities resulted in slow accumulation of pyruvate and lactate in the organs of both size groups. The elevation in anaerobic glycolysis, however, decreased at hours 36 and 48 in small animals and 48 hours in large animals exposed to lethal concentration. This revealed that the animals relied initially on energetically less efficient glycolysis to meet the required energy demands; but on long-term exposure the significant decrease in the generation of ATP by the suppressed glycolysis and TCA cycle could lead to the death of the snails under acute nickel stress. In sublethal concentration, the rate of oxygen consumption and SDH activity decreased on days 5 and 10 in the organs of small animals, but on further exposure to days 20 and 30, regression in the decrease was observed. In the organs of large animals more suppression of oxygen consumption and SDH activity especially at day 30 indicated the inability of this size group of animals to recover their level of energetics from the state of suppression. Thus, the small animals exhibited greater metabolic efficiency by elevating their level of energetics necessary to detoxify and/or to eliminate the toxic ions. With the elevation of oxidative metabolism, the rise in the level of anaerobic glycolysis gradually reached to normal in small animal; but it maintained a progressive elevation in the organs of large animals.

6. Changes in protein metabolism can be considered as one of the important diagnostic tools in evaluating the toxicity of any metal. Soluble and structural proteins decreased over time of exposure in the organs of both size groups of snails exposed to lethal concentration with the increase in the levels of free amino acids and protease activity. The results indicated severe
proteolysis in the organs of snails exposed to lethal concentration of nickel. The increase in the activity of proteases could be due to the damage caused to lysosomal membranes, and also due to cellular destruction and decreased protein synthetic potentials. The steep increase in the rate of nickel accumulation could be the primary reason for protein degradation and destabilization, which was greater in hepatopancreas. In sublethal concentration the soluble protein levels exhibited a decrease at day 5 in the organs of small animals and at days 5 and 10 in those of large size groups. Later an elevation was observed that in protein levels at day 10 followed by a regression of it in small animals; whereas in large animals the initial decrease at day 5 was followed by a gradual elevation at remaining chronic exposure periods. Elevation of protease activity at days 5 and 10, then suppression was noticed in both sizes exposed sublethal concentration. Free aminoacid levels, however, are decreased in small snails whereas increased upto day 20 and then decreased at day 30 in large ones. These results suggested an increase in the protein synthetic potentials in snails exposed to lower nickel concentrations, probably to synthesise the enzymes required for detoxification and to stabilize the tissue organization for prevention of speedy diffusion of nickel ions. Small animals though initially exhibited a little susceptibility but could activate protein synthetic potentials on prolonged exposure; a delay in such initiation was noticed in large animals.

7. Suppression in the protein synthetic potentials had been evident in the organs of snails of both size groups at lethal concentration by a decrease in the activities of AAT and AIAT at 36 and 48 hours of exposures. Contrary to it, progressive increase of GDH activity indicated deamination. An elevation in AAT and AIAT activities at 12 hours in the organs of small animals and
upto 24 hours of exposure in those of large animals indicated a preparatory phase for the synthesis of necessary proteins to meet the toxic stress. However, decrease in protein synthetic phase at 36 and 48 hours of exposure to lethal concentration was evident by the suppression of AAT and AIAT activities. With the decrease in oxidative metabolism some of the amino acids might have been channeled to gluconeogenic pathway to provide metabolites for the release of energy through anaerobic glycolysis. Rest of the amino acids, which did not undergo deamination, had accumulated in the cells and lead to hyperaminoacidemia, which could disturb the acid–base balance of the cell. Elevation in GDH activity indicated active deamination in the organs of snails under acute toxic stress. The magnitude of it was greater in the organs of small animals than in those of large ones. In sublethal concentration the AAT and AIAT as well as GDH activities increased over time of exposure. It indicated the reorganization of amino acids for the synthesis of necessary proteins to meet toxic stress. In addition, as evident by the increase in GDH activity some of amino acids might have been deaminated and incorporated into TCA cycle for the release of necessary energy to meet the demand. The intensity of above changes was greater in small animals than in large ones.

8. In protein metabolism ammonia production and its detoxification are the major events. Elevation in deamination in the organs of snails of both size groups exposed to lethal concentration resulted in a significant increase in the level of ammonia. Operation of purine nucleotide cycle could be responsible for the increase in ammonia level. This leads to development of added ammonia toxicity in addition to the nickel. The main basic mechanism to detoxify ammonia is its conversion into urea by the enzymes of urea cycle.
Eventhough an initial increase in the activity of arginase was observed but it decreased on long-term exposure. It revealed the conversion of ammonia to urea upto 12 hours of exposure but due to impairment of urea cycle under high concentration of nickel, ammonia levels elevated progressively. In sublethal concentration a decrease in the level of ammonia with an increase in the levels of urea and activity of arginase suggested the activation of compensatory mechanisms in those snails with rapid conversion of ammonia formed during transdeamination to less toxic substance like urea. The urea thus formed also was useful for the maintenance of osmo-concentration of body fluids. Due to the conversion of ammonia to urea, these two levels gradually reached normalcy on prolonged exposure in the organs of small animals, but in large animals accumulation of ammonia dominated over its conversion to urea.

9. Histological changes in tissues of animals under lethal and sublethal concentrations of nickel stress provide support to the shifts in protein metabolism. In the lethal concentration of nickel mantle appeared with peeling up of inner and outer epithelial layers, greater dissolution of fibrous tissue leading to formation of more intercellular spaces with scattered secretary granules, atrophy of pallial muscle fibers and pyknotic nuclei leading to the impairment in shell secretion and respiration. The degenerative changes observed in hepatopancreas like degeneration of tubular epithelium, intertubular connective tissue, extrusion of cytoplasmic material into hemocelic spaces, and atrophy with the maximum disintegration of gland tubules could cause impairment in all its metabolic functions including detoxification. The total architecture of the foot muscle fibers projected a heavy vacuolization, severe degree of muscular atrophy,
sarcoplasmolysis, and loss of muscular integrity. The muscle bundles became highly wavy and there was total disappearance of the nuclei in pedal sinuses. The intensity of these changes was greater in the small animals than in those of large ones. These pathological changes confirmed the severe proteolysis and decreased protein content of these organs leading to the structural disruption and disintegration by high nickel concentrations. Above changes in the organs of snails exposed to sublethal concentration were mild in degree. However, moderate degenerative changes were noticed in the mantle, hepatopancreas and foot at day 5 in small and at days 5 and 10 in large animals, which also moved support for the decrease in the protein levels in these organs. Eventhough, a few mild degenerative changes were observed at day 5, on further exposures the organs regained normalcy in structural organization, as evident by the increase in soluble and structural proteins, in the organs of small size group. But, there was a little degree of regeneration in the tissue organization at day 30 of exposure in the organs of large size animals. This proved that small animals are metabolically more active than large snails.

10. On the whole, the biochemical and histological responses of the freshwater snail *Pila globosa* exposed to nickel were dependent on the concentration of the metal, period of exposure, specificity of the organ and size of the animal. High concentrations of the metals severely suppressed most of the biochemical activities of the cell on prolonged exposure. It was well evident by an irrecoverable histological damage to the organs of both size groups at lethal exposure. The small snails appeared more sensitive to the lethal concentration of nickel than the large size groups. The sublethal concentration of nickel initially suppressed some of the biochemical activities.
in the organs of both the size group of snails and caused cellular damage to mild to a moderate degree. However, small animals could overcome this stress by activating the compensatory mechanisms with the metabolic reorganization and elevation in their protein synthetic potentials. Such efficiency was relatively less in the large size group, as the compensatory mechanisms of them were geared up only after long period of exposure. It can be concluded that the freshwater snails can serve as good biological monitors as they could concentrate nickel in their tissues in large amounts. But the response patterns differed in them which not only dependent on the concentration of the metal in the ambient medium but also on the specificity of the organ, the duration of exposure to the toxic metal and size of the animal.