CHAPTER 6

FORAMINIFERAL RESPONSE TO DIFFERENT HEAVY METAL POLLUTANTS
Foraminiferal response to heavy metal pollutants

"Great things are not done by impulse, but by a series of small things brought together."
- Vincent van Gogh

6.1. Introduction

With the dawn of industrialization, human ways of life changed manifolds. Ways became more consumerist and materialistic, with proliferation of gadgets at its peak. Manufacturing units boomed up. Production increased with demand and thus pollution became rampant. The land on which humans lived, the air breathed were all gradually polluted one after the other. Then, it was water, the cradle of life. Waste, be it solid or liquid; from all the sources, land, water and air finds its abode in the sea. The saga continues. Pollution occurs when concentrations of various chemical or biological constituents exceed a level at which a negative impact on the ecosystem or human health can occur. Pollution results primarily from human activities. More specifically, in the marine context "Pollution of marine environment means the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such a deleterious effect as to harm living resources and marine life, hazardous to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of seawater and reduction of amenities" (http://www.helcom.fi/Convention/en_GB/text). Although marine pollution has a long history, significant international laws to counter it were enacted in the twentieth century. Marine pollution was a concern during several United Nations Conferences on the Law of the Sea beginning in the 1950s. The oceans have long been considered to have limitless capacity to receive and absorb all types of wastes. Since the 1950's many scientists began to warn that this limitless capacity is running out and the very survival of the marine environment was in danger. Marine pollution can be demarcated into deep sea and coastal pollution. Trade and commerce, merchandise, navigation, fisheries, marine resources etc. have always kept coastal areas bustling with human activities; and so are many reasons for coastal pollution. Moreover, coastal areas also serve as inlets for waste generated on inland regions. Most sources of marine pollution are land based. The pollution often comes
from non-point sources such as agricultural runoff and windblown debris. The sources of contaminants include sewage, urban run-off, industrial wastes, coastal development, and shipping activities. The chemical or biological constituents creating pollution are known as contaminants. More specifically, they can be divided into inorganic contaminants, such as metals; organic contaminants such as pesticides, PCB or petroleum hydrocarbons; Biological contaminants such as coliform bacteria and other pathogens.

Presently the harmful effect of heavy metals as environmental pollutants is widely known and is a matter of concern for the environmentalists because of its lasting effects on biota. Heavy metals are metallic chemical elements that have a relatively high density and are toxic or poisonous at low concentrations. All natural metals occur in sea water in greater or lesser amounts. Some, such as iron, copper, cobalt and zinc are essential in small quantities for the healthy growth of marine organisms. Others, such as mercury, cadmium and lead have no known biological role. All metals are toxic if present in excess but the most important marine contaminants are generally considered to be amongst the non-essential elements. Such toxins can accumulate in the tissues of many species of aquatic life in a process called bioaccumulation. They are also known to accumulate in benthic environments, such as estuaries and bay muds: a geological record of human activities of the last century.

Because they do not degrade or cannot be destroyed, heavy metals are persistent in all parts of the environment. Human activity affects the natural geological and biological redistribution of heavy metals through pollution of the air, water, and soil. The primary anthropogenic sources of heavy metals are point sources such as mines, foundries, smelters, and coal-burning power plants, as well as diffuse sources such as combustion by-products and vehicle emissions. Humans also affect the natural geological and biological redistribution of heavy metals by altering the chemical form of heavy metals released to the environment. Such alterations often affect a heavy metal's toxicity by allowing it to bioaccumulate in the food chain, thereby causing the vivid aftereffects to organisms at different levels of the food chain.

The international community is beginning to recognize the adverse health effects of heavy metals. In 1998, the United Nations proposed the protocol to the convention on long-range trans-boundary air pollution on heavy metals. This protocol is designed to reduce worldwide air emissions of mercury, cadmium and lead, but is yet to be officially adopted (http://www.answers.com/topic/heavy-metals).
This chapter deals with the effect of heavy metals mercury and cadmium on the most popular and widely studied marine protists — foraminifera and the following sections contain some background information on these two heavy metals as pollutants.

6.1.1. Mercury (Hg)

Among the heavy metal pollutants, Mercury (Hg) ranks as the most harmful toxin to the living organisms. In oxygenated marine sediments bacteria may convert the less toxic, inorganic form of the metal to the more toxic, organic form of methyl mercury. This chemical form is relatively mobile in the environment and tends to accumulate in fish. In the most disastrous case of Hg pollution ever reported, over 3000 people suffered from diverse disorders and even died, due to methyl mercury intoxication, which was later known as Minamata disease, from Minamata Bay, Japan. This was reportedly due to the consumption of fish contaminated with heavy concentrations of inorganic mercury discharged into the Minamata bay as waste water by a petrochemical company from 1932–1968. This episode raised a worldwide concern towards the harmful effects of environmental pollution and efforts were initiated towards progress in environment protection measures.

Mercury is an important industrial chemical used, for example, in the chlor-alkali industry and in the manufacture of small batteries. Though the reported amount of dissolved mercury in the marine water sounds negligible, i.e. less than 2 ng/l in open oceans and 50-1000 ng/l of the suspended matter along the continental margins (Mason & Fitzgerald, 1996), the concern lies in the fact that it gets biomagnified in the food chain, resulting in higher concentration of this toxic metal in higher level organisms up in the food chain. Through different edible seafood, such as fish and bivalves, Hg finds its way into human beings. Mercury which is a neurotoxin can lead to multifold impacts in humans like memory loss, impaired coordination, vision disturbances, cardiovascular problems, etc. It also affects the thyroid gland, digestive system, liver and skin.

Despite these alarming effects and worldwide concern, as yet no significant effort has been made in India to prevent this heavy metal from reaching the water sources. A news report based on the first global study on mercury by UNEP, states that India may become a hotspot for mercury poisoning owing to the upsurge in gold mining over the last three decades, as reported in “The Times of India” (Mago, 2003). Although there are some reports on Hg pollution, few researchers have studied concentration of mercury in waters off the western coast of India (Singbal et al., 1978; Krishnakumar & Bhat, 1998;
Kaladharan et al., 1999). A minimum of 26 ng/l and a maximum of 187 ng/l of mercury in the regions Off Goa along the west coast of India have been reported (Singbal et al., 1978). Although, based on field studies, a few attempts were made to document benthic foraminiferal response to trace metal pollution along the Indian coasts (Naidu et al., 1985; Sivakumar et al., 2008), harmful effects of Hg were not discussed.

6.1.2. Cadmium (Cd)

Cadmium (Cd), like mercury, is an industrially important metal which is used directly in plastics and electroplating and is also found in applications associated with zinc and phosphorus. Although cadmium has no known biological role it is taken up by marine phytoplankton; apparently by the same mechanism employed for the uptake of the essential nutrient element phosphorus. It has been demonstrated to stimulate phytoplankton growth and photosynthesis up to surprisingly high levels. Even in quite heavily contaminated marine systems cadmium is very unlikely to cause acute toxic effects to marine animals but there have been at least two instances where cadmium pollution has apparently been responsible for adverse effects to humans. The best known example of this was the outbreak of itai-itai disease in Japan which affected a village on the Jintsu river in Japan. The use of irrigation water, contaminated with cadmium from a zinc processing plant, in paddy fields was originally identified as the cause of the problem but other factors such as malnutrition and vitamin deficiency have also been cited as major contributory parameters. Itai-itai disease is characterized by brittle bones and considerable pain (the name means 'ouch-ouch') and the symptoms can be alleviated by the administration of large doses of vitamin D. The other well known instance of cadmium poisoning was caused by the consumption of contaminated Tasmanian oysters which led to nausea and vomiting in the victims.

Various chemical, physical, physiological as well as biological methods have been formulated and adopted to detect marine pollution. Conventional biological methods of pollution detection have included the estimation of levels of pollutants in both micro and macro marine organisms. Marine organisms like bivalves, fishes etc have the potential to sequester toxic pollutants in their tissues and gradually, these toxicants get biomagnified as they traverse the higher trophic levels. These organisms can detect and incorporate pollution signatures of a very short time span in them, and cannot be representative of the past, either remote or near.
Marine pollution is manifest when organisms of the higher trophic levels are affected, so a base level detection of pollutants is not achieved. Many species of marine microorganisms like bacteria have been extensively used to detect marine pollution on a base level but since bacteria lack fossilization potential they are again of limited use in recording the marine environment of the distant or the near past.

An effective tool to curb the problem of time series pollution detection is by using foraminifera as proxy for pollution detection. Foraminifera are microscopic, unicellular, almost exclusively marine organisms with a hard test. Foraminifers occupy the base level of the food chain. They are abundantly distributed vertically as well as geographically. Being microscopic they have a very short life span. They are highly sensitive to the changes, which occur in their ambience. These changes get incorporated in their test. The test has fossilization potential and the changes taking place in its environment gets preserved in their test even after the death of the organism. Thus foraminifer's fossils from a given region serve as an effective tool to detect the quality of the marine environment (including pollution) of that region at that given time as well as of the past.

6.1.3. Benthic foraminifera in marine pollution studies

Benthic foraminiferal distributions in polluted areas have been investigated for more than four decades and several workers have pointed out that these organisms provide one of the most sensitive markers available for inferring the deterioration of marginal marine environments (Alve, 1995). After pioneering work on the effects of pollution on benthic foraminifera by Zalesny (1959), pollution studies using benthic foraminifera as proxy indicators were started by Resig (1960) and Watkins (1961). Since then numerous workers focused on the effects of various pollutants on biota from different marginal environments. Initially, most of such studies dealt with the effects of organic waste contamination (Bandy et al., 1964a, 1964b; Seiglie, 1968; Schafer, 1973, Schafer et al., 1975), and little attention was paid to understanding the response of foraminifera to heavy metal pollution (Alve, 1991; Nigam et al., 2006b).

The majority of the above-mentioned studies reported variation in species abundance in the polluted areas. Also, there were reports of deformed tests from the polluted sites. Although morphological deformities in fossil and recent foraminiferal tests from polluted sites have been noticed since the 19th century (Carpenter, 1856; McCrone & Schafer, 1966; Setty & Nigam, 1984; Naidu et al., 1985; Bhalla & Nigam, 1986; Stouff et al.,
1999a; Yanko et al., 1999; Naidu et al., 2000; Debenay et al., 2001; Frontalini & Coccioni, 2008; Romano et al., 2008), the interpretation of these results was doubted when similar deformities were reported from areas subjected to natural stress like abnormal salinity (Freudenthal et al., 1963; Hofker, 1971; Brasier, 1975; Reiss & Hottinger, 1984; Nigam et al., 2006a), change in nutrient levels (Murray 1963) and rapid change in environmental conditions (Scott & Medioli, 1980). Boltovskoy & Wright (1976) opined that abnormalities in foraminiferal test can also be due to mechanical damage. If such ambiguities are to be solved, there should be some standard results in controlled conditions wherein the effect of different parameters can be studied in different combinations and with which the field results can be compared.

Laboratory culture experiments provide such continuous and accurate observations on the foraminiferal response under controlled conditions. Here a single parameter can be altered, keeping the rest constant in order to observe the foraminiferal response to variation in a particular parameter. In this way, foraminiferal response to specific parameters can be characterized, adding credibility to the field based observations (Nigam et al., 1996a; 1996b; Saraswat et al., 2004; Filipsson, 2008).

In the light of the discussion above, the effect of varying concentrations of heavy metals mercury and cadmium on benthic foraminiferal species *Rosalina leei* and *Pararotalia nipponica* respectively was studied through laboratory experiments and the same has been described in the following segments of this chapter.

Samples containing live foraminifera for the experiments were collected from the shallow-water areas off Goa, the central west coast of India near to National Institute of Oceanography, Goa as per the methods described in chapter 3.

### 6.2. Gradual increase in mercury concentrations: Effect on benthic foraminifera *Rosalina leei*

#### 6.2.1. Experimental setup

The juvenile specimens of *Rosalina leei* were subjected to different concentrations of mercury prepared by dissolving water soluble mercuric chloride in seawater. One set was maintained without any mercury and served as control. A total ten sets of media were prepared with different mercury concentrations ranging from 20 ng/l to 180 ng/l at 20 ng/l intervals. In order to avoid a sudden shock to the organisms, the Hg concentration was increased gradually at every alternate day keeping one set at each concentration (Fig. 6.1). Two specimens each were maintained in all mercury
concentrations prepared. To find out the maximum Hg tolerance limit of this species, the specimens kept at 180 ng/l were later subjected to mercury concentrations up to 260 ng/l. Measurements such as the maximum diameter and number of chambers were taken at every alternate day. Additional observations like the pseudopodial activity, shape and orientation of newly added chambers were also made. Other parameters including salinity (35 %), temperature (Room Temperature), feed (*Navicula*) and light (12 hr light- 12 hr dark) were maintained uniform throughout the experiment 6.2.2. Results

The specimens showed extensive pseudopodial activity at the onset of the experiment. With gradual increase in the mercury concentration, the pseudopodial activity started declining and almost ceased in 4-5 days in case of specimens subjected to 100 ng/l. The specimens were still alive and were accumulating food near the last chamber very slowly. Similar trend was observed in the growth rate also. The maximum growth in diameter attained by the specimens showed an inverse relation with the mercury concentration in the medium (Fig. 6.2), i.e. specimens kept at higher Hg concentration attained comparatively less growth. Growth nearly ceased after 40 days at all concentrations, but the specimens continued to live. The specimens were alive even when the concentration was increased to 260 ng/l. The maximum size attained by the experimental specimens was less than the normal size of the field specimens. Most importantly, it was found that chambers added to the specimens kept at higher concentrations of mercury, were abnormally large and with unusual orientation.
Trays with gradually increasing Hg concentration (ng/l) with days of observation

Fig. 6.1: Set up of Experiment to decipher the effect of gradual additions of mercury on Rosalina leci:
Days = days of observation; Trays (represented by the rectangles) are labeled with their respective concentrations of mercury (in ng/l) on each day of observation
Fig. 6.2: The maximum growth attained at different concentrations of mercury in the experiment in which the amount of Hg was increased gradually

6.3. Sudden increase in mercury concentrations: Effect on benthic foraminifera *Rosalina leei*

6.3.1. Experimental Setup
Juvenile specimens of *Rosalina leei* were subjected to different concentrations of mercury prepared by dissolving water soluble mercuric chloride in seawater. One set was maintained without any mercury and served as control. A total twelve sets of media were prepared with different mercury concentrations from 25 ng/l to 300 ng/l at 25 ng/l intervals. In order to see the response of benthic foraminifera to sudden stress, the organisms were directly exposed to the respective concentrations (Fig. 6.3). Five specimens each were maintained in all mercury concentrations prepared. Other parameters such as salinity (35 %), temperature (25 °C), feed (*Navicula*) and light (12 hr light - 12 hr dark) were maintained uniform throughout the experiment. The specimens were observed every third day and their responses were observed.
Fig. 6.3: Set up of Experiment to decipher the effect of sudden additions of mercury on *Rosalina leei*:
Trays (represented by rectangles) are labeled with their respective concentrations of mercury (ng/l) during the experiment.
6.3.2. Results

For the first 40 days of the experiment, the specimens did not show any visible change in morphology, but later morphological abnormalities started to appear. The abnormalities included larger than normal size of the last chambers, and abnormal orientation and shape of the new chambers (Plate 6.1). When the percentage of specimens deformed at each concentration is plotted, it showed that up to 150 ng/l, 20% - 75% of the specimens were deformed, whereas at Hg concentrations above 150 ng/l and up to 275 ng/l, all the specimens showed deformation (Fig. 6.4). Specimens kept at 300 ng/l died after an exposure of 19 days to the mercury concentration. None of the control specimens showed any morphological deformation throughout the experiment.

Apart from the morphological deformation irregular reproduction was also noticed in the experimental specimens (Fig. 6.5). As compared to the control specimens, a higher number of specimens subjected to Hg concentration reproduced.

Fig. 6.4: Percentage of deformed specimens at different mercury concentrations in the second set of experiment where Hg was suddenly introduced.
Fig. 6.5: (a) Reproduction in specimens kept in media without Hg (b) Reproduction in specimens kept in higher Hg concentrations
Plate 6.1: Different types of abnormalities (B-M) reported in specimens subjected to higher concentrations of Hg. Fig A shows a normal specimen. Scale bar = 100 μm
At lower concentrations (25 ng/l, 50 ng/l), none of the specimens reproduced throughout the experiment, but at 75 ng/l to 225 ng/l, reproduction was reported at all seven concentrations. In specimens subjected to 75 ng/l, 100 ng/l, 175 ng/l, 200 ng/l and 225 ng/l, the number of juveniles produced after reproduction varied from 6-12 but in case of specimens subjected to 125 ng/l and 150 ng/l Hg concentration, the number of juveniles were 23 and 20 respectively. The juveniles died within a day or two in all the cases. At still higher concentrations 250 ng/l and 275 ng/l, juveniles did not come out of the mother specimen at all. On the contrary, reproduction in the control specimens produced a minimum of 30-35 juveniles per mother specimen.

6.4. Gradual increase in Cadmium concentrations: Effect on benthic foraminifera *Pararotalia nipponica*

6.4.1. Experimental Setup

For the experiment, cadmium chloride monohydrate (atomic weight 201.32) was used to prepare a stock solution of 500 µg/l concentration by dissolving CdCl₂·H₂O in filtered seawater (CdCl₂ being the soluble form of cadmium). The range of cadmium concentration was decided on the basis of previous literature. According to Krishnakumar *et al.*, (2004), the mean cadmium concentration in seawater samples collected from west coast of India was 2.95 µg/l, However, at a few stations the Cd Concentration reached even up to 8.15 µg/l. Based on these records it was decided to subject the foraminifers to Cd concentration range from 2 µg/l to 14 µg/l with an interval of 2 µg/l. In order to avoid a sudden shock to the foraminifers the cadmium was added gradually in to the media starting from 2 µg/l till the desirable concentration is reached as per the setup (Fig. 6.6.

A set of four specimens was maintained at each concentration with replicates. Before the onset of the experiment, all the specimens were photographed and measured for the maximum diameter and number of chambers using the inverted Microscope (software ACT-2U). Prior to addition of every higher Cd concentration, the specimens were photographed and its maximum diameter was measured in order to observe and record the changes brought about by Cd administration. After adding the Cd solution of highest concentration (14 µg/l), the concentration was further increased in order to understand the tolerance of *P. nipponica* to cadmium.
Fig. 6.6: Set up of Experiment to decipher the effect of gradual additions of cadmium on *P. nipponica*

Days = days of observation; Trays (represented by the rectangles) are labeled with their respective concentrations of cadmium (in μg/l) on each day of observation.
The set-up of the experiment is explained in figure 6.6, where the rectangles indicate the experimental trays where cadmium concentrations were gradually increased from 2 µg/l to 14 µg/l. Specimens were monitored throughout the experiment for their response to cadmium in terms of growth and morphological changes.

6.4.2. Results
At the onset of the experiment all specimens were healthy and showing extensive pseudopodial activity and actively feeding up on the diatoms. With the progressive addition of cadmium into the media the specimens other than the control specimens showed reduction in their pseudopodial activity and developed visibly abnormal chambers. This phenomenon was common at all cadmium concentrations right from the lowest 2 µg/l to the highest 14 µg/l; only the degree of deformity in the specimens varied. The specimens at higher concentrations showed severe deformities compared to the ones kept in lower concentrations (plate 6.2).

Fig. 6.7: The maximum growth attained at different concentrations of cadmium in the experiment in which the amount of Cd was increased gradually
An interesting observation regarding the pattern of deformation in the specimens grown at high Cd concentration are the changes in coiling direction. The abnormal chambers formed during the experiments were added in to a different plane as evident from the comparison between the control specimen and experimental specimens given in plate 6.2.

Due to the same reason, the growth could not be measured effectively and the changes in the maximum diameter of the specimens during the experiment do not show any trend with the increase in concentration of cadmium in to the media (Fig. 6.7). But compared to the controlled specimens, all the experimental specimens show less growth. While the control specimens grown in field conditions without any cadmium showed an average growth of 124.31μm, all the experimental specimens subjected to various cadmium concentrations from 2μg/l to 14 μg/l showed growth varying from 112.69 μm to 44.02 μm. All the experimental specimens were alive in all the concentrations during the span of the experiment. The concentration of cadmium was further increased beyond 14 μg/l to 16 μg/l in the final set; but the specimens continued to survive though remained inert.

6.5. Discussion

Heavy metals certainly have deleterious effect on benthic organisms in general (Aschan & Skullerud, 1990) and foraminifera in particular (Yanko et al., 1998). Much has been discussed on the controversies surrounding the use of morphological deformities of foraminiferal tests as a tool to identify the presence of various pollutants. The concern is to characterize and differentiate between the foraminiferal response to natural stress (such as temperature, salinity changes) from that to anthropogenic pollutants through a series of field and laboratory culture studies (Nigam et al., 1996a, b; Yanko et al., 1998; Samir, 2000; Scott et al., 2001; Geslin et al., 2002, Saraswat et al., 2004; Romano et al., 2008). Working towards this objective, laboratory culture experiments were attempted to study the response of the benthic foraminifer -

- *Rosalina leei* to varying (gradual as well as sudden changes) concentrations of heavy metal mercury
- *Pararotalia nipponica* to varying concentrations of heavy metal cadmium; which are discussed separate in the coming sections for effective and clear conveying of the ideas.
Plate 6.2: Different types of abnormalities (B-M) reported in specimens subjected to different concentrations of cadmium. Fig A shows a normal specimen. Scale bar = 100 μm
6.5.1. Effect of heavy metal mercury on *Rosalina leei*

A distinct difference is noted in the response of *R. leei* to gradual and sudden increase in Hg concentration. The results of the first experiment show that gradual exposure to mercury concentrations affected the normal growth of the specimens and the growth was inversely proportional to Hg concentration. Although deformation was reported, but only in specimens subjected to higher Hg concentrations and the number of abnormal specimens was very low. Contrastingly, in specimens subjected to sudden stress, the percentage of deformed specimens was very high (75-100% of the total specimens per conc.). Abnormalities included change in the plane of addition of new chambers, leading to no net increase in the maximum diameter of the specimen. As, in order to measure incremental and overall growth, size in terms of maximum diameter of the individual specimen has to be measured, such measurements were not possible in highly deformed specimens under sudden exposure to different Hg concentrations. Therefore, percentage of deformed specimens was plotted which shows a good correlation with different Hg concentrations. Although the number of deformed specimens was more while being subjected to sudden stress, the test abnormalities were similar as that in the earlier experiment, i.e. abnormal size, shape and orientation of newly added chambers. A significant effect on growth but low number of deformities in specimens subjected to gradually added Hg, while a large number of deformities in specimens subjected to sudden Hg exposure, probably indicate the adaptive capability of *R. leei*. In the experiment wherein Hg was added gradually, probably no severe damage was done to the normal physiology of the *R. leei* specimens and enough time was available to adapt as per the changed condition by way of lowering the growth rate. Whereas, in specimens directly subjected to different concentrations of Hg, irreparable damage to the soft tissue probably leads to increased abnormalities.

The decline in the pseudopodial activity at the early stages of both the experiments indicates that the cytoplasm is immediately affected by the presence of Hg in the medium. Similarly the inverse relationship between Hg concentration and growth indicates that the presence of heavy metal in the medium inhibits the normal metabolic activity of the organism. The decreased metabolic activity slows down the growth, as a result of which, the specimens subjected to different mercury concentrations attain lower average size as compared to the field specimens. This explains the occurrence of stunted specimens at polluted sites (Yanko *et al.*, 1994, 1998; Samir & El-Din, 2001).
The findings confirm the views of Boltovskoy & Wright (1976) who noted that 'the presence, absence, disequilibria or inter-relations of some of the trace elements in individual organisms can retard or stop normal growth, can provoke abnormal development (monstrosities) and can even induce death'.

The addition of abnormal chambers at higher mercury concentrations and morphological abnormalities after prolonged exposure to the pollutant, explains the occurrence of abnormal tests in areas subjected to various pollutants as reported for decades by a number of previous workers (Watkins, 1961; Lidz, 1965; Seiglie, 1971, 1975; Bhalla & Nigam, 1986; Sharifi et al., 1991; Alve, 1991a; Yanko et al., 1998; Geslin et al., 1998; Samir, 2000; Debenay et al., 2001; Samir & El din, 2001; Scott et al., 2005, Bergin et al., 2006, Frontalini & Coccioni, 2008). Since, during the experiment, all the parameters other than the Hg concentration were kept constant, the response can be attributed to the effect of mercury.

Increased instances of stress induced reproduction were one of the main effects of sudden addition of Hg, as seen in second experiment. Specimens subjected to all but 25 ng/l and 50 ng/l Hg concentrations, reproduced under sudden addition of Hg into the medium. Additionally, reproduction was significantly different in specimens subjected to sudden Hg stress, than that in control sets. The specimens subjected to sudden addition of Hg, produced less number of juveniles and they could not survive for more than 2 days in the medium. Additionally, in specimens subjected to the highest Hg concentration, juveniles could not come out and died within the mother cell. Although reproduction was also noted in earlier experiment (gradual addition of Hg), but only a few specimens subjected to comparatively high Hg concentration reproduced.

Reproduction in foraminifera is controlled by the environmental factors. The optimum range of environmental conditions required for the successful reproduction in foraminifera is very narrow compared to the optimum range for their survival (Murray 1963). A deviation from the optimum conditions cause variations in the reproductive behaviour in foraminifera. Although few in number, there are previous reports addressing this particular aspect of the ecological preferences of foraminifera. Myers (1935b) and Bradshaw (1957) reported that lower temperature leads to a delayed reproduction in foraminifers. In a subsequent study Bradshaw (1961) reported that higher temperatures lead to quick reproduction in foraminifera. Ross (1977) maintained that reproduction is linked to the seasonal changes in food. Hemleben and Kitazato (1995)
reported that the culture maintained without food survived for longer duration but reproduced less than the ones maintained under continuous food supply. This shows the influence of food on the normal reproduction in foraminifers. Like the natural stresses, anthropogenic pollutants also control the reproduction in foraminifers. Moodley et al. (1998a) reported that sulphidic conditions resulted in total lack of reproduction in foraminifera. According to Cadre and Debenay (2006), copper contamination resulted in delayed reproduction, whereas in another recent study by Ernst et al. (2006), few species reproduced quickly under induced oil pollution. In the light of these previous studies it can be concluded that foraminiferal response varies with stresses. In the present study, It was observed that number of reproduction in specimens subjected to the increased concentration of Hg is more as compared to the control specimens. The observed differences in reproduction and the number of juveniles produced, in specimens maintained with and without Hg, conclusively show that heavy metal pollutants affect the normal growth as well as reproduction in Rosalina leei. The death of the juveniles soon after the reproduction, in the specimens kept at high Hg concentration, may be because of the fact that the juveniles from this abnormal reproduction were probably not healthy to cope with the sudden stress in the form of high Hg content. In the light of the findings, this study reinforces the views expressed by Alve (1991b) that “in extreme cases of heavy metal pollution, the organism devotes it energy to protect itself. As a result, such an individual has little ability left for protein synthesis. This inhibits the energy budget, reproduction cycle, and also harms the cytoskeleton”. This may also be a probable explanation for the reduced number or gradual absence of some species of benthic foraminifera from the areas subjected to pollution. As there are not many previous reports in this line, the present attempt is significant in characterizing the foraminiferal response to different heavy metal pollutants.

6.5.2. Effect of heavy metal cadmium on Pararotalia nipponica

The decline in the pseudopodial activity in the experimental specimens other than the control specimens suggests that the cytoplasm is immediately affected by the addition of cadmium in to the media and is explained on the basis of the studies by Bresler and Yanko (1995), which says that there is significant biological influence of heavy metals on foraminiferal cytoplasm.
The lesser growth attained by the specimens grown in media with cadmium compared to the specimens grown without cadmium indicates that the presence of cadmium in the medium inhibits the normal metabolic activity of the organism thereby slowing down the growth, resulting in stunted specimens. This explains the occurrence of stunted specimens at polluted sites (Yanko et al., 1994, 1998; Samir & El-Din, 2001).

The addition of abnormal chambers during the course of the experiment in the specimens subjected to cadmium concentrations suggest that the heavy metal cadmium is adversely affecting the *P. nipponica* specimens and since all parameters other than the cadmium concentrations were maintained constant, the response can be attributed to the presence of cadmium in the media. The development of deformities in all the specimens subjected to different Cd concentrations right from the lowest 2 µg/l to the highest (14 µg/l) indicates the toxic effect of the heavy metal cadmium even at lower concentrations. The change in the coiling plane due to the addition of abnormal chambers to a plane different than the normal specimens is a peculiar observation made from the experiment. The reduction in growth by the cadmium addition and the development of abnormalities are in a way similar to the effect of mercury and once again confirm the views of Boltovskoy & Wright (1976) who noted that 'the presence, absence, disequilibria or inter-relations of some of the trace elements in individual organisms can retard or stop normal growth, can provoke abnormal development (monstrosities) and can even induce death' and once again explains the occurrence of abnormal tests in areas subjected to various pollutants as reported for decades by a number of previous workers (Watkins, 1961; Lidz, 1965; Seiglie, 1971, 1975; Bhalla and Nigam, 1986; Sharifi et al., 1991; Alve, 1991b; Yanko et al., 1998; Geslin et al., 1998; Samir, 2000; Debenay et al., 2001; Samir & El din, 2001; Scott et al., 2005, Bergin et al., 2006, Frontalini and Coccioni, 2008) as explained in the section above.

### 6.6. Conclusions

#### 6.6.1. Effect of heavy metal mercury on Rosalina leei

- The response of benthic foraminifera *Rosalina leei* is different and distinct to gradual and sudden stress conditions.
- On gradual increase in the mercury concentrations, specimens showed lesser growth with increasing Hg concentration.
- Sudden addition of mercury increased abnormal reproduction which is significantly different from the normal pattern of reproduction in this species.
- Morphological abnormalities developed in both gradual as well as sudden additions of mercury; but percentage of abnormalities was negligible in gradual addition of mercury as compared to that in sudden addition where 75% (at 100-150 ng/l) – 100% (at 175-275 ng/l) of the specimens were reportedly deformed.
- Though the number of deformed specimens vary considerably in both experiments, the main type of morphological abnormalities remain similar which is significant to characterize the response of *R. leei* to this particular pollutant.

6.6.2. Effect of heavy metal cadmium on *Pararotalia nipponica*
- The specimens subjected to various cadmium concentration attained smaller size than the control specimens.
- Morphological abnormalities developed in all concentration of cadmium; severity of deformation increased with increase in cadmium concentration
- Change in coiling direction was peculiar to the type of morphological deformation of the specimens.

6.7. Significance of the study
Deformities in foraminiferal tests from the polluted environments have been one of the important aspects of pollution monitoring studies utilizing foraminiferal characteristics. Despite the large number of studies monitoring increased deformities in tests from polluted environments, this characteristic is still to attain the status of an effective proxy in pollution monitoring due to the prevailing reports of abnormal tests from naturally stressed environments. There has been the need to differentiate and characterize the benthic foraminiferal response to natural as well as anthropogenic stresses. Laboratory culture studies under controlled conditions where foraminiferal response to single parameters can be monitored effectively is the best solution to address this problem.

Present study is an attempt towards this direction where foraminiferal responses to two heavy metals are discussed. The findings confirm the fact that foraminiferal responses can be of significant use to field based studies to decipher marine pollution which is very significant as early warning signals of pollution.