

Effects of sudden stress due to heavy metal mercury on benthic foraminifer *Rosalina leei*: Laboratory culture experiment

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Abstract

Laboratory culture experiments were carried out to understand the response of benthic foraminifer *Rosalina leei* to gradual as well as sudden addition of heavy metal mercury into the media. When mercury was added suddenly, specimens did not show any change in morphology during the initial 40 days. However, later on, out of all the specimens subjected to mercury concentrations upto 150 ng/l, 75% developed deformities, whereas all the specimens subjected to 150-275 ng/l Hg concentrations, had deformed chambers. All specimens kept at 300 ng/l Hg concentration died within 20 days. In addition to this, irregularities were also observed in the rate of reproduction, number of juveniles produced and the survival rate of the juveniles. Where as in an earlier experiment where Hg concentration was increased gradually, irregularities in the newly added chambers were noticed only in case of specimens subjected to very high (180 ng/l) Hg concentration. However, during this experiment, growth was found to be inversely proportional to the mercury concentration.

Keywords: Hg, Pollution, Benthic Foraminifera, Culture Experiment, Abnormalities, *Rosalina leei*

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1. Introduction

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 (e.g. sewage, paper and pulp mills), 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., 1999; 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).

Presently the harmful effect of heavy metals as environmental pollutants is widely known and is a matter of concern for the environmentalists because of their lasting effects on biota. A few elements, notably mercury (Hg), cadmium (Cd) and lead (Pb) have been reported in anomalously high concentrations from marine environments. Among these, Hg ranks as the most harmful toxin to the living organisms. 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. 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, 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.

In the light of the discussion above,, we studied the effect of varying concentrations (both gradual as well as sudden additions) of heavy metal mercury on the benthic foraminiferal species *Rosalina leei* in laboratory culture.

2. Materials and Methods

2.1 Selection of species and environmental set up at sampling location

Samples containing live foraminifera were collected through diving in shallow-water areas off the central west coast of India near to National Institute of Oceanography, Goa. The sampling location was selected on the basis of availability of live specimens and proximity to the laboratory for regular sampling and supply of seawater (Fig. 1). The material included both sediment samples as well as marine algal samples. The samples were collected in pre-labeled polythene bags containing seawater. Once brought to the shore, the samples were transferred to plastic tubs filled with filtered seawater and shaken vigorously to detach the foraminifera from the substrate, and then sieved through a stack of 2 sieves (800 μm and 63 μm). The sieve kept on the top with mesh size 800 μm was used to get rid of the extraneous material whereas the lower sieve (63 μm size) was used to concentrate foraminifers. The >63 μm sample was collected in beakers along with sea water and brought to the laboratory.

The material so collected, was observed under reflected light stereo zoom microscope for live specimens. Out of the various species available in the study area, *Rosalina leei* was chosen because of its high abundance in the collected samples and also as they are easily cultured in the laboratory (Nigam & Caron, 2000). The first results of this study were reported by Saraswat et al. (2004) and this study is in continuation of the previous experiment. Live specimens of *R. leei* were picked and kept under observation for few days, in multi-well culture dishes. The living status of such specimens was confirmed under the inverted microscope by observing the pseudopodial movements, food capture etc. As it is necessary to study the optimum requirements of the species in order to successfully grow it in the laboratory cultures, the live specimens were kept under

different combinations of environmental parameters such as salinity, temperature, etc. This was done in accordance with the seasonal variation of this species in the field (Table 1). In foraminifers, both optimum growth and reproduction range of physico-chemical parameters are variable. The latter is comparatively a narrow one; the preliminary experiments were conducted to study their optimal conditions for growth as well as reproduction under laboratory conditions. Once the specimens reproduced, the juveniles were maintained in the same conditions and were subjected to further experiments.

2.2 Experiment 1 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. 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 (See Saraswat et al., 2004 for further details).

2.3 Experiment 2 Setup

Similar to the first experiment, 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. 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.

3 Results

3.1 Experiment 1 (Gradual Addition of Mercury)

The specimens showed extensive pseudopodial activity for 12-15 days 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. Maximum growth in diameter attained by the specimens showed an inverse correlation with the mercury concentration in the medium (Fig. 2), i.e. specimens kept at higher Hg concentration attained comparatively less growth (Saraswat et al., 2004). 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 larger and with unusual orientation.

3.2 Experiment 2 (Sudden Addition of Mercury)

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 (Plate1). When the percentage of specimens deformed at each concentration is plotted, it showed that up to 150 ng/l, 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. 3). 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 we noticed irregular reproduction in the experimental specimens (Fig. 4a, 4b). As compared to the control specimens, a higher number of specimens subjected to Hg concentration reproduced. 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.

4. 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, we attempted laboratory culture experiments to study the response of the benthic foraminifer *Rosalina leei* to varying (gradual as well as sudden changes) concentrations of heavy metal mercury.

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, 1991; 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 (Table 2) 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 (1935) 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 & 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. (1998) reported that sulphidic conditions resulted in total lack of reproduction in foraminifera. According to Le Cadre & 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, we observed higher rate of reproduction in specimens subjected to the increased concentration of Hg 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 (1991) that “in extreme cases of heavy metal pollution, the organism devotes its 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.

5. Conclusions

From the present study we conclude that the response of benthic foraminifera *R. leei* is different and distinct to gradual and sudden stress conditions. When subjected to gradual stress, growth of the specimens showed an inverse relation to the Hg concentration, but percentage of abnormalities was negligible as compared to that in sudden stress where 75-100 % of the specimens were reportedly deformed. Though the numbers of deformed specimens vary considerably in both experiments, the main type morphological abnormalities remain similar which is significant to characterize the response of *R. leei* to this particular pollutant. Sudden stress also caused increased number of stress induced

reproduction which is significantly different from the normal pattern of reproduction in this species. It is evident from the results that heavy metal pollution influences size of foraminifera and abundance of abnormal specimens in population. The findings once again confirm the fact that foraminiferal responses can be of significant use to field based studies to decipher marine pollution.

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Legend to Figures:

Figure 1: Sampling station near National Institute of Oceanography, Goa.

Figure 2: The maximum growth attained at different concentrations of mercury in the experiment in which the amount of Hg was increased gradually (Modified after Saraswat *et al.*, 2004).

Figure 3: Percentage of deformed specimens at different Hg concentrations in the second set of experiment where Hg was suddenly introduced.

Figure 4: Reproduction in specimens kept in media without mercury (a) and at higher Hg concentrations (b). In case of reproduction in control specimens (without Hg) juveniles came out, whereas in the specimens with high amount of Hg in the media, juveniles did not come out of the specimen.

Legend to Tables:

Table 1: Details of physico-chemical parameters at the sampling station, taken from previous reports (Rodrigues, 1984) and collected as part of the present study.

Table 2: Details of physico-chemical conditions in the second set of experiment wherein *R. leei* was subjected to 12 different Hg concentrations varying from 25 ng/l to 300 ng/l at an interval of 25 ng/l. All the specimens were subjected to a constant 35 ‰ salinity and 25° C temperature.

Legend to Plate:

Plate 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

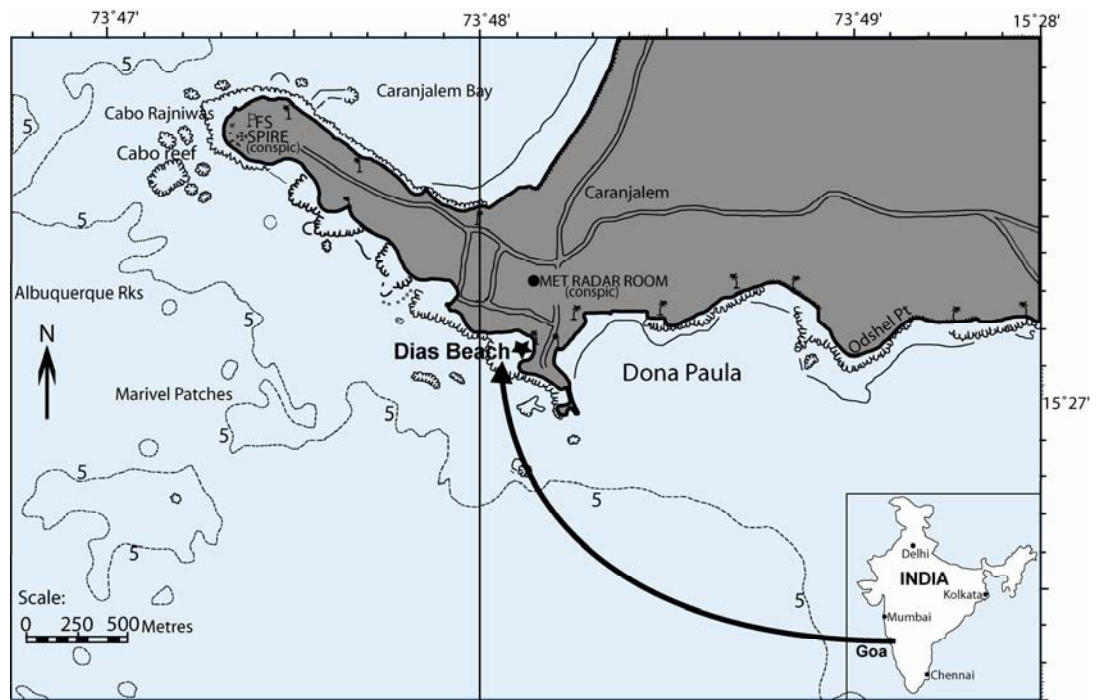


Fig. 1.

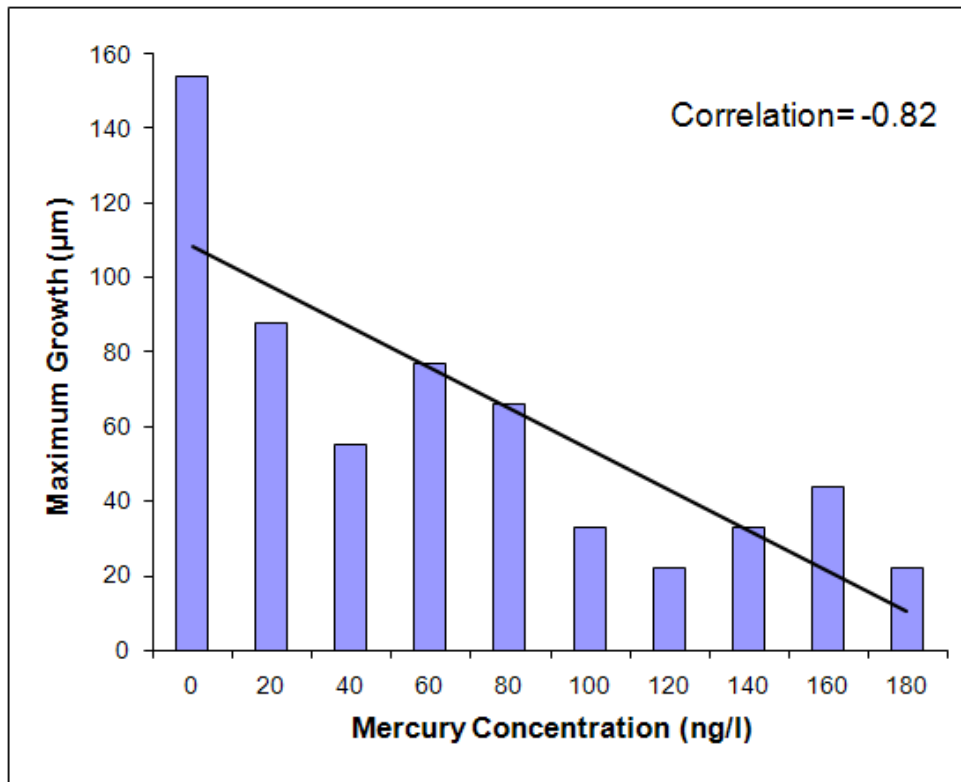


Fig. 2.

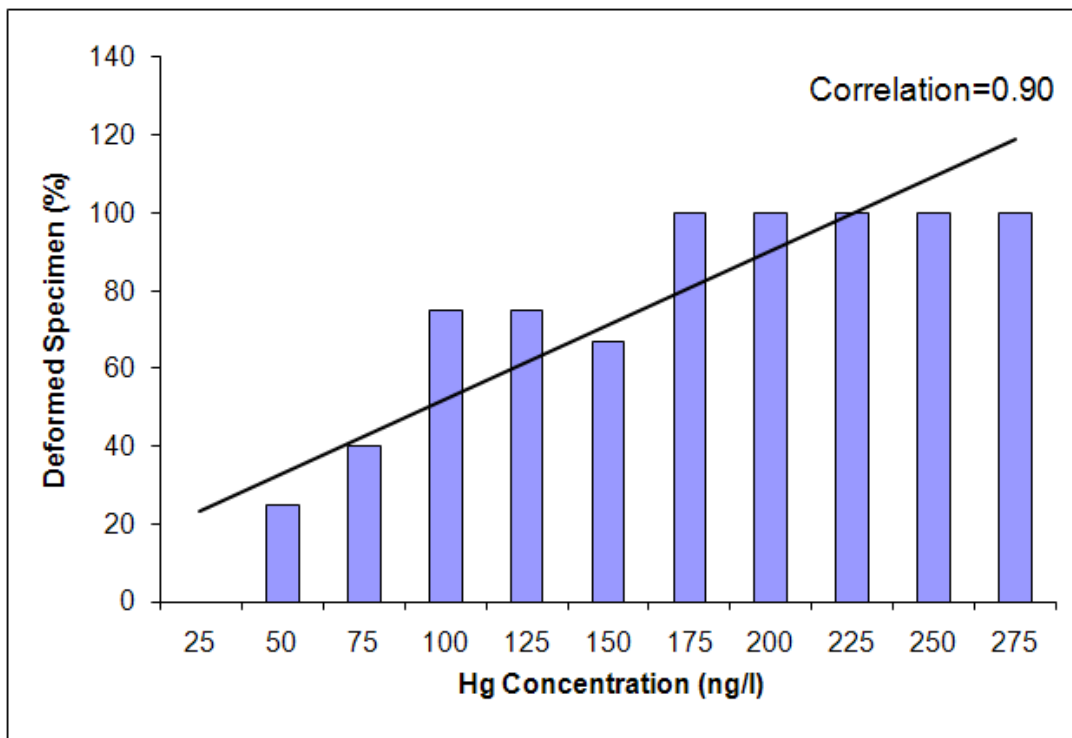


Fig. 3.

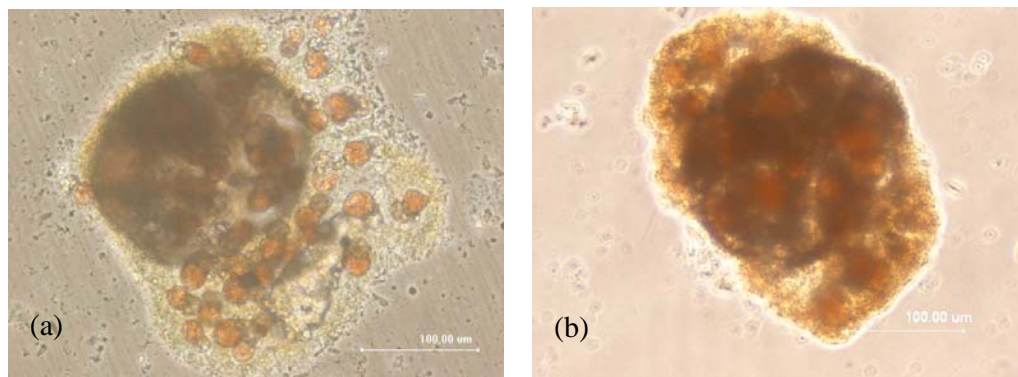


Fig. 4.

TABLE 1

		1981-1983 (Rodrigues, 1984)	2005-2007
Temperature (°C)	Pre-monsoon	26.6 ± 0.5 to 30.2 ± 0.3	27 - 31
	Monsoon	26.1 ± 0.4 to 29.2 ± 0.2	26 (Aug)
	Post-monsoon	24.8 ± 0.4 to 30.6 ± 3.91	26 - 30
Salinity (‰)	Pre-monsoon	30.84 ± 1.34 to 36.57 ± 3.91	33 - 37
	Monsoon	11.76 ± 1.45 to 30.53 ± 1.06	10 - 35
	Post-monsoon	28.52 ± 1.72 to 34.00 ± 0.77	32 - 36
pH	Pre-monsoon	7.65 ± 0.04 to 8.14 ± 0.09	7.90 - 8.22
	Monsoon	7.74 ± 0.08 to 7.89 ± 0.07	7.20 - 8.04
	Post-monsoon	7.69 ± 0.03 to 7.95 ± 0.02	7.83 - 8.01

Premonsoon: February-May; Monsoon: June-September; Postmonsoon: October-January

TABLE 2

Hg Concentration	pH
25 ng/l	7.72
50 ng/l	7.73
75 ng/l	7.72
100 ng/l	7.74
125 ng/l	7.78
150 ng/l	7.75
175 ng/l	7.76
200 ng/l	7.75
225 ng/l	7.76
250 ng/l	7.74
275 ng/l	7.74
300 ng/l	7.73

Initial Seawater Salinity – 35 ‰, pH – 7.73 and Dissolved Oxygen – 6.41 mg/l

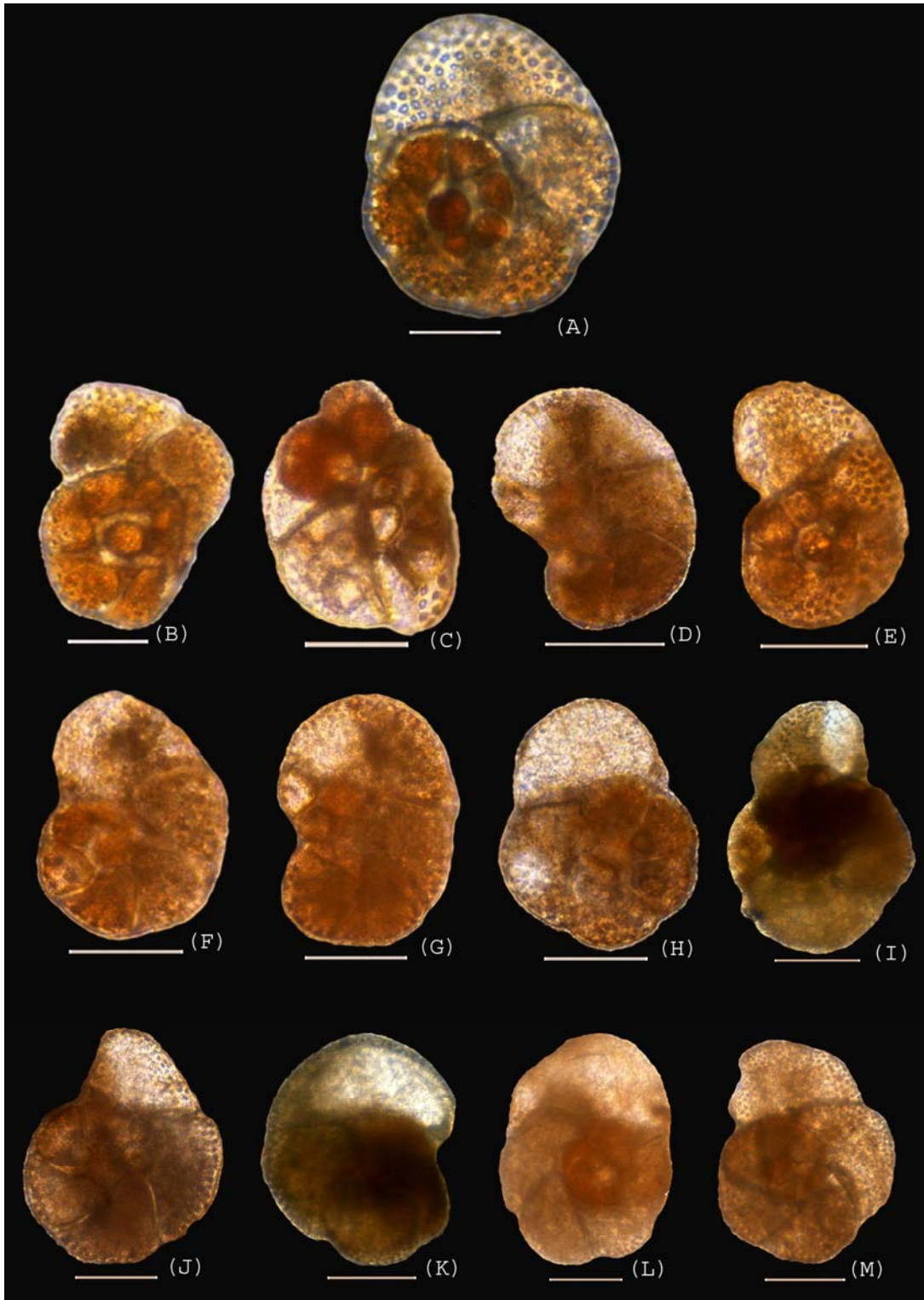


Plate 1