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EXPERIMENT TO DECIPHER THE EFFECT OF HEAVY METAL CADMIUM ON COASTAL BENTHIC FORAMINIFER *PARAROTALIA NIPPONICA* (ASANO)

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ABSTRACT

The response of benthic foraminifer *Pararotalia nipponica* to heavy metal cadmium is observed in laboratory experiment. The results indicate that under a given set of conditions, heavy metal cadmium is deleterious to benthic foraminifera. Gradual increase in cadmium (Cd) concentration mainly affected normal growth and caused morphological abnormalities which were consistent from 4 μg/l to 14 μg/l. The intensity of deformation was severe exhibiting a peculiar change in coiling direction with increase in cadmium concentration. The presence of >50% deformed specimens at the lowest cadmium concentration (2 μg/l) indicates that the threshold of Cd concentration which is detrimental to *P. nipponica* is <2 μg/l.

Keywords: Benthic foraminifera, *Pararotalia nipponica*, cadmium, laboratory experiment, morphological abnormalities

INTRODUCTION

The heavy metals are among the most widespread and hazardous environmental pollutants that have contaminated the surface, ground and coastal marine waters (Martinez-Colon *et al*., 2009). These metals are present in small amounts in the environment, but if present in excess, they are toxic and can cause acute or chronic toxicity. The harmful effect of heavy metals is widely known and is a matter of concern for the environmentalists because of its lasting effect on biota (IOSHIC, 1999; Mami *et al*., 2011). Among the metal pollutants, aluminum, arsenic, cadmium, copper, lead, nickel, mercury, selenium, silver and zinc are of particular concern (Frontalini and Coccioni, 2011). Out of these, mercury, cadmium, chromium and lead are reported to be very toxic to aquatic organisms and humans. Because heavy metals can biomagnify and reach the higher organisms in the food chain, they ultimately affect the humans through contaminated food. For the same reason, a base level detection of pollutants is essential to prevent the harmful effects reaching the higher organisms. 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. Many species of marine micro-organisms such as bacteria have been extensively used to detect marine pollution on base level (Rasmussen and Sorenson, 1998; Ramaiah and De, 2003; Jiya *et al*., 2011). However since bacteria lack fossilization potential they are of limited use in discerning historical pollution changes.

An effective tool to address the problem of time series pollution detection, in absence of any baseline data, is by using organisms with fossilization potential such as foraminifera as proxy. Foraminifera are microscopic, unicellular, almost exclusively marine organisms with a hard test. They are abundantly distributed vertically as well as geographically. They are highly sensitive to the changes in their ambience. These changes get incorporated in their test. The test has fossilization potential and the changes taking place in its environment get preserved in their test even after the death of the organism. Thus foraminifera serve as an effective bio-indicator of changes in the environmental conditions especially with respect to pollution levels of a given region and help to compare it with historical levels of the said pollutant, in spite of absence of any baseline data.

Studies on benthic foraminiferal response to various pollutants were initiated by Zalesny (1959), Resig (1960) and Watkins (1961). Thereafter numerous workers started focusing on the benthic foraminifera as sensitive markers to report the deterioration of marginal marine environments (Bandy *et al*., 1964; Seiglie, 1968; Schafer, 1973; Schafer *et al*., 1975; Alve, 1991a; 1991b; Nigam *et al*., 2006b). Majority of such studies were based on the fossil evidences from polluted areas where the reported abnormalities were attributed to the presence of a known pollutant (Carpenter, 1856; McCrone and Schafer, 1966; Setty and Nigam, 1984; Naidu *et al*., 1985; Bhalla and Nigam, 1986; Stouff *et al*., 1999a; Yanko *et al*., 1999; Naidu *et al*., 2000; Debenay *et al*., 2001; Frontalini and Coccioni, 2008; Romano *et al*., 2008; Martins *et al*., 2010). But the reports on similar deformities from areas subjected to natural stresses like extreme salinity (Freudenthal *et al*., 1963; Hofker, 1971; Brasier, 1975; Reiss and Hottinger, 1984; Nigam *et al*., 2006a), change in nutrient levels (Murray, 1963) and rapid change in environmental conditions (Scott and Medioli, 1980) forced the researchers to rethink about the use of foraminiferal proxies for pollution studies. As the credibility of field-based interpretations was debated, there was a need of some standard results in controlled conditions wherein the effect of specific pollutants can be studied.

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; Cadre and Debenay, 2006; Nooijer *et al*., 2007; Filipsson, 2008, Linshy *et al*., 2009; Kurtarkar *et al*., 2011; Frontalini and Coccioni, 2012). In the present study, an attempt is made to study the effect of heavy metal cadmium (Cd) on coastal benthic foraminiferal species *Pararotalia nipponica* in a laboratory experiment.

Cadmium as a pollutant

Cadmium is a non-essential, non degradable element reported as a major contaminant that can cause adverse effects on aquatic system (Rasmussen and Anderson, 2000; Adami *et al.*, 2002; Fillipovic and Raspor, 2003). It is an industrially used substance with long lasting negative effects on human health (Jarup, 2003). Till date, cadmium is not known to have any physiological function in the human body and is considered as a biohazardous metal. The ill effects of cadmium pollution were evident and started getting serious attention after the epidemic itai-itai disease reported from Jinzu river basin, Japan in 1940s, where people were severely affected by the consumption of rice grown on fields irrigated with highly cadmium polluted water (Godt *et al.*, 2006). Cadmium is known to cause several health hazards including kidney damage (Jarup *et al.*, 1998; Bernard, 2004), bone damage (Kazantzis, 2004), acute respiratory diseases (Barbee and Prince, 1999), gastro-intestinal problems (Nordberg, 2004) and even cancer in humans (Il'yasova, 2005).

Cadmium finds various anthropogenic pathways to reach the environment since it is widely used in various industries (as anticorrosive agent, stabilizer in PVC products, a colour pigment, in nickel cadmium batteries, in nuclear power plants as a neutron absorber and in phosphate fertilizers and various pesticides). Reported total global emission of cadmium amounts to 7000 tons/year (Stoeppler, 1991). The careless dumping and incineration of various cadmium polluted wastes ends up polluting different compartments of environment (Hutton, 1983; Clark, 1992; EPA, 2011; Rubinelli, 2002; Jarup, 2003; Godt *et al.*, 2006). Excess of cadmium in the soil has reportedly disrupted the natural terrestrial as well as aquatic ecosystems (Gardea-Torresdey *et al.*, 1996; Meagher, 2000). The fact that the natural cycling of harmful metals released from industrial, domestic and urban effluents like Pb, Zn, Cd, Hg and Cu is affected due to anthropogenic activities is a growing concern (Schindler, 1991). Since oceans provide a vital sink for terrestrial pollutants, ultimately the harmful heavy metals and their compounds find their final destination in the coastal waters and sediments.

Typical maximum cadmium concentrations in bays and estuaries range from 0.3 to 862 ppm (Förstner and Wittmann, 1983), although the typical Cd concentration range for relatively uncontaminated marine sediments lies between 0.1 to 0.6 ppm (Warren, 1981). Along the Indian coast, published records on the concentration of dissolved metals are rather limited and are confined to a few selected areas (Rokhade, 2009). Nakatsuka (2007) reported 0.17 µg/l of dissolved cadmium from the open waters of the Indian ocean whereas Sanzgiri *et al.* (1981) had reported an overall average of 0.15 µg/l dissolved Cd from northern Indian ocean. In a recent study Sankar *et al.* (2010) reported that cadmium concentration varies from 2.25 µg/l to 10.06 ug/l in seawater samples along the east coast. Along the west coast of India, the highest amount of cadmium (80 µg/l) is reported off Mumbai (Mohapatra and Rajendran, 1999-2000). Ouseph (1992) reported a mean dissolved Cd concentration of 1.8-4.2 µg/l from the Cochin estuary. Krishnakumar *et al.* (2004) reported the mean cadmium concentration in seawater along west coast of India as 2.95 µg/l, the highest Cd concentration (8.15 µg/l) was reported off Kochi; Jiya *et al.* (2011) reported 50 mg/l cadmium from the estuarine waters of Kochi. Based on these records it was decided to subject the foraminifers to Cd concentration from 2 µg/l to 14 µg/l at an interval of 2 µg/l.

MATERIALS AND METHODS

Both sediment and algal (floating as well as the ones attached to rocks) samples were collected in pre-labeled polythene bags containing seawater. Once brought to the shore, the algal samples were transferred to plastic tub filled with filtered seawater and were shaken vigorously to detach the foraminifera from the substrate, and then sieved over 2 sieves with different mesh sizes, kept one over the other. The sieve kept on the top has a mesh size of 800 µm and is used to get rid of the large extraneous material whereas the lower sieve has a mesh size 63 µm that is used to concentrate the foraminifera. The plus 63 µm sample was collected in beakers along with seawater and was brought to the laboratory. Similarly, the sediment samples were sieved over 63 µm sieve and the filtrate (> 63 µm fraction) was collected in beakers filled with seawater and brought to the laboratory. Seawater was also collected from the same area as it was used as media for culturing foraminifera.

Live foraminifers were separated with the help of pipette controller under reflected light stereozoom microscope (Olympus SZX 12). Later on, these specimens were observed under inverted microscope (Nikon TE 2000 U) to confirm their live status by observing protoplasm, pseudopodial activities like movement, food capture, etc. The healthy specimens of *Pararotalia nipponica* were separated and their growth pattern was observed constantly. The healthy foraminifers thus maintained in the laboratory were used for further experiments.

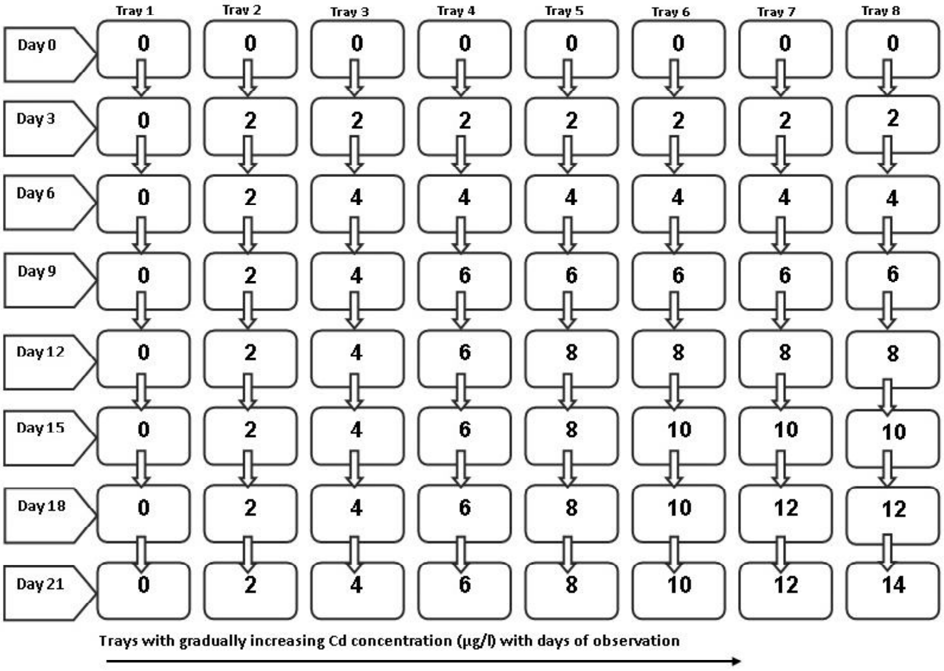
Experimental Set-up

The cadmium chloride monohydrate was used to prepare a stock solution of 500 µg/l concentration by dissolving $CdCl_2.H_2O$ in filtered seawater ($CdCl_2$ being the soluble form of cadmium). In order to avoid a sudden shock to the foraminifers the cadmium was added gradually into the media starting from 2 µg/l till the desirable concentration is reached as per the experimental set-up (Fig. 1)

A set of five 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 the highest concentration (14 µg/l), the concentration was further increased in order to understand the tolerance *of P. nipponica* to cadmium. All the specimens were maintained at a constant temperature (27°C) and salinity (35 psu), throughout the experiment. The culture media was changed every third day and with every media change an equal quantity (25 µl) of diatom *Navicula* sp. was fed to the specimens. Specimens were monitored throughout the experiment for their response to cadmium in terms of growth and morphological changes.

RESULTS

At the onset of the experiment all specimens were healthy and showing extensive pseudopodial activity and were actively feeding. With the progressive addition of cadmium into the media, the specimens other than the controlled specimens



	Tray 1	Tray 2	Tray 3	Tray 4	Tray 5	Tray 6	Tray 7	Tray 8
Day 0	0	0	0	0	0	0	0	0
Day 3	0	2	2	2	2	2	2	2
Day 6	0	2	4	4	4	4	4	4
Day 9	0	2	4	6	6	6	6	6
Day 12	0	2	4	6	8	8	8	8
Day 15	0	2	4	6	8	10	10	10
Day 18	0	2	4	6	8	10	12	12
Day 21	0	2	4	6	8	10	12	14

Fig. 1. Set-up of experiment to decipher the effect of gradual additions of cadmium on *P. nipponica*: Days = days of observation; Rectangles indicate the experimental trays where cadmium concentrations were gradually increased from 2 µg/l to 14 µg/l.

showed reduction in their pseudopodial activity and developed visibly abnormal chambers. Morphological deformities were observed at all cadmium concentrations right from the lowest (2 µg/l) to the highest (14 µg/l). It was observed that at the lowest concentration (2 µg/l), ~50% of the total specimens developed morphological deformities, whereas at all other concentrations thereafter (4 µg/l–14 µg/l), ~80% of the total number of experimental specimens showed morphological deformation (Fig. 2). None of the control specimens showed any morphological deformation throughout the experiment. Though the morphological deformation was consistent from 4 µg/l Cd onwards, the degree of deformity (intensity of deformation) in the experimental specimens varied. The specimens showed severe deformities with each increase in cadmium concentration. (Specimens B-I in Fig. 4). An interesting observation regarding the pattern of deformation in the specimens grown at high cadmium concentration was the change in their 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 (specimen A) and experimental specimens (specimens B to I) in Fig. 4. Due to the same reason, the growth of the experimental specimens could not be measured effectively. Hence the pattern of changes in the growth (in terms of maximum diameter) of the specimens during the experiment do not show any trend with the increase in concentration of cadmium into the media (Fig. 3). But as compared with the controlled specimens, the growth in all the experimental specimens was less. While the growth in control specimens grown without any cadmium was 145±16 µm, all the experimental specimens subjected to various cadmium concentrations grew from 119±24 µm to 27±10 µm. All the experimental specimens were alive in all the Cd concentrations throughout 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.

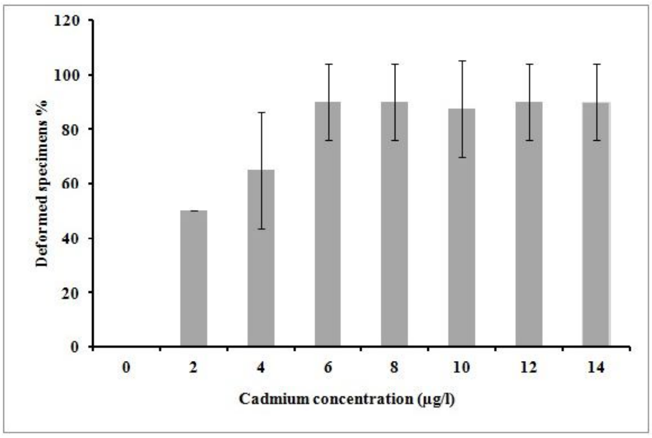


Fig. 2. Percentage of deformed specimens of *P. nipponica* at different concentrations of cadmium.

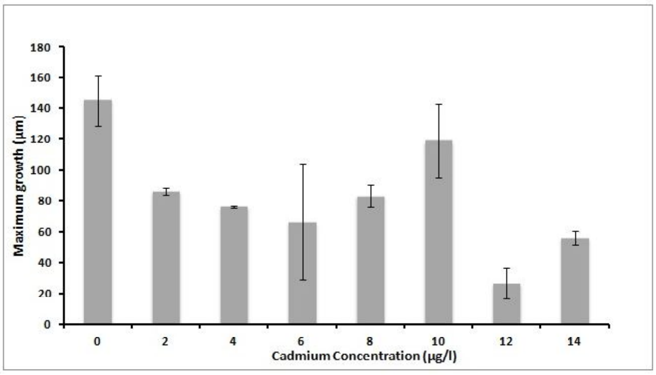


Fig. 3. The maximum growth attained by *P. nipponica* at different concentrations of cadmium.

DISCUSSION

Number of studies focusing on benthic foraminiferal response to trace metal pollution has markedly increased over

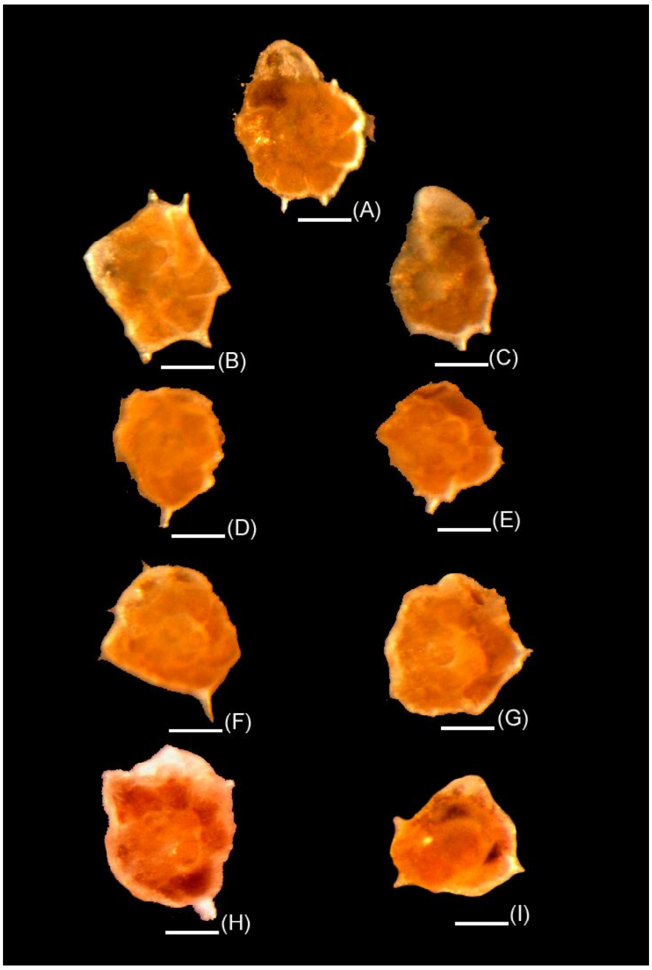


Fig. 4. Different types of abnormalities (B-M) reported in specimens subjected to different concentrations of cadmium. A shows a normal specimen. Scale bar = 100 µm.

the last few decades (Alve, 1991b; Yanko *et al.*, 1994; 1999; Stouff *et al.*, 1999a,b; Coccioni, 2000; Samir and El-Din, 2001; Elberling *et al.*, 2003; Châtelet *et al.*, 2004; Coccioni *et al.*, 2003; Ferraro *et al.*, 2006; Frontalini and Coccioni, 2008; Frontalini *et al.*, 2009; Frontalini and Coccioni, 2012). Regardless of the different environmental settings, all the studies report deleterious effects of such pollutants on ecosystem and refines the use of foraminifers as effective bio-indicators of marine pollution. The adverse effect on benthic foraminifers include changes in population density and diversity, assemblages, structure, test morphology, size, proloculus size and morphology, test ultra structure, test abnormality and chemistry (Alve, 1991b, 1995; Yanko *et al.*, 1994; 1999; Geslin *et al.*, 1998, Armynot du Chatelet and Debenay, 2010 for review).

In the present study where coastal benthic foraminiferal species *Pararotalia nipponica* was subjected to different concentrations of cadmium, comparable observations were made. During the course of the experiment, the pseudopodial activity in the experimental specimens showed a decline compared to the control specimens suggesting that the cytoplasm is immediately affected by the addition of heavy metal cadmium. Earlier, Bresler and Yanko (1995) had reported a significant biological influence of heavy metals on foraminiferal cytoplasm. Boltovskoy and Wright (1976) had clearly 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'. Lesser growth attained by the specimens grown in media with cadmium compared to the specimens grown without cadmium indicate that the presence of cadmium in the medium inhibits the normal metabolic activity of the organism. The physiological disturbance caused by the presence of heavy metal slows down the growth (Samir and El-Din, 2001), as a result of which, the specimens subjected to different cadmium concentrations attain lower average size as compared to the field specimens. This is in line with the previous reports that trace metal pollutants result in pathological processes in foraminiferal cell and trigger changes in abundance, diversity, maximum growth attained at maturity and mainly cause test abnormalities (Coccioni, 2000; Coccioni *et al.*, 2009), and explains the occurrence of stunted specimens at polluted sites (Yanko *et al.*, 1994; 1998; Samir and El-Din, 2001). The net adverse effect of Cd on the growth rate is, however, significantly less than that of the Hg. A comparison of the net reduction in growth of the specimen with unit increase in heavy metal concentration shows that for every 10 µg/l increase in Hg, growth decreased by ~7-8 µm (Saraswat *et al.*, 2004), whereas for every 1 µg/l increase in Cd, the growth decreased by ~6 µm. Here, we would, however like to mention that we have considered a linear decrease in growth with increasing concentration of pollutant and a uniform response of both the *P. nipponica* and *Rosalina leei*. The relative susceptibility of benthic foraminifera to heavy metal pollution will most likely be species specific.

Test abnormalities in benthic foraminifera from different environment settings is undoubtedly a general feature, but following Alve (1991b), we can consider 1% of abnormal tests as an effective threshold in a non-stressed environment. Stouff *et al.* (1999b) confirmed the same through culture experiments solving the ambiguities on using the test abnormalities to monitor marine pollution (Geslin *et al.*, 2002). In the present study, the addition of abnormal chambers during the course of the experiment in the specimens subjected to cadmium 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 cadmium 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. Similarly Stouff *et al.* (1999b) reported development of abnormalities in *Ammonia beccari* in response to copper concentration as low as 10 µg/l. The presence of >50% deformed specimens at the lowest Cd concentration indicates that the threshold Cd concentration which is detrimental to *P. nipponica* is <2 µg/l.

Similarly, two species of *Ammonia* exhibited test deformation in all treatment sets which tend to increase with increasing copper concentrations in the media under culture condition (Cadre and Debenay, 2006) which was confirmed by Frontalini and Coccioni (2012). Frontalini and Coccioni (2008) also reported that higher trace metal contents result in abnormalities in foraminiferal tests and abnormal specimens increase with higher trace element concentrations.

Abnormalities were also reported in *Rosalina leei* (Saraswat *et al*., 2004) subjected to different mercury concentrations and further confirmed by Nigam *et al*. (2009) that sudden additions of mercury had acute effects compared to gradual additions. Apart from the trace metals, foraminifera are reported to have developed abnormalities responding to the induced oil pollutants (Morvan *et al*., 2004; Ernst *et al*., 2006).

A comparison of percentage of deformed specimens with the concentration of different pollutants also indicates that Cd is a less effective pollutant than Hg and Cu. Nearly all the specimens of *Rosaline leei* developed deformities when subjected to 175 µg/l of Hg (Nigam *et al*., 2009), whereas such a complete deformation of all the specimens of *P. nipponica* was reported only at 6 µg/l Cd concentration. As compared to Cd and Hg, benthic foraminifera can sustain very high levels of another metal, namely Copper as only 57% deformed specimens of two species of *Ammonia* were noted at as high Cu concentration as 200 µg/l (Cadre and Debenay, 2006). It should, however, be noted that *P. nipponica* and *Ammonia* are comparatively thick walled than *R. leei*, which might explain a part of the higher susceptibility of *R. leei* to Hg than that of *P. nipponica* to Cd and still lower of *Ammonia* to copper. Interestingly, the susceptibility limit of benthic foraminifera to different heavy metals also depends on whether they are directly exposed to heavy metal or in presence of sediments. The heavy metal susceptibility limit is much higher when benthic foraminifera are subjected to pollutants in presence of sediments, due to bioavailability of the pollutants (Frontalini and Coccioni, 2012).

The change in the coiling plane due to addition of abnormal chambers to a plane different from the normal one is a peculiar observation made from the experiment. There was a consistent morphological deformation in the specimens subjected to cadmium concentrations from 4 µg/l to 14 µg/l, but the intensity of deformation in the experimental specimens increased with increasing cadmium concentration which shows the sensitivity of *P. nipponica* to the heavy metal cadmium. According to Alve (1991b), the organism responding to extreme cases of heavy metal pollution, will devote its energy to protect itself and, as a result, will have little ability left for protein synthesis. This inhibits the energy budget, reproduction cycle, and also harms the cytoskeleton. Samir and El-Din (2001) had reported that living deformed foraminiferal specimens had higher levels of heavy metals (Pb, Zn, Cu, Cr and Cd) compared to non-deformed specimens and forms with twisted, compressed and abnormal growth characterize higher values of heavy metals.

In the light of the findings from the present study and the views expressed by previous workers, the development of morphological abnormalities in experimental specimens subjected to various concentrations of cadmium, reveal the sensitivity of benthic foraminifera to heavy metal pollutants which is expressed by a modification of their test structure. It further explains the noticeably increased proportion of deformed tests in areas subject to pollution, including heavy metals (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 and El din, 2001; Scott *et al*., 2005, Bergin *et al*., 2006, Cadre and Debenay, 2006; Frontalini and Coccioni, 2008).

CONCLUSIONS

Based on the laboratory culture experiment to understand response of shallow water benthic foraminifera *Pararotalia nipponica* to heavy metal Cd, we report that the specimens subjected to various cadmium concentration attained smaller size than the controlled specimens. Morphological abnormalities developed at all cadmium concentrations; but there was a consistent increase in the degree of morphological deformation in the specimens subjected to cadmium concentrations above 4 µg/l. The intensity of deformation in the experimental specimens increased with increasing cadmium concentration. The presence of deformed specimens at the lowest Cd concentration indicates that the threshold Cd concentration detrimental to *P. nipponica* is <2 µg/l. Change in coiling direction was peculiar to the type of morphological deformation of the specimens. The study indicates that the heavy metals are detrimental for the foraminifera and further that the fossil foraminifera can be used to infer historical pollution levels in absence of any baseline data.

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