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ORIGINAL RESEARCH ARTICLE

Toxicity Study of *Ammonia tepida* (Cushman) Under Laboratory Conditions with Concentration of Heavy Metal

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ABSTRACT

The benthic foraminifera *Ammonia tepida* has an experimental culture due to the heavy metal concentrations it can vary in gradual as well as addition metals of mercury. Initially adding in mercury, the subjected specimens did not show any change in morphologically during the early 38 days. After the subjected specimens adding different concentrations of mercury upto 145 ng/l, 70% developed deformities and had deformed chambers 155-270 ng/l. After that the mercury concentration 305 ng/l adding all the specimens died within 19 days. The results were observed irregularities performance and increased the rate of reproduction. Large quantity of juveniles produced and rate of the juveniles has a survival. Where as in a first experiment where concentration of mercury increased gradually and observed irregularities in newly added chambers, it's only in case of specimens subjected to very high (175 ng/l) mercury concentration. Since the inversely proportional growth was found during this experiment.

Keywords: *Ammonia tepida*, mercury toxic, experimental culture, pollution indicators.

INTRODUCTION

Order Foraminiferida belongs to the Kingdom Protista, Subkingdom Protozoa, Phylum Sarcomastigophora, Subphylum Sarcodina, Superclass Rhizopoda, Class Granuloreticulosea, species *Ammonia tepida* are found in all marine environments, they may be planktic or benthic in mode of life [1]. *A. tepida* are game for many small marine invertebrates and fish, that the foraminifera are a key group in the marine food chain; they feed on small prey habitually inaccessible for the bigger size fauna. The planktonic forms generally inhabit the open ocean and seldom live in coastal waters, while benthonic foraminifera exist on substrates from abyssal plains to high intertidal areas. Diversity of foraminifera is highest in tropical waters and sub tropical regions. Not evenly occur in distribution of foraminifera, since the environmental parameters which influence the distribution and abundance of benthic forms. *A. tepida* respond to pollution as well as to the environmental gradients and also changes either due to density and diversity of the assemblage [1, 2]. The pollution indicators species for foraminiferas are high in marine environment. The pollution can affect on foraminifera in a different ways. Few studies of foraminifera, in controlled laboratory environments, provide very little information regarding trophic strategies but much has been inferred by relating test morphology to habitat. Previously reported that the studies of pollution and heavy metals affected in foraminifera species, [3] has been studied the cadmium, zinc and barium in foraminifera tests, [4] has been reported that the experimental determination of cadmium uptake in shells of planktonic foraminifera *Orbulina universa* and *Globigerina bulloides*, implications for surface water paleo reconstructions, [5] has been studied the morphological deformities of benthic foraminiferal tests in response to pollution by heavy metals, implications for pollution monitoring, [6] has been studied the foraminifers as indicators of marine pollution, a culture experiment with *Rosalina leei*, [7] has been reported that the morphological and cy- tological responses of *Ammonia* sp to copper

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contamination, implication for the use of foraminifera as bioindicators of pollution, [8] has been studied the effects of sudden stress due to heavy metal mercury on benthic foraminifera *Rosalina leei*, laboratory culture experiment, [9] has been studied the evaluation of the ecological effects of heavy metals on the assemblages of benthic foraminifera of the canals of aveiro. 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. Even though there are some reports on mercury pollution, few researchers have studied concentration of mercury in waters off the western coast of India [10, 11, 12]. 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 [10]. Although, based on field studies, few attempts were made to document benthic foraminiferal response to trace metal pollution along the Indian coasts [13], harmful effects of mercury 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 *A. tepida* under the laboratory culture conditions.

MATERIALS AND METHODS

Selection of species and environmental set up at sampling location

The live foraminifera were collected from shallow water of Ennore Coastal waters. The sampling location was selected on the basis of availability of live specimen 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 shaked 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 foraminifera. The >0.063 µm sample was collected in beakers along with sea water and brought to the laboratory.

Fig 1: Sampling station near Ennore from Chennai

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, *A. tepida* was chosen because of its high abundance in the collected samples and also as they are easily cultured in the laboratory [14]. The first results of this study were reported by [6] and this study is in continuation of the previous experiment. Live specimens of *A. tepida* 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., these was done in accordance with the seasonal variation of this species in the field. In foraminifers, both optimum growth and reproduction range of physicochemical 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.

Experiment 1 Setup

The juvenile specimens of *A. tepida* 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 25 ng/l to 175 ng/l at 25 ng/l intervals. In order to avoid a sudden shock to the organisms, the mercury concentration was increased gradually at every

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alternate day keeping one set at each concentration. To find out the maximum Mercury tolerance limit of this species, the specimens kept at 175 ng/l were later subjected to mercury concentrations up to 255 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 ‰) and temperature (room temperature) (Fig. 5), pH and dissolved oxygen (Fig. 6), feed (*Navicula*) and light (12 hr light- 12 hr dark) were maintained uniform throughout the experiment.

Experiment 2 Setup

Similar to the first experiment, juvenile specimens of *A. tepida* 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 30 ng/l to 305 ng/l at 30 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 ‰) and temperature (25.5 °C) (Fig. 5), pH and dissolved oxygen (Fig. 6), 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.

RESULTS

Experiment -1 (Gradual Addition of Mercury)

Experimental periods the specimens showing that the pseudopodial activity for 10-14 days, at the same time the concentration mercury gradually increases. The activities slowly decrease within 3-5 days in case of specimens subjected to 100 ng/l. The specimens were still moving and accumulating food near the last chamber very slowly. Its similar activity was observed in the growth rate also. The maximum growth was observed by the specimens showing an opposite correlation with the mercury concentration in the medium (Fig. 2) and also the specimens kept at higher mercury concentration attained comparatively less growth [6]. After 40 days at each concentration growth nearly ceased at the same time the specimens continued to live. The specimens were alive even when the concentration was increased to 255 ng/l. when compare normal size due to experimental size specimens was less. The significantly, it was found that chambers added to the specimens kept at higher concentrations of mercury, were abnormally larger and with remarkable orientation.

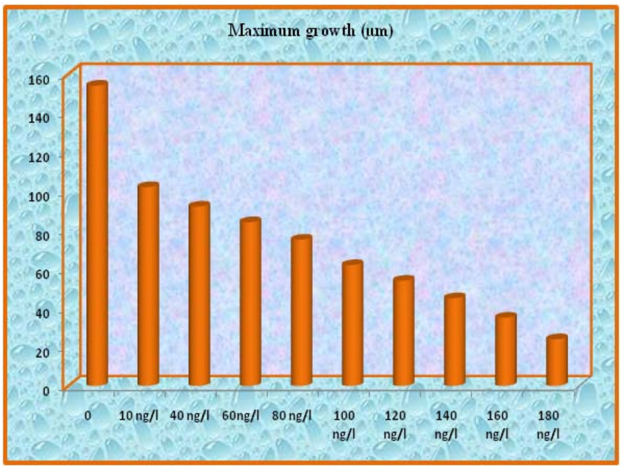

Fig 2: The maximum growth attained at different concentrations of mercury in the experiment in which the amount of mercury was increased gradually

Experiment 2 (Sudden Addition of Mercury)

The period of the experiment within 45 days, due to the specimens did not show any remarkable changes in morphology, and later the specimens morphological abnormalities started to appear (Fig. 7). The abnormalities included larger than normal size of the last chambers, and abnormal orientation and shape of the new chambers (Fig. 5). The specimens percentage completely destroyed at each concentration is plotted, it showed that up to 145 ng/l, 70% of the specimens were deformed, whereas at Mercury concentrations above 145 ng/l and up to 270 ng/l, all the specimens showed deformation (Fig. 3). The specimens kept at 305 ng/l died after an exposure of 18.5 days to the mercury concentration. None of the control specimens showed any morphological deformation throughout the experiment.

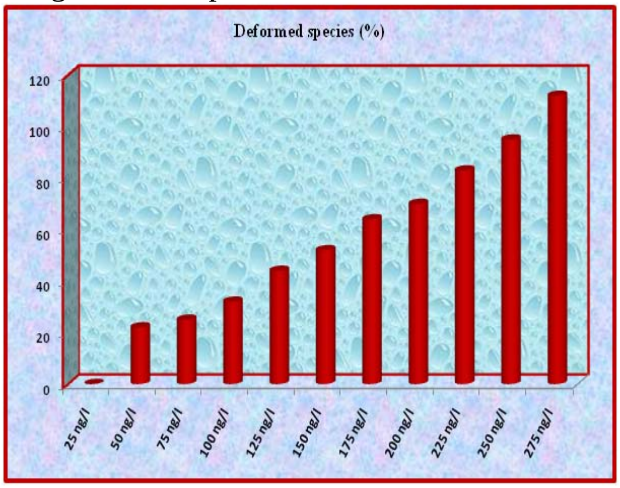

Fig 3: Percentage of deformed specimens at different mercury concentrations in the second set of experiment where mercury was suddenly introduced

The experimental specimens there indicate irregular reproduction apart from the morphological deformation (Fig. 4). Due to mercury concentrations reproduced higher when compare to the control specimens. At lower concentrations (30 ng/l, 55 ng/l), none of the specimens reproduced throughout the experiment, but at 70 ng/l to 220 ng/l, reproduction was reported at all seven concentrations. In specimens subjected to 70 ng/l, 105 ng/l, 180 ng/l, 205 ng/l and 230 ng/l, the number of juveniles produced after reproduction varied from 7-14 but in case of specimens subjected to 120 ng/l and 155 ng/l mercury concentration, the number of juveniles were 24 and 19 respectively. The juveniles died within a day or two in all the cases. At still higher concentrations 255 ng/l and 270 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.

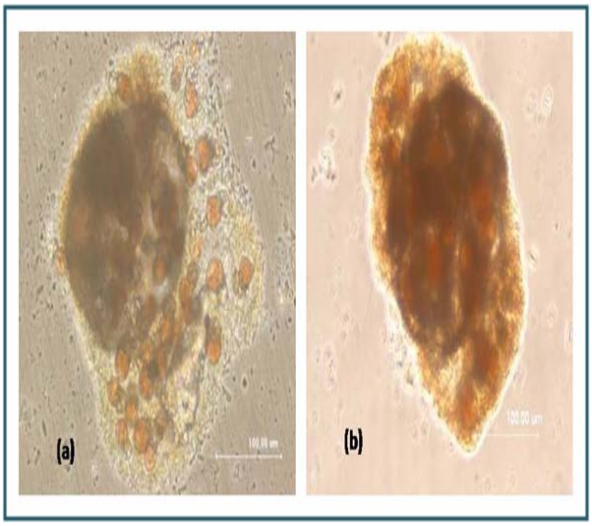

Fig 4: (a) In case of reproduction in control specimens (without Mercury) juveniles came out, (b) whereas in the specimens with high amount of Mercury in the media, juveniles did not come out of the specimen

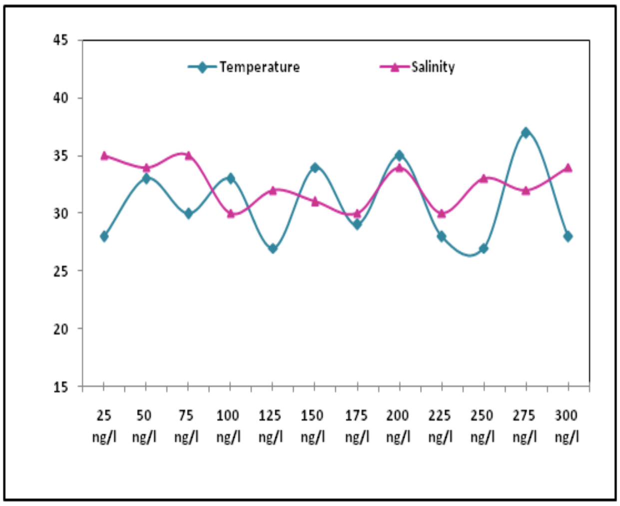

Fig 5: Temperature and salinity during the experiment

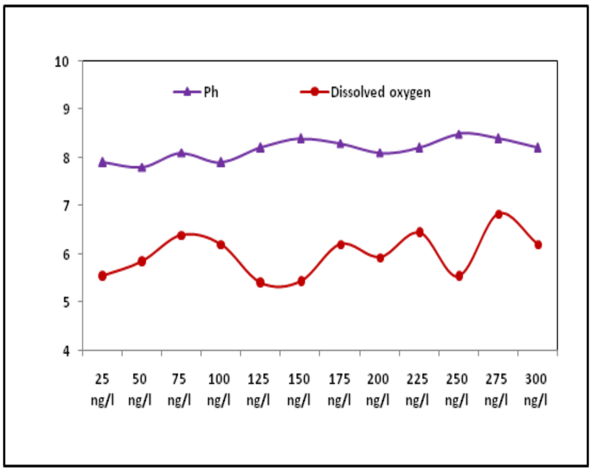

Fig 6: pH and dissolved oxygen concentrations during the experiment

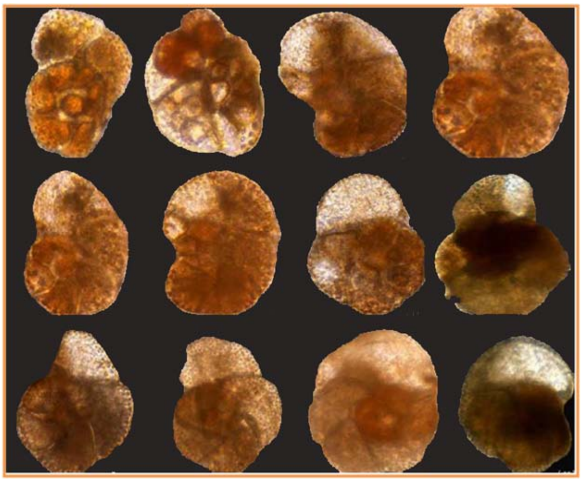

Fig 7: Morphological abnormalities of *A. tepida* in contaminated culture

DISCUSSION

The culture an experimental set up deformation in foraminifera is known from the biological record. In order environments, deformation occurs more frequently in polluted that in non-polluted areas. However the different kinds of test deformation develop under pollution versus naturally induced stress and what kind of stress properties cause deformations have not yet been established [15,16]. In the present study, *A. tepida* are gradual and sudden increase at the time of mercury concentration. The first experiment indicated that the gradual exposure to mercury concentrations can affect the normal growth of specimens as well as the growth was inversely proportional to mercury concentration. Finally the reported on deformation, since the subjected specimens to higher in mercury concentrations and the small number of abnormal specimens were observed. Similarly due to specimens subjected to sudden stress, the percentage of deformed specimens was very high (70-100%). The 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

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incremental and overall growth, size and also the individual specimen has to be measured, such measurements were not possible in highly deformed specimens under sudden exposure to different mercury concentrations. However the deformed specimens were plotted and shows are similar correlation with dissimilar mercury concentrations. Much number of deformed specimens while being subjected to sudden stress, the test abnormalities was similar as that in the previous experiment such as the abnormal size, shape and orientation of newly added chambers. The major fact on growth still little numbers of deformities specimens subjected to gradually added mercury; since a many numbers of deformities specimens were subjected to unexpected on mercury exposure, almost certainly indicate that the adaptive capability of *A. tepida*. Finally in this experiment in which mercury were added gradually, not observed in various damage was done to the normal structure in the specimens of *A. tepida* and sufficient instance was obtainable to adapt as per the changed condition by way of lowering the growth rate [1, 2, 17,18]. Whereas the specimens straight subjected to different concentrations of mercury, irreparable damage to the soft tissue probably leads to increased abnormalities. The first stage on the pseudopodial activity of both experiments indicates that the cytoplasm is suddenly affected by the presence of mercury in the medium. Much the relationship as inverse between mercury concentrations and the growth indicates that the presence of heavy metal in the medium inhibits the usual metabolic activity on its organism. The metabolic activity decreased the growth slowly down. Here it would be concluded that the specimens subjected to different mercury concentrations reach small average size when compared as the field specimens. Similar study agree with previous studies, [16] has been reported that the cadmium partition coefficients of cultured benthic foraminifera *A. beccarii*, [17] has been reported that the impact of salinity on the Mg, Ca and Sr, Ca ratio in the benthic foraminifera. *A. tepida*[18] has been studied the experimental determination of barium uptake in shells of planktonic foraminifera *Orbulina universa*,.

Since, the second experiment observed that the subjected specimen increased instances of stress due to stress have a high reproduction, its can induced on medium mercury. The specimens subjected to all but 20 ng/l and 55 ng/l mercury concentrations, reproduced under sudden addition of mercury into the medium. And also the control sets can significantly vary in specimens subjected to sudden mercury stress. The specimens subjected to sudden addition of mercury, its produced juveniles very less number and finally it can be survive more than three days. Additionally, in specimens subjected to the maximum concentration of mercury conditions, the juveniles could not come out and many of them died within the mother cell. The reproductions are high since the gradual addition of mercury, as well as few specimens subjected to comparatively high mercury concentration reproduced. The environmental parameter is controlled by the foraminifera reproduction. Therefore, the optimum range of environmental conditions necessary for the successful reproduction in foraminifera because it’s very narrow compared to the optimum range for their survival. The variable of optimum condition fact that the foraminifera reproductive performances. The number of publication dealing with pollution’s effects on foraminifera assemblages and fact of reproductive strategies, [17] has been reported that the cadmium, zinc, copper, and barium in foraminifera tests, [15] has been reported that the benthonic foraminiferids as pollution indicators in Southampton water from England, [5] has been reported that the response of benthic foraminifera to various pollution sources. [1] have been reported that the heavy metal incorporation in foraminiferal calcite from multi-element enrichment culture experiments with *A. tepida*,[2] have been reported that the benthic foraminifera as bio-indicators of pollution.

In the present investigations, clearly indicate that the subjected specimens the rate of reproduction are high the reason of increased concentration of mercury, it can compared to the control specimens. The experimentally has been observed the reproduction difference and the produced number of juveniles, which maintained the specimens with or without mercury, finally show that the pollution of heavy metal affect on the normal growth and have a reproductions in *A. tepida*. After the reproduction the juveniles can death very soon, subjected specimens at high rate of mercury concentrations, in the fact can the juveniles from this abnormal reproduction almost certainly it’s not healthy to cope with the unexpected stress in the form of high mercury content. In the present study, observed that the pollution occurs when harmful can affects animal behavior, food chain and abundance species can be reduced or destroyed. This experimental study indicates the gradually increased mercury concentration can affect due to subjected specimens metabolic activities [1]. Due to the high amount of mercury in the experimental conditions, *A. tepida* have been affected and the changing whole behavior especially reproduction as an observed this experimental periods, the infertility, reproductive failure, birth defects and anoxic conditions. The harmful contents such as mercury that the ocean animals breathe and eat can be deadly. Many species of foraminiferas freely floating in marine environment it can increased or decreased depends the ecological conditions.

The experiment investigation here it would be concluded that the benthic foraminifera *A. tepida* is different to gradual and sudden stress conditions. It can subjected to gradual stress, the specimens growth showed that an inverse relation to the mercury concentration, however the percentage of abnormalities not an important, when correlate to that in sudden stress anywhere 70-100% of the specimens were reportedly deformed. While the deformed specimens lot it be different considerably in each experiment. The morphological structures on abnormalities stay similar which is most important to characterize the response *A. tepida* to this particular contaminant. The fact on *A. tepida* sudden stress due to the numbers have been increased and induced reproduction which can significantly different from the normal specimen of reproduction in the particular species. The results evidence of the pollution of heavy metal influences size of foraminifera and increased in the specimens from the population. Experiment abnormalities are commonly used as indicators of pollution, while they may be caused by natural environmental factors. *A. tepida* are more tolerant and increase in share with rising pollution. It may be easily used as bioindicators to monitor heavy metal pollution. The experiment results showed is not an again show enough for once since following further steps, the foraminifera are helpful for this type of study because of their wide distribution in marine environments, taxonomic diversity and high sensitivity to environmental variations.

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