Effects of mercury on embryonic development and larval growth of the sea urchin *Echinometra mathaei* from the Persian Gulf

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Abstract

This study investigated the effects of increasing mercury (Hg) concentration on early developmental stages of sea urchin, *Echinomethra mathaei,* as a bioindicator. The toxicity test was carried out after the gamete released induction and fertilization in six concentrations of mercury within the range of 4, 8, 16, 32, 64 and 128 µg/L. Embryos samples were incubated for 30 h in control and test solutions. After incubation, the percentage of developed 4-arm pluteus larvae was recorded in each group and embryonic abnormalities were studied by a microscope. Results of this study indicate that exposure of embryos to increasing mercury concentrations lead to abnormalities such as changes to shape and size of pluteus larval arms and also arrested development in early embryonic stages. Furthermore, embryos were analyzed to determine mercury absorption by cold vapour atomic absorption spectrometry method (AAS). The median effective concentration (EC50) value calculated for mercury was 17/42 µg/L.

Keywords: Mercury, Sea urchin, Toxicity test, *Echinometra mathaei*, Persian Gulf

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Introduction

Heavy metals are important and stable environmental contaminants and the main sources of their input into the marine environment are domestic and industrial wastes (Nagajyoti et al., 2008; Othman, 2008). The presence of heavy metals in the atmosphere, soil and water causes serious problems for all organisms and the bioaccumulation of which in the food chain is very dangerous (Celechovska et al., 2008). These contaminants have different effects on aquatics, including growth reduction, behavior, morphologic abnormalities and genetic changes (Gopalakrishnan et al., 2007; Nasehi et al., 2012). Mercury is one of the most important pollutants, particularly in the environments affected by the human-beings' activities (Islam et al., 2007). Based on the chemical form, the dose and level of exposure, mercury and its compounds cause a great variety of toxicities (Satoh, 2003; Roos et al., 2012). Chemical analyses determine the nature and degree of pollution, but do not provide any information about the effects of these substances on organisms (Fernandez and Beiras, 2001). So bioassays that the basis of which is measuring biological responses of marine organisms in early life stages have been used to study these effects of environmental pollutants (His et al., 1999). Many studies have shown that among the sea invertebrate, sea-urchins extensively are used as standard organisms for doing these ecotoxicological tests (Cesar et al., 2004; Hovanec et al., 2005). Sea urchins are sensitive to marine pollutants in the early life stages and provide a useful assay for determining the toxicity of pollutants such as heavy metals (Eriksen et al., 2001; Bielmyer et al., 2005). Fernandez and Beiras (2001) have reported on sensitivity of the *Paracentrotus lividus* sea urchin embryo to mercury in the range of 0.01–0.10 mg/L. In this study, early developmental stages of sea urchin, *Echinometra mathaei* from the Persian Gulf were used as bioindicators to determine the toxicity effects and thresholds of mercury.

Materials and methods:

Sample collection

Adult sea urchins (*E. mathaei*) were collected from the subtidal rocky shore at Bostane port (26˚31'N, 54˚39'E). Then, samples were transferred to the Persian Gulf and Oman Sea Ecological Research Institute laboratory and were maintained in tanks which contained sea water.

Preparation of metal solutions

Test solutions were prepared by dissolving $Hg(NO3)_2$ in filtered (0.5 & 1 μm filters) sea water in the range 4, 8, 16, 32, 64 and 128 μg/L concentrations. Filtered natural sea water was used as the control group.

Toxicity test

Initially, spawning was inducted by the injection of 1 mL KCl 0.5M into the celomatic cavity. Sperms and eggs were collected in separate dishes containing filtered sea water and for an integrity check gametes were observed under the microscope. In order to fertilize, a little amount of sperm solution was transferred to egg solution. After the first cleavage, embryos at the two-cell stage were added to 500 mL of test and control solution to

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attain a final density of 20 embryos/mL. Three replicates were used for test and control solution, and the experiment was maintained at 29 °C and salinity of 39 g/L for 30 h. These conditions allowed complete development of embryos into pluteus larvae. After the incubation period, samples were fixed with 4% formalin. Afterwards, in each replicate the percentage of 4-arm pluteus larvae was recorded and the percentages of normal and abnormal larvae were determined. In addition, the samples for chemical analysis were collected.

Chemical analysis

All reagents were of analytical reagent grade: nitric acid 10% (Merck, Germany); stannous chloride 99% (Merck, Germany). Deionized water was used for the preparation of all solutions (Milli-Q System, Millipore). Mercury stock standard solution (1000 mg/L) was prepared by dissolving 1.354 g of $HgCl_2$ in 10% HNO_3 in a 1000 ml glass volumetric flask and kept in the refrigerator (+4 °C). The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution through intermediate solutions using 10% HNO_3. Stannous chloride solution (1.1% v/v) was prepared by dissolving the salt in 1000 ml of 3% HCl. Standard solutions and samples were stabilized by adding 1–2 drops of a 5% (w/v) $KMnO_4$ solution.

The reliability and consistency of Hg level in samples; analysis was confirmed by using certified reference materials (CRMs) which were analyzed in parallel with the samples.

In order to measure the concentration of mercury absorbed by the samples, embryos preparation was performed in different concentrations (4, 8, 16, 32, 64 and 128 µg/L) based on Moopam method (1999). Mercury was analyzed by automated cold vapour atomic absorption spectrophotometry (Fernandez and Beiras, 2001). Mercury analysis was done by atomic absorption spectrophotometry (AAS-Thermo-M series).

Statistical analysis

We used Kruskal-Wallis and Dunnett tests to evaluate the effects of mercury on development of embryos. Furthermore, the median effective concentration (EC50) for mercury, i.e. the metal concentration reducing embryogenesis success to 50% of the control values, was obtained by linear regression of the larval percentage data against the logarithm of concentration (µg/L of metal ion).

Results

Embryogenesis success

Gradually by increasing concentrations of mercury, percentage of pluteus larvae development decreased and the percentage of the other developmental stages increased (Fig. 1).

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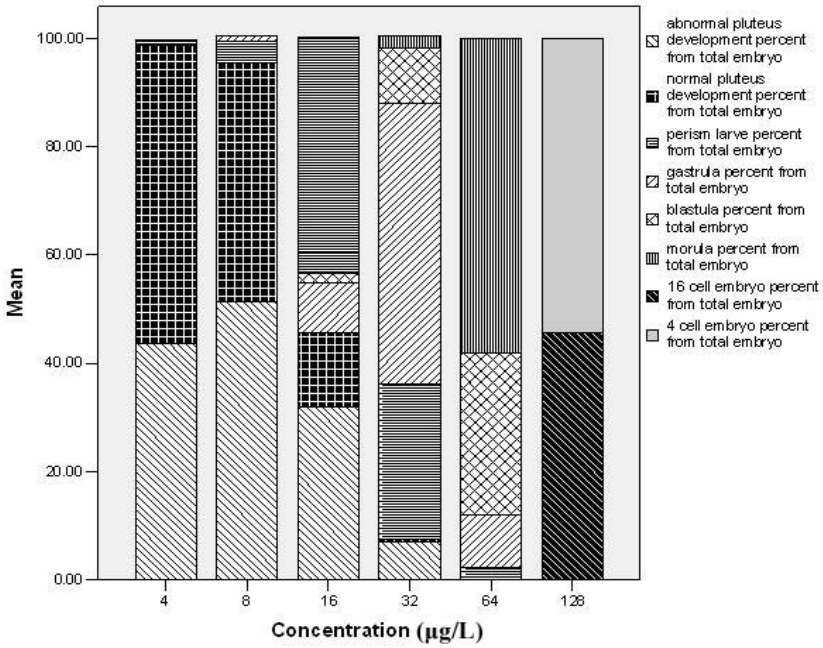


Figure 1: The percentage of *Echinometra mathaei* developmental stages at different concentrations of mercury

As illustrated in the concentration-response curves (Fig. 2), increasing mercury concentration inhibited embryogenesis success (defined as the percentage of developed 4-arm pluteus larvae). The results of Kruskal-Wallis tests showed significant difference in mean percentage of pluteus larval development in different mercury concentrations ($P<0.01$).

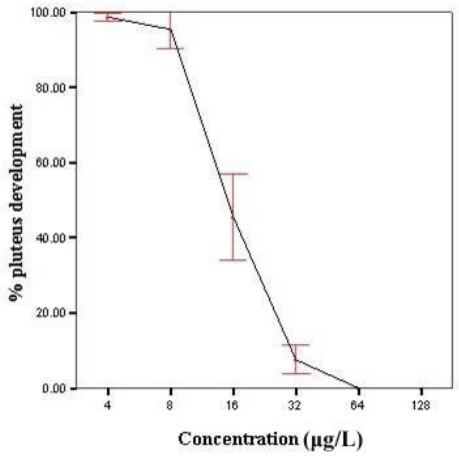


Figure 2: Mean percentage of pluteus larval development at different concentrations of mercury, each data point represents the mean of three replicates. [Error bars=SD]

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Furthermore with increasing mercury concentrations, the percentage of normal larvae reduced and the percentage of abnormal larvae increased, as at concentration 32 µg/L almost all larvae had abnormal development (Fig. 3). The EC50 value (the metal concentrations reducing embryogenesis success to 50% of the control values) calculated for mercury was 17/42 µg/L in this study.

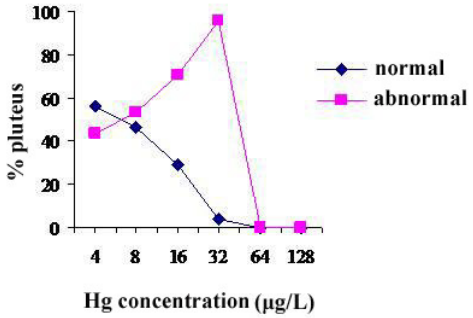


Figure 3: Percentage of normal and abnormal pluteus larvae development after 30 h of exposure to the mercury

Morphological studies

The morphological results showed that at 4 µg/L mercury concentration approximately all of embryos reached to pluteus larval stage (98.68%) and the majority of them (54.98%) had normal development (Fig. 4A). At the concentration of 8 µg/L, nearly 51.33% of embryos had abnormal development. Larval abnormalities included changes to shape, size and number of pluteus larval arms (skeletal abnormalities) (Fig. 4B). At the concentration of 16 µg/L, less than half of sample (45/54%) reached to pluteus larval stage and the majority of reminders were at perism stage (Fig. 4C). At 32 µg/L, nearly half of the embryos (51.67%) were found at gastrula stage (Fig. 4D). At 64 µg/L, due to increase in mercury accumulation in embryo and increase in toxic effects, no pluteus larva was formed and most of embryos development was arrested at morula and blastula stages (Fig. 4E). At the concentration of 128 µg/L of mercury, development was arrested at earlier cleavage (Fig. 4F). The EC50 value (the metal concentrations reducing embryogenesis success to 50% of the control values) obtained for mercury was 17.42 µg/L.

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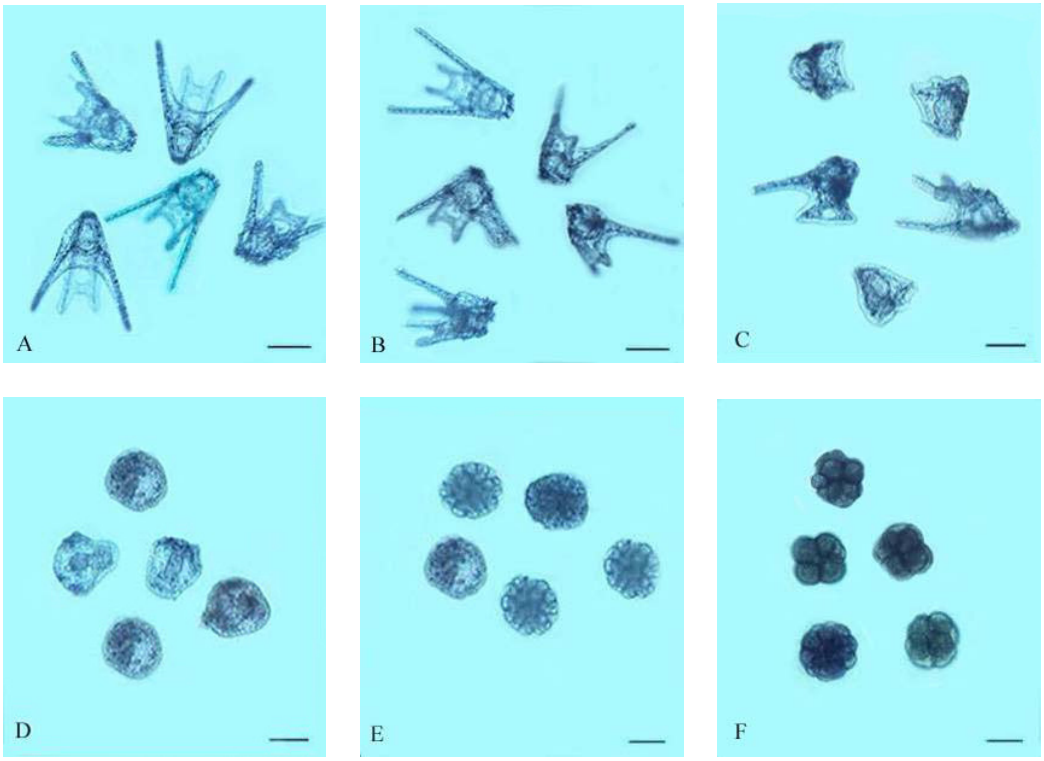


Figure 4: Effect of different concentrations of mercury (induced in two-cell stage after 30 h of development) on early embryonic development and pluteus larvae. A-C: show abnormal pluteus development at 4, 8 and 16 µg/L of mercury respectively. D: 32 µg/L mercury, showing developmental arrest of half of the embryos to the gastrula stage. E: 64 µg/L mercury, showing developmental arrest in morulae stage in the majority of embryos. F: 128 µg/L mercury, showing developmental arrest at 8 and 16 cell stages. Scale bar = 100 µm for A-C; Scale bar=50 µm for D-F.

Chemical analyses

The measured absorbed mercury in the embryos showed that mercury absorption increases by increasing mercury concentration. Any value in Figure 5 shows the amount of absorbed mercury per 30,000 embryos (in 3 replicates) in each concentration.

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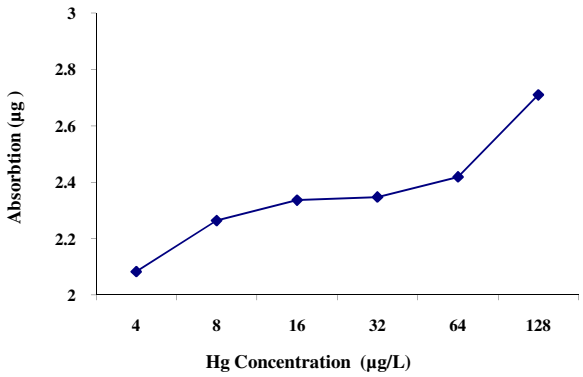


Figure 5: Absorbed mercury measured from embryos at concentrations of 4,8,16,32,64 and 128 µg/L mercury. Each point represents average of three replicates.

Discussion

Early developmental stages of some invertebrates such as sea urchin have extensively been used to determine the effects of environmental contaminants (Simon and Laginestra, 1997). The toxicity of mercury on embryonic development of sea urchin *E. mathaei* was explored in this study. The results of this research revealed that early life stages of sea urchin are really sensitive to mercury and the absorption of mercury by the sea urchin embryos, *E. mathaei* caused many abnormalities. The exposure to mercury during early developmental stages lead to arrested development and morphologic abnormalities in embryo and larvae. Such abnormalities were also reported by Fernandez and Berias (2001). Similarly Eissa (2007) reported that mercury causes different abnormalities including, exo-gastrola (in which archenteron is not formed inside the embryo), Mesenchyme- filled blastulae and gut or skeletal abnormalities in larvae. The larval skeleton of the sea urchin contains an embedded proteinaceous matrix and calcium carbonate ($CaCO_3$). Since the presence of Ca ++ ion is necessary for spicule formation, it seems possible that the ability of the Hg ions in disturbing the ionic balances (e.g., Ca++) is the probable reason for the formation of skeletal abnormalities (Walter et al., 1989; Warnau et al., 1996; Gillot et al., 1999). The EC50 (Median Effective Concentration) values for mercury measured in this study were 17.42 µg/L while the EC50 values for mercury on *Paracentrouts lividus* reported 21.95 µg/L (Fernandez and Beiras, 2001), compared to 20-40 µg/L (Warnau et al., 1996) and 4-8 µg/L (His et al., 1999). The comparison of the present results and other studies shows the existence of some differences in the EC50 values. Some of the possible reasons for these differences might be related to the differences in the used species, their habitats and exposures time. Different species show different resistance to contaminants. Also, the amount of time to reach a particular developmental stage (exposures time in toxicities tests) differs between various species coming from different latitudes (King and Riddle, 2001; Prato et al., 2006). Indeed, the difference in environmental factors such as

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temperature, salinity, pH and reaction of pollutants to environmental compounds including organic and non-organic (which are naturally found in sea environments) can affect the results of toxicities tests (Bay et al., 1998; Jackson et al., 2005; Prato et al., 2006). These findings show that to do toxicity test, it is necessary to consider environmental factors and the amount of organic and non-organic substances in the environment. In conclusion, the maximum concentration of mercury in the Persian Gulf was reported 13.0 µg/L (Ropme, 1999). Our study indicated that 17.42 µg/L of mercury reduced 50% the development of the test population, so the measured concentration of mercury is lower than the effective concentration. But it always seems necessary that increasing inputs of pollutants such as heavy metals to this ecosystem increases their levels to effective levels, following that the life of different species residing in this ecosystem can be exposed to serious dangers.

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References

Bay, S.M., Greenstein, D.J., Jirik, A.W. and Brown, J.S., 1998. Southern California bight 1994 pilot project: VI. Sediment toxicity. Southern California Coastal Water Research Project,Westminster, CA.

Bielmyer, G.K., Brix, K.V., Capo, T.R. and Grosell, M., 2005. The effects of metals on embryo-larval and adult life stages of the sea urchin, *Diadema antillarum*. *Aquatic Toxicology*, 74(**3**):254-263.

Celechovska, O., Malota, L. and Zima, S., 2008. Entry of heavy metals into food chains: a 20-year comparison study in northern Moravia (Czech Republic). *Acta Veterinaria*, 77(**4**):645-652.

Cesar, A., Marin, A., Marin-Guirao, L. and Vita, R., 2004. Amphipod and sea urchin tests to assess the toxicity of Mediterranean sediments: the case of Portman Bay. *Scientia Marina,* 68,205-213.

Eissa, S.H.H., 2007. The toxicity of mercury on the sea urchin gametes and embryos. *Egyptian Journal of Experimental Biology*, 3,37-42.

Eriksen, R.S., Nowak, B. and Van Dam, R.A., 2001. Copper speciation and toxicity in a contaminated estuary. Supervising Scientist Report 163P.

Fernandez, N. and Beiras, R., 2001. Combined toxicity of dissolved mercury with copper lead and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea-urchin. *Ecotoxicology,* 10(**5**):263-271.

Gillot, I., Payan, P., Girard, J.P. and Sardet, C., 1999. Calcium in sea urchin egg during fertilization. *The International Journal of Developmental Biology*, 34, 117-125.

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[DOR: 20.1001.1.15622916.2013.12.4.13.2]

Gopalakrishnan, S., Thilagam, H. and Raja, P.V., 2007. Toxicity of heavy metals on embryogenesis and larvae of the marine sedentary polychaete *Hydroides elegans*. *Archives of Environmental Contamination and Toxicology*, 52(**2**):171-178.

His, E., Heyvang, I., Geffard, O. and De Montaudouin, X., 1999. A comparison between oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassay for toxicological studies. *Water Research*, 33(**7**):1706-1718.

Hovanec, T.A., Toy, E.L., Westerlund, J. and Coshland, J.L., 2005. The toxicity of synthetic sea salts and natural development of white sea urchin (*Lytichinus pictus*) larvae. *Advanced Aquarist's Online Magazine*. 4.

Islam, E., Yang, X.E., He, Z.L. and Mahmood, Q., 2007. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *Journal of Zhejiang University. Science. B*, 8(**1**):1-13.

Jackson, R.N., Baird, D. and Els, S., 2005. The effect of the heavy metals lead (Pb^{2+}) and zinc (Zn^{2+}) on the brood and larval development of the burrowing crustacean, *Callianassa kraussi*. *Water SA*, 31(**1**):107-116.

King, C.K. and Riddle, M.J., 2001. Effects of metal contaminants on the development of the common Antarctic Sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids. *Marine Ecology Progress Series*, 215,143-154.

MOOPAM, 1999. Manual of oceanographic, observation and pollutant Analysis Method, ROPME.

Nagajyoti, P.C., Dinakar, N., Prasad, T.N.V.K.V., Suresh, C. and Damodharam, T., 2008. Heavy metal toxicity: industrial effluent effect on groundnut (*Arachis hypogaea L.*) Seedlings. *Journal of Applied Sciences Research,* 4(**1**):110-121.

Nasehi, N., Monavari, M., Naderi, G., Vaezi, M.A. and Madani, F., 2012. Investigation of heavy metals accumulation in the sediment and body of carp fish in Aras River. *Iranian Journal of Fisheries Sciences*, 12(**2**):398-410.

Othman, M.S., 2008. Metals concentrations in the sediments of Richard lake, Sudbury, Canada and sediment toxicity in an amphipod *Hyalella azteca*. *Environmental Science and Technology*, 1(**1**):34-41.

Prato, E., Biandolino, F. and Scardicchio, C., 2006. Test for acute toxicity of copper, cadmium, and mercury in five marine species. *Turkish Journal of Zoology*, 30,285-290.

Roos, D., Seeger, R., Puntel, R. and Vargas Barbosa, N., 2012. Role of calcium and mitochondria in MeHg-Mediated cytotoxicity. *Journal of Biomedicine and Biotechnology,* 2012,248764.

ROPME, 1999. Regional Report of the State of the Marine Environment .Regional organization for the protection of the marine environment (ROPME) Kuwait, 220P.

Satoh, H., 2003. Behavioral teratology of mercury and its compounds. *The Tohoku Journal of Experimental Medicine,* 201(**1**):1-9.

Simon, J. and Laginestra, E., 1997. Bioassay for testing sublethal toxicity in effluents, using gametes of the sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program.

Walter, P., Allemand, D., De Renzis, G. and Payan, P., 1989. Mediating effect of calcium in $Hgcl_2$ cytotoxicity in sea urchin egg: role of

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mitochondria in Ca2+ mediated death. *Biochimica Et Biophysica Acta*, 1012(**3**):219-226.

Warnau, M., Iaccarino, M., De Biase, A., Temara, A., Jangoux, M., Dubois, P. and Pagano, G., 1996. Spermiotoxicity and embryotoxicity of heavy metals in the echinoid *Paracentrotus lividus*. *Environmental Toxicology and Chemistry/ SETAC,* 15(**11**), 1931.

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