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Evaluation of the toxicity of metal pollutants on embryonic development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata Echinoidea)

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ABSTRACT Bioassays are frequently used to evaluate biological effects of pollutants on marine organisms. The objective of such tests is the detection of toxic effects on populations that are representative of a given ecosystem. Sea urchin is a model organism employed in the field of environmental toxicology due to its sensitivity towards various pollutants, particularly heavy metals. Preliminary bioassay tests on embryos and/or larvae of *Paracentrotus lividus* (Lamark, 1816) from Madagh (Oran, Algeria) were used to assess the potential toxicity and determine the LC_{50} of four metal pollutants, Cadmium, Copper, Lead and Zinc.

KEY WORDS Bioassays; LC_{50}; Madagh; Heavy metals; *Paracentrotus lividus*.

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INTRODUCTION

Inland aquatic and marine systems represent containers for virtually all contaminants, via direct and indirect contributions (Peijnenburg et al., 1997). Research on the action of heavy metals on the development of the sea urchin eggs represent an important contribution to the progress of knowledge in the field of embryonic determination.

Sea urchin is a preferred model in such a research, due to a number of reasons including external growth of embryos, rapid cell division rate and cell transparency, thus being commonly employed in the field of Environmental Toxicology (Guillou & Michel, 1993; Quiniou et al., 1997). Bioassays or bio-tests frequently use several techniques to measure, predict and control the effect of the release of toxic substances on marine organisms.

Present study examines the impact of four heavy metals, Cadmium, Copper, Lead and Zinc, on the embryonic development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata Echinoidea).

MATERIALS AND METHODS

We investigated the site of Madagh bay (Oran, Algeria: 35°37'952" N; 000°104'243" W) (Fig. 1), a non-impacted area, closed at its ends by two small caps reducing the action of winds. Moreover, the proximity of the Habibas island, which is considered a marine protected area, could make this site a reference station for comparative studies regarding the monitoring of pollution impacts in the coastal marine ecosystem of western Algeria, a site rich in algae and *Posidonia* meadows (Benghali, 2006).

Collection of sea urchins was carried out during the period March-June 2010, when spawning is at its peak in this echinoid species. Spawning was induced by injection of 0.5 ml of 0.5 M KCl into the

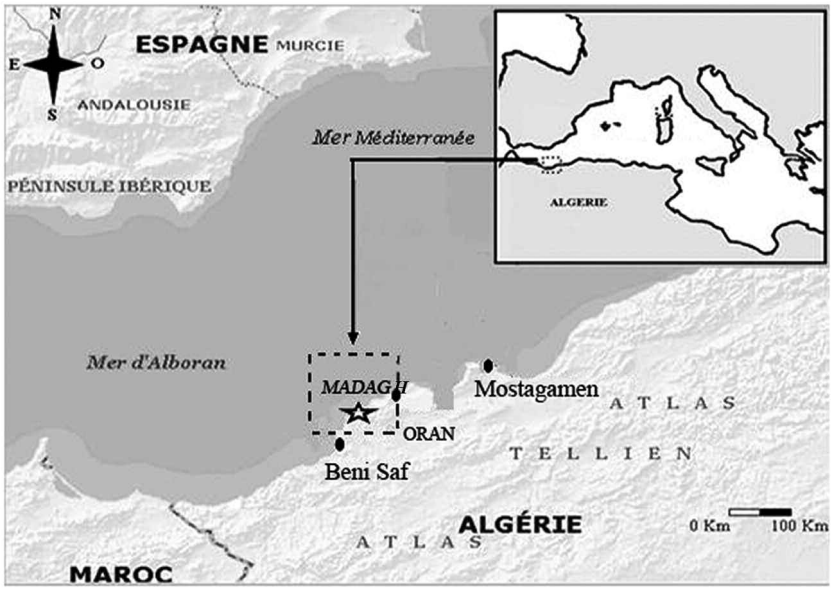

Figure 1. Sampling site: Madagh bay, Oran, Algeria.

coelomic cavity of the sea urchin (Harvey, 1940); the male sex products were recovered "dried" and stored in melting ice. Moreover, sperms of several males were pooled. Females were placed in a 250 ml Erlenmeyer flask containing filtered seawater (FSW) so that the genital pore was in contact with the surface of the water. After spawning, eggs were sieved with a 160 micron sieve and collected in a test tube. Volume was adjusted to 500 ml using FSW and homogenized. Subsequently, the first 100 ml of the solution (containing eggs) was removed and replaced by FSW. This operation was repeated a second time (Dinnel et al., 1988; Quiniou et al., 1999; Guillou et al., 2000).

Once suspensions of gametes were obtained, eggs and sperms were recovered separately in 2 ml of FSW. Fertilization was performed in beakers containing 1500 to 2000 oocytes to which 250 µl of sperm was added. After one hour of contact, we checked the fertilization success under a light microscope. Bioassays were carried out according to Coteur et al. (2003). A 15 well plate was used for each metal pollutant (Cd, Pb, Cu and Zn).

Four different increasing concentrations (10 µg/l; 50 µg/l; 100 µg/l; 200 µg/l) and a control solution (FSW), employed as blank, were used. Each well contained 10 ml of each solution, then 250-300 embryos were transferred to each well and incubated for 72 hours at 21 ± 1 °C.

At the end, larval development was stopped by adding neutral formalin (35%) and the percentage of anomalies was determined according to the criteria of Klöckner et al. (1985). Number of malformed eggs/larvae, expressed in percentage, was assessed under the optical microscope by scanning slides containing about 100 eggs each. The number of dead cells was adjusted by the formula: % mortalities corrected = (Po – Pt)/(100 – Pt), where Po is the percentage of mortalities observed and Pt is the percentage of mortalities in controls (Abbott, 1975).

Five replicates were performed for each concentration and each metal. The statistical treatment of experimental data was performed by the probit method (Bliss, 1935), which is useful for experiments with reduced number of animals and particularly suitable for research on marine invertebrates, as confirmed by Bendimerad (2000).

RESULTS

The mean percentages of embryonic abnormalities observed in each heavy metal solution ± standard deviation are shown in Table 1.

As shown in Table 1, concentrations of 10 µg/l and 50 µg/l determined minor negative effects on larval development. At 100 µg/l, the malformation percentage is about double or more (respect to 50

[µg /l] Metals	10	50	100	200
Cd	7.31 ± 1.84	18.26 ± 2.97	79.32 ± 15.38	88.00 ± 14.60
Pb	5.00 ± 1.68	12.20 ± 7.85	22.40 ± 7.97	40.33 ± 4.04
Cu	5.93 ± 1.76	28.93 ± 4.52	41.91 ± 4.13	52.66 ± 24.61
Zn	10.60 ± 2.70	17 ± 3.98	36.73 ± 10.32	40.5 ± 24.02

Table 1. Mean percentages of abnormal embryos ± standard deviation observed in the sea urchin *P. lividus* from Madagh bay (Oran, Algeria) treated with four heavy metal solutions at different concentrations.

µg/l) depending on the metal, in particular, it resulted 79.32% for Cd, 41.91% for Cu, 22.40% for Pb and finally 36.73% for Zn (Figs. 2-5).

Graphs show results we partly expected: the more the concentration increases, the more the percentage of malformations is important, although, surprisingly, percentage of malformations observed after treatment with Cadmium at 200 µg/l is higher (88%) (Fig. 2) than that detected with the other metals: 52.66% (Copper, Fig. 3), 40.33% (Lead, Fig. 4) and 40.50 % (Zinc, Fig. 5).

LC_{50}, calculated according to the method of Bliss (1935), resulted 61.65 µg/l for Cd, 158.48 µg/l for Cu, 389.04 µg/l for Zn and 446.68 µg/l for Pb.

DISCUSSION

Geffard (2001), using Pb solutions at 10 µg/l and 50 µg/l, obtained, as percentages of malformations, 14.8 ± 6.7% and 17.2 ± 3.9%, respectively; whereas, 100% of larvae with abnormalities were observed at 1200 µg/l. The LC_{50} was 482.0 ± 101.0 µg/l. The LC_{50} value we found for Pb is 446.68 µg/l. When comparing the two values, they appear to be very similar.

In a previous study, carried out on the same species and in the same biotope, Dermeche (2010) reported, for Cd and Pb solutions at 10 µg/l, percentages of malformations of 8.33 ± 0.47% and 10.66 ± 0.47%, respectively. At 200 µg/l the percentages passed to 82.33 ± 0.94% and 40.67 ± 0.94% with a LC_{50} of 69.18 µg/l for Cadmium and 436.51 µg/l for Lead. Once again, these values are close to the values discussed in the present paper (61.65 µg/l for Cd and 446.68 µg/l for Pb). Many authors use Zinc and Copper to determine the LC_{50} by using the sea urchin larvae. According to Hall & Golding (1998), Ghirardini et al. (2005) reported that a concentration of 30 µg/l of Copper shows a negligible effect, while it takes a concentration of 50 µg/l to observe the first malformed larvae. These results are consistent with those obtained by His et al. (1999) who observed developmental defects after treatment with a Copper solution at 60 µg/l.

Our study gives a percentage of 28.93 of malformations at a concentration of 50 µg/l, a result that remains consistent with results obtained by different authors. Bougis & Corre (1974) suggested that the effect of Copper varies depending on the quality of brood stock. This would explain different results obtained. It is likely that gametes of poor quality are more sensitive to a toxic agent.

Although echinoderms are capable of removing accumulated contaminants, the residence time in the body and the principal mode of elimination appear to depend on the metal (Warnau et al., 1997; Mannaerts, 2007). According to Basuyaux et al. (2009) and Pétinay et al. (2009), larvae can develop up to a Copper concentration of 90 µg/l but malformations start appearing at 50 µg/l. Copper leads to a significant reduction in growth at 30 µg/l with larvae showing spicules reaching 464 ± 7 microns while normal ones generally are up to 495 ± 9 µm. Bielmyer et al. (2005) noted that abnormalities in larval development and, above all, the stop at pluteus stage were manifested at concentrations ranging from 40 to 80 µg/l.

According to Fernandez & Beiras (2001), Cd causes 100% of arrest of development of the

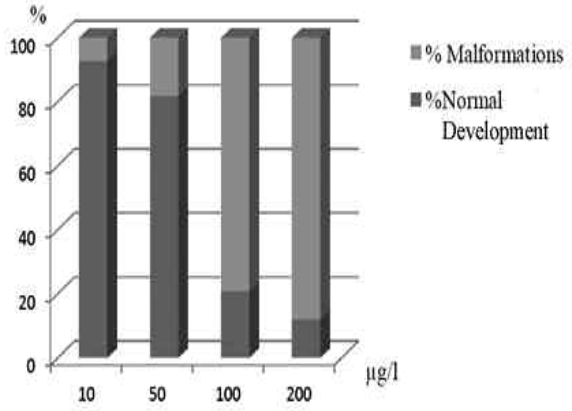

Figure 2. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after tretament with Cadmium solutions.

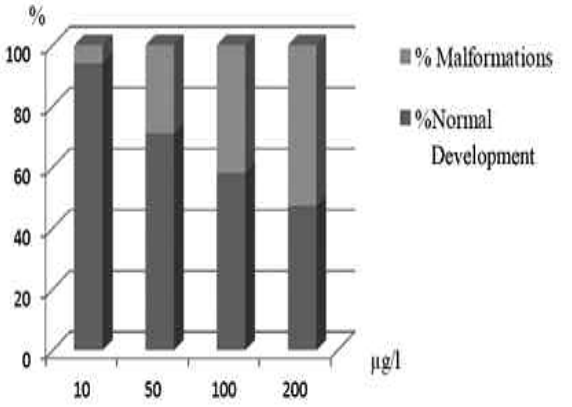

Figure 3. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after tretament with Copper solutions.

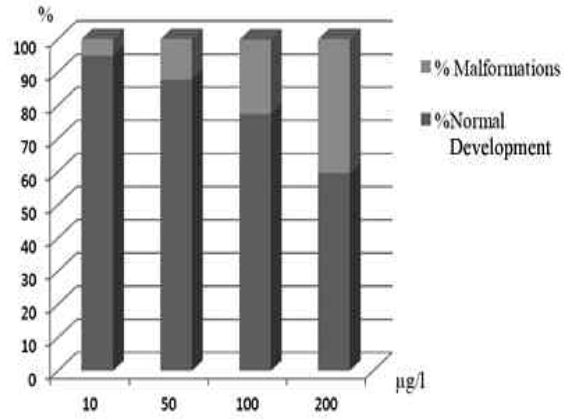

Figure 4. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after treatment with Lead solutions.

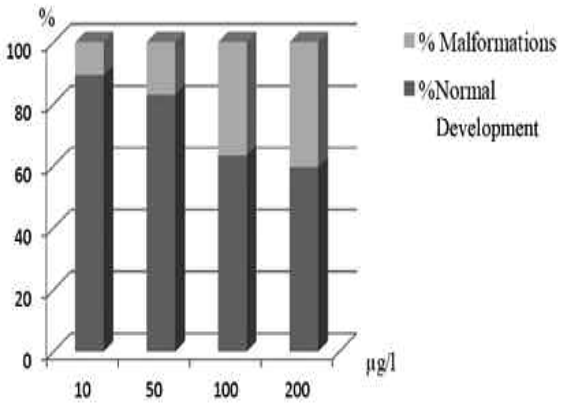

Figure 5. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after treatment with Zinc solutions.

pluteus at 16 µg/l and the stop at blastula and gastrula stage at concentrations from 32 to 64 µg/l, respectively. Several studies demonstrated sensitivity of sea urchin embryos to heavy metals solutions in the range of 0.01-0.1 mg/l for Hg and Cu, and 0.1-10 mg/l for Cd and Pb (Waterman, 1937; Kobayashi, 1981; Carr, 1996; Warnau et al., 1996).

In a Cu solution at 64 µg/l, embryonic development was arrested at gastrula stage, and at morula stage at 128 µg/l. The toxic effects of Zinc on the larval development of sea urchin were as follows: the highest concentration used, 480 µg/l, completely inhibited the embryonic development; at very low concentrations (7.2 µg/l) no inhibitory effects were observed at first cleavage or at pluteus formation; exogastrula and Apollo-like gastrula were observed at concentrations ranging from 14 to 58 µg/l. At higher concentrations (120 to 240 µg/l), embryonic development and the elevation of the fertilization membrane showed signs of delay and even malformations, as well as polyspermies, permanent blastula, or exogastrula (Kobayashi & Okamura, 2006).

Other metals known to cause exogastrulation in echinoids are: sodium selenite, cobalt chloride, zinc chloride or acetate, nickel, mercury chloride or acetate, the trivalent chromium and manganese chloride (Rulon, 1952; 1956; Timourian, 1968; Timourian & Watchmaker, 1970; Kobayashi, 1971; 1990; Murakami et al., 1976; Pagano et al., 1982;

Species	Cu	Cd	Pb	Zn	References
Paracentrotus lividus	<32 µg/l	> 11 µg/l	----	----	Pagano et al., 1986
	----	----	----	> 33µg/l	Ramachandra et al.,1997
	----	----	0.21 - 0.26 µg /l	----	Warnau & Pagano, 1994
	----	----	482.68 µg /l	----	Geffard, 2001
	158.48 µg/l	61.65 µg/l	446.68 µg/l	389.04 µg/l	present study
Strongylocentrorus purpuratus	6.3 µg/l	----	<9.7 µg/l	----	Dinnel, 1990
	----	0.5 µg/l	----	23 µg/l	Ramachandra et al.,1997
Strongylocentrorus intermedius	----	0.5 to 2.5 µg/l	----	----	Gnezdilova et al., 1985
Arbacia lixula	----	----	----	10-100 µg/l	Castagna et al., 1981

Table 2. Toxicity (LC_{50}) of heavy metal (Cd, Cu, Pb, Zn) solutions at different concentrations for different sea urchin species from the Mediterranean.

Mitsunaga & Yasumasu, 1984; Vaschenko et al., 1994); according to Lallier (1955) and Timourian (1968), skeletal malformations of pluteus were caused by Zinc whereas delay in skeletal development was always caused by Cadmium and Cobalt (Kobayashi, 1990; Mannaerts, 2007).

King & Riddle (2001) showed that exposure of *Sterechinus neumayeri* embryos to various concentrations of Copper caused significant damages to the development at different stages and, particularly, at the stage of blastula; moreover, significant abnormalities were observed at a concentration of 4-5 µg/l. High mortality of embryos was estimated at a concentration of 16 µg/l, and abnormalities were observed at a concentration of 32 µg/l; a Copper concentration of 11.4 µg/l caused 50% of developmental abnormalities after 6 to 8 days of exposure.

Radenac et al. (2001) reported, for Cu solutions at 50 µg/l, about 36.9% of malformations which approximates the results (28.93%) observed, for the same concentration, in this study; however, at 100 µg/l this rate reached 99.80% which exceeds our result (41.91%); 100% of larval malformations were obtained at 250 µg/l while our study showed 52.66% of malformations at 200 µg/l. For Pb solutions, at 250 µg/l, a high percentage (96.6%) of malformation was reported. On the contrary, in our study, at 200 µg/l, we obtained only 40.33% of malformations. These results seem to suggest a different sensitivity of different species to heavy pollutants; concerning Zn, our results are coherent with those of Radenac et al. (2001).

CONCLUSIONS

Considering different stages of development of *P. lividus*, embryos and larvae were found to be the most sensitive and best suited to study heavy metal toxicity. Moreover, they can be used when testing short (embryotoxicity) and long-term (larval growth) issues. Information reported in this study show that responses are indicative of embryo sensitivity. *P. lividus* fulfills the characteristics of a good indicator species as accumulative bio-indicator of the health of a given environment.

Cadmium, Copper, Lead and Zinc are considered among the most toxic and persistent pollutants, with a very long biological half-life (16 to 33 years) leading to accumulation in organs (Guthrie & Perry, 1980). It is therefore an urgent need for further research and adequate scientific-environmental strategies to encourage studies of this type and use the results for the management of harmful polluting sources.

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