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Cadmium, Copper and Tributyltin effects on fertilization of *Paracentrotus lividus* (Echinodermata)

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ABSTRACT - Marine environments are continuously being threatened by a large number of xenobiotics from anthropogenic sources. The effect of chemical pollution on living organisms are numerous and may impair reproductive success of adults species of marine invertebrate and vertebrate through effects on gamete quality. Echinoderms are characterized by external fertilizzation and gametes, free of any type of protection, may be in contact with toxic substances so the reproductive success depends largely on the environment conditions. The purpose of this work is to assess the effects on the *in vitro* fertilization of exposure of sea urchin *Paracentrotus lividus* gametes to xenobiotic substances as $CuSO_4$, $CdCl_2$ and TBTCl. The effect of contaminant were assessed by two experimental set in which gametes were treated with different concentration (0, 10^{-3}, 10^{-5} 10^{-7} 10^{-9} M) of different substances as $CdCl_2$, $CuSO_4$ and TBTCl. The effects were evaluated as percentage of fertilization. The results showed that the gametes exposure to xenobiotic decreased the percentage of fertilization and that more sensitive to treatment were the sperm cells, propably because the toxic effect affected the motility of the sperm. In conclusion, the absence of fertilization (spermiotoxicity) may submit the toxic effects of these substances to the level of body and may candidate the sea urchis as biosensors for the evaluation of environmental quality.

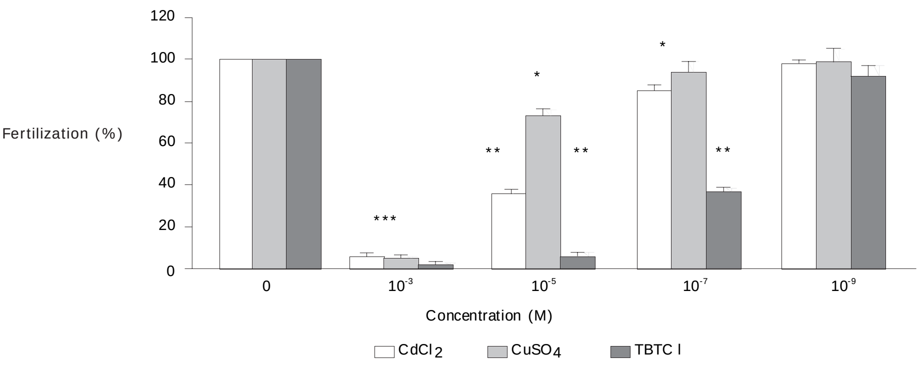
Key words: Paracentrotus lividus, Cadmium, Copper, TBT, Embryotoxicity, Biomarker.

Introduction - The chemical pollution of marine coast environments by the vast number of xenobiotics has increased during the last decade as a direct consequence of a wide variety of anthropic activities, becoming one of the most critical environmental problems. Among pollutants more widespread, heavy metals are predominant. They caused severe pollution because are non-degradable, they tend to accumulate in organisms' tissues and can be passed along food-chains, becoming toxic at high concentrations (Hopkin *et al*., 1989). Toxic effects can occur at all levels of biological organization, with toxins influencing ecological interactions such as predation, parasitism, competition and the structure of communities and ecosystems (Hoffman and Parsons 1994; Walker *et al.* 2001). In particular, pollution may impair reproductive success of organisms through decreasing the quality and/or quantity of gametes, which in turn may affect fertilization success, embryo development, larval viability and subsequently species fitness and survival. Sea urchins play a key role in controlling rocky subtidal community structures in coastal waters (Pringle *et al*., 1982; Fletcher, 1987) and changes in sea urchin populations have led to major alterations in marine community structure (Witman, 1985; Scheibling, 1986). Sublethal exposure to heavy metals (e.g. Cu, Zn and Cd) in the laboratory has led to poor embryonic and larval development in the sea urchin *Strongylocentrotus intermedius* (Khristoforova *et al*., 1984; Durkina, 1994), *Sphaerechinus granularis* (Lucu *et al*., 1991) and *Arbacia punctulata* (Bowen and Engel, 1996). In this study, we examined *in vitro* the effects of acute exposure to $CdCl_2$, $CuSO_4$ and TBTCl of sea urchin *Paracentrotus lividus* reproduction, in which was assessed 1. Morphology and heath status of gametes after treatment with xenobiotics; 2. percentage of fertilization in presence of xenobiotics using non exposed and exposed gametes, 3. Cleavage and embryo morphology. The results of this study allow a better understanding of the effects of cadmium and cuprum pollution on the reproductive success of sea urchins, and permit an extrapolation to predict population effects on this ecologically important species.

Material and methods - Sea urchin *Paracentrotus lividus* 8 – 10 cm diameter, were collected by SCUBA from subtidal zones of a pristine site of Trapani. Prior experiments specimens were acclimated for 10 days at 15°C in marine aquaria equipped with artificial sea water (425 mM NaCl; 9 mM KCl; 9.3 mM $CaCl_2{\cdot}2H_2O$; 25.5 mM $MgSO_4{\cdot}7H_2O$; 23 mM $MgCl_2{\cdot}6H_2O$; 2 mM $NaHCO_3$), biological and physical filters and fed with commercial invertebrate food (Azoo, Taikong Corp. Taiwan). To induce spawn individuals were injected with 1 ml/each 0.5 M KCl through the peristomal membrane. The sperm/eggs collected from each individual were stored separately in micro-tubes, and used for experiments within the same day. For determing the fertilization capability and embryotoxicity of treated gametes, sperm (ca. 0.5 ml) from male sea urchins were individually collected, divided in 0.1 ml aliquots ($4.5x10^5$ sperm/ml). The collected eggs after washing were divided in aliquots (150 eggs/ml). In order to test relative sensitivity to $CdCl_2$, $CuSO_4$ and TBTCl, each aliquots of sperm and eggs were exposed (1 hour at 19°C) to 0, 10^{-3}, 10^{-5} 10^{-7} 10^{-9} M for $CdCl_2$, $CuSO_4$ and TBTCl, than mixed in different combination with a ratio of 3000:1. Two experimental set were performed: #1. Gametes after exposure were washed and utilized for fertilization; #2. Fertilization was performed in presence of various concentrations of xenobiotics. Parameters measured were fertilization and embryo morphology. Each experiment was performed in triplicate. The values were the mean of three assays ±SD. Significance was determined by using the Student's t test and differences were considered significant at $P<0.05$.

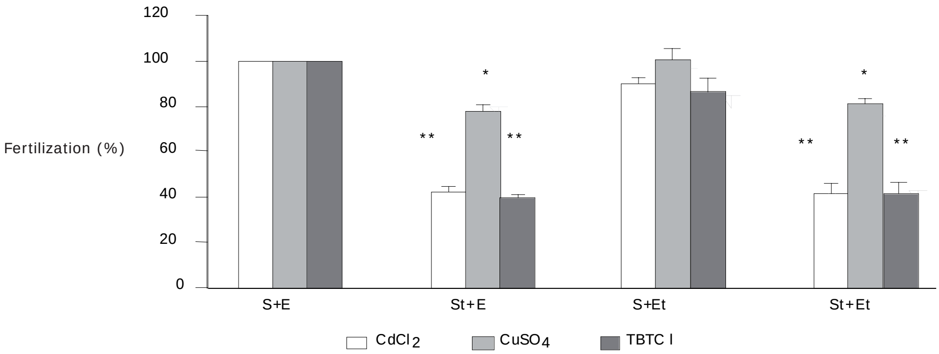
Results and conclusions - Pretreatment of gametes with concentration of xenobiotics ($CuSO_4$ 10^{-3} M, $CdCl_2$ 10^{-3} M and TBTCl 10^{-5} M) caused a severe dose-dependent toxic effecs on the fertilization. The impairment was more evident in the experimental set #2 in which the fertilization was performed in presence of xenobiotics concentrations of (Figure 1).

Figure 1. Fertilization of *P. lividus* gametes in presence of xenobiotics.



The TBT seemed to be more efficient than metals to disturb the fertilization. At concentration of 10^{-5} M was still able to inhibit significantly the fertilization (6.5%). At the concentration of 10^{-9} M the inhibitor effects of xenobiotics, were not more obvious. A smaller effect was recorded in a #1 experimental set (data not shown). The largest decrease is attributed mainly to the treatment of male gametes as showed by the experiments in wich were compared gametes xenobiotic treated ($CuSO_4$ 10^{-5} M, $CdCl_2$ 10^{-5} M and TBTCl 10^{-7} M) with non treated ones. When sperm preincubated for 1 h with xenobiotic were put with non treated eggs was observed an inhibition on fertilization (Figure 2). Such alterations may indicate that xenobiotics have caused structural impairments to sperm during development (Au *et al*., 2000). Propably dependenting from the low and imprecise mobility of sperm (Au *et al*., 2001a; b). The present findings is in agreement with the proposition that a decrease in fertilization capacity of *Paracentrotus lividus* sperm may be related to toxic effect of xenobiotic on sperm motility. Further studies will be necessary to determine the effects in subsequent stages of development and evaluate the levels of embryotoxicity of xenobiotic substances. These results propose the sea urchins *Paracentrotus lividus* as biosensors for the evaluation of environmental quality.

Figure 2. Effect of xenobiotics exposure to *P. lividus* gametes. S=untreated sperm; E=untreated egg; St=sperm incubated for 1h with xenobiotics; Et=egg incubated with xenobiotics for 1h.



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