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Bioavailability of Sea Urchin to Aquatic Toxicity Tests

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Abstract

Echinoderms a wide range of valuable biological processes can be considered and can be relevant for Eco toxicological analyses. As is well known the effects of toxic chemicals and complex mixtures on early developmental stages of aquatic organisms are of great importance in the protection of the natural population's health. The sea urchin is one of the most sensitive and suitable test organisms for acute bioassay of pollution. The use of sea urchin embryos and gametes in testing developmental, reproductive and cytogenetic effects of chemicals and complex mixtures has been successfully developed by a number of laboratories worldwide. The use of multispecies tests capable of accounting for the differences in sensitivity of different species to different contaminants.

Keywords

Sea urchin; Ecotoxicology; Aquatic pollution

Introduction

There are numberless pollutants in the aquatic environment that compromising survival of the organisms, altering their physiologies. Consequences caused by these pollutants may remain recessive for several generations or may exhibit major effects in the population [1]. Pollution in the aquatic environment causes multiple damages in the organisms, at the level of population and ecosystem, as in organ function, reproductive stages, and biological diversity. Aquatic organisms expose too many xenobiotic during their lifespan both from the water and through aquatic food chain. Studies reveal the fact that a number of chemicals contaminated to the environment have toxic and developmental effects. The major sources for these chemicals substances are industrial and agricultural activities. Xenobiotic from those sources ultimately contact the aquatic ecosystems. The degree of contamination has traditionally been appraised by chemical analyses that provide no estimate of the deleterious effects on the biota. An evaluation of the complex actions and bioavailability of contaminants and the determination of the biological effects of unidentified substances require bioassays with live organisms [2-9]. In studies carried out to date it has been attempted to demonstrate the effects of pollution on living creatures by using macro- and micro-organisms [10-12].

From this point of view echinoderms to be good candidates for being selected as marine target macro invertebrates and useful test species in ecotoxicology. Brusca and Brusca had reported that they are key-components of the marine ecosystems, where they can contribute to more than 90% of the benthic biomass and their benthic habits make them easy targets of environmental contamination, with particular reference to micro pollutants stored in marine sediments [13, 14]. Additionally, many echinoderm species are second or third level predators, and therefore they are susceptible to biomagnifications processes and their developmental biology is well-characterized [15]. In addition, echinoderms should be considered particularly as relevant test species because of their key phylogenetic position: they are deuterostomians and therefore they are closely related to vertebrates. For this reason they show some similarities with vertebrates in terms of physiological processes and hormonal pathways and, possibly, they might share also similar targets at both the organism and tissue/cell level in terms of response to environmental contamination [16, 17].

As is well known the effects of toxic chemicals and complex mixtures on early developmental stages of aquatic organisms are of great importance in the protection of the natural population's health. The sea urchin is one of the most sensitive and suitable test organisms for acute bioassay of pollution. The use of sea urchin embryos and gametes in testing developmental, reproductive and cytogenetic effects of chemicals and complex mixtures has been successfully developed by a number of laboratories worldwide [6-9, 18-20]. Criteria for the choice of organisms for bioassays have been established by Stebbing et al. who recommend the use of multispecies tests capable of accounting for the differences in sensitivity of different species to different contaminants [3]. The embryos and larvae of marine organisms are generally more sensitive to toxic substances than adults, and gametes and embryos of sea urchins *P. lividus* and *A. lixula* have been recognized as valuable tools in toxicological studies since Lillie first studied Arbacia [21]. The effects of numerous factors which cause change in seawater quality upon early development, reproduction and genetics are being researched in many laboratories across the world using sea urchin embryos and gametes, including those of *P. lividus* [18]. In contrast, *A. lixula* has not been widely used for toxicity testing, although in some studies it has been used in conjunction with *P. lividus* [6, 22-25]. Sea urchins constitute the group of creatures most preferred for use in many ecotoxicological studies because of the advantages such as; a) they provide an opportunity to work with a metazoan organism, b) test organisms are available throughout virtually the whole year, c) the tests give speedy, easy and sensitive responses in a very short time, d) cost is low, and e) they enable the sub-lethal effects to be determined [26].

Paracentrotus lividus Lamarck 1816 (rock sea urchin) is a species commonly used in marine toxicity tests. A characteristic of this species as bioindicator is its wide distribution, throughout the Mediterranean Sea [27]. *Arbacia lixula* Linnaeus 1758 (black sea urchin) has been found to share habitat with *P. lividus* in several zones, both in Mediterranean and Atlantic coasts, Therefore, it may also be a good candidate for toxicity testing. Despite these species can share habitat (shallow waters on rocky shores), their preferring different substrate for feeding [27-31]. *P. lividus* feed mainly on algae, sea grass and particulate matter [27]. *A. lixula,* on the other hand, has been reported to graze encrusting coralline algae and possibly sessile invertebrates [27, 30, 31]. Embryo-larval bioassays have proved to be very sensitive indicators of seawater contamination because larvae represent critical stages of life [6, 32, 33]. The use of fertilization and embryo-larval development toxicity bioassays to assess marine sediments and seawater pollution in a monitoring procedure in coastal areas requires analysis of the sensitivity of native sea urchin bioindicator species, together with standardization and optimization of the toxicity tests. Fertilization and larval development tests with *P. lividus* have shown good conformity between tests results and pollutant contents [6, 8, 9]. In contrast, *A. lixula* has, not been widely used for toxicity testing [6, 22, 30], although in some studies it has been used in conjunction with *P. lividus* [23-25, 34]. No comparative study has been made of the differences in sensitivity between the two species.

A large data set exists on the toxicity of several chemicals on the developmental stages of *P. lividus* [6, 8, 9, 35-37]; Pagano et al. had reported toxic effects of sediments and waters from two river (Sarno River and Volturno River) in Italy using sea urchin *P. lividus* embryotoxicity and fertilization test and found that the Sarno River should be regarded as an indicator of the poor environmental conditions of these water bodies [26]. Also, they had suggested that sea urchin is a sensitive tool for evaluating biological quality of contaminated sediments and waters.

Roepke et al. had reported that as one of two invertebrate deuterostome phyla, echinoderms exhibit a developmental pattern that is similar to chordates, thus providing a valuable tool for studies of development [38]. That is why the sea urchin is used as one of the most suitable test organisms for acute bioassays of marine pollution reported the strategic advantages of testing animals different from vertebrates [5, 7], whose employment is often restricted by ethical and practical reasons, and emphasizes how valuable and useful model echinoderms can be for ecotoxicological tests. Toxicity bioassays using the sea urchin gamete and embryos appear to be quite sensitive and informative, offering wide range endpoints. Sea urchin bioassays are now widely used in studies involved in the toxicological characterization of heavy metals and xenobiotics in environmental monitoring [6, 18, 20, 39]. A large data set exists on the toxicity of several chemicals on the developmental stages of *P. lividus* [6-9, 35-37]. In contrast, *A. lixula* has not been widely used for toxicity testing [6, 22].

Warnau and Pagano noted that $PbCl_2$ does not exert any detectable spermiotoxic effect on *P. lividus* fertilization [36], while larval malformations significantly increased at the 10^{-7} M $PbCl_2$ through embryogenesis. Atkinson et al. reported that the reproductive hormone, estradiol and its synthetic counterpart, ethynylestradiol, inhibited normal embryonic development in the sea urchin embryo and larvae at concentrations relevant to those present in the environment (1×10^{-3} ng/ml to 10 ng/ml) [40].

In the study by Ghirardini et al. using *Paracentrotus lividus,* in order to determine the spermiotoxic test's sensitivity and selectivity the non-ionic surface active substance Linear alkyl benzene sulphonate (LAS) and nonylphenol polyethoxylates (NPEs) compounds as well as biotransformation products (NP) were used [41]. The impact of NP and NPEs on fertilization was determined with a spermiotoxicity test, for *P. lividus* the EC_{50} value of NP and NPEs was reported as 0.27 mg-NP/L and 1.94 mg-NPEs/L respectively. At the same time, as a result of the chronic toxicity test, NP was reported to have no chronic effect on reproduction of *P. lividus* [41]. Roepke et al. using two different sea urchin species (*Strongylocentrotus purpuratus* and *Lytechinus anamesus*) exposed endocrine disrupting compound OP (1, 2, 3, 4 and 5 ng/ml), at fertilization it was reported that 96 h later at the pluteus stage it had retarded development and caused a decrease in normal pluteus frequency. OP was also found to cause developmental anomalies at a concentration of 1-100 ng/ml [38, 42, 43]. Kyomoto et al. showed that the effect of estrogenic compounds on sea urchins, *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus,* ontogeny differs according to the developmental stage [44]. According to their results, the exposure to EER disturbed early embryogenesis, but had little effect on later larval development, even at relatively high concentrations, whereas for the juvenile stage it promoted growth. However, BPA suppressed juvenile growth, even at 0.5 µM, which was too low to affect early embryogenesis. This reveals that the dose dependence and even the mode of effect of these compounds can change during the animal development.

Ghirardini et al. investigated that the capacity of two toxicity bioassays (fertilization and embryo toxicity tests) to discriminate sediment toxicity using the sea urchin *Paracentrotus lividus* in five stations with different levels of pollution in the Lagoon of Venice [45]. Results of this investigation showed that both higher embriotoxicity than spermiotoxicity in all stations and also researcher reported that a good fit was found between ecotoxicological data and chemical contamination levels.

The objective of the study by Dinnel et al. was to compare the sensitivity of a standardized sea urchin sperm/fertilization assay to the responses of embryo, larval, and adult marine organisms to metals (Ag, Cd, Cu, Pb, Zn) and pesticides (DDT, Dieldrin, Endrin, Endosulfan) added to natural seawater [46]. The results, although highly variable, generally showed that sperm/fertilization and embryo assays were quite sensitive to the metals tested, but that the larval and adult assays were more sensitive to the pesticides. These comparative data, together with other studies of complex effluents, show that the standardized sperm/fertilization bioassay is an especially quick and useful tool for biomonitoring of marine waters.

Study by Novelli et al. sperm cell and embryo toxicity tests was performed using the Mediterranean sea urchin *Paracentrotus lividus* to assess the toxicity of tributyltin chloride, bis(tributyltin)oxide, triphenyltin acetate, and triphenyltin hydroxide [47]. In this experiment mean effective concentration values (EC_{50}) reported as ranged from 2.97 to 18.5 µg/L for sperm cells and from 1.11 to 2.62 µg/L for embryos. Besides that the two tributyl compounds was significantly found toxic greater than that of two triphenyl compounds for sperm cells and for embryos, the triphenyl compounds found more toxic.

Castagna et al. reported the action of various concentrations of zinc on different phases of development of *Arbacia lixula* [22]. They were used in the experiment 0.01, 0.1, 1 and 10 mg/L zinc concentrations which were tested on unfertilized eggs, sperms, fertilized eggs and adult individuals. It was found that after 96 h a 0.01 mg one to one concentration reduces the mobile sperm percentage compared with the control group. The results show that to be possible in the case of zinc: in fact, the percentages of mobile forms of sperms after 72 h at concentrations of 0.1, 1 and 10 mg one to one are considerably lower than those found in the control group and at 0.01 mg one to one, discriminating, of course, between the concentrations that are harmful even to the eggs in development. Castagna et al. results confirm the fact that the embryonal stages have a higher sensibility to toxicity than adult organisms of the *Arbacia lixula* [22]. In the study of Pagano et al., the action of Cr6+ (as chromate) and Cr3+ (as sulfate and nitrate) on fertilization and development has been investigated in sea urchins *Paracentrotus lividus* and *Sphaerecinus granularis* [39]. They are reported that, the differentiation of the gut and skeleton was severely affected in the presence of chromate (5×10^{-5} to 5×10^{-4} m) by rearing the embryos. And also this study showed that the treatment of sperm before fertilization with $CrO^{2-}{}_4$ (10 to 30 min, 10^{-4} to 10^{-2} m) resulted in a number of abnormal larvae, depending on the length of exposure and the $CrO^{2-}{}_4$ concentration. At the end of this report, Researcher were reported that this kind of toxicological study remarkable for environmental contamination.

Maisano et al. reported the embryotoxicity of CuO Nanoparticles in the black sea urchin *Arbacia lixula* embryos [48]. Fertilized eggs were exposed to five doses of CuO NPs ranging from 0.07 to 20 ppb, until pluteus stage. In their study developmental delay and morphological alteration, including skeletal abnormalities, were observed, as well as impairment in cholinergic and serotonergic nervous systems. These findings suggest the potential of CuO NPs to interfere with the normal neurotransmission pathways, thus affecting larval morphogenesis. The study by Maisano et al. showed that, the embryotoxicity tests are effective for evaluation of nanoparticle effects on the health of aquatic biota [48]. Furthermore, as the black sea urchin *A. lixula* demonstrated to be vulnerable to NP exposure, it may be a valid bioindicator in marine biomonitoring and Eco toxicological programmes. And also researcher reported that the black sea urchin may be proposed as a valid bioindicator in marine biomonitoring and Eco toxicological programmes. As echinoderms are a sister group to the chordates in which humans are classified, investigation on sea urchins may provide new insights on the potential similarities between invertebrate and vertebrate systems on their biological responses.

In scientific literature it may be attained some investigations having test with two species comparatively. Carballeira et al. reported some adverse effects of salinity on eggs of *P. lividus* and *A. lixula* and found that fertilization of *A. lixula* and *P. lividus* was not found to be affected by salinity [49]. In another study by Carballeira et al., embryos of two species of sea urchin (*P. lividus* and *A. lixula*) were exposed to antibiotics and disinfectants and the abnormalities in larval development, and the effective concentrations (ECs) were calculated to evaluate the toxicity [49]. This study reported that both species showed similar sensitivities to all substances tested. One of the research show similar sensitivity of *P. lividus* and *A. lixula* when exposed to samples with high levels of ammonium and cadmium, ammonium chloride, zinc sulfate and sodium dodecyl sulfate [25, 50]. Previous studies have also shown that different sea urchin species display similar sensitivities when exposed to sediment and effluent samples, reference toxicants, metals and mixtures of metals [23, 25, 30, 50].

Arslan et al. carried out a research previously with *Paracentrotus lividus* and *Arbacia lixula* to determine the effects of the NP and OP (concentrations ranging from 0.937 to 18.74 µg/L), and octylphenol (concentrations ranging from 5 to 160 µg/L) on embryonic development shows that *A. lixula* is more resistant than this species [7]. In the study of Arslan and Parlak, embryotoxicity and spermyotoxicity of Bisphenol-a was tested on both species of sea urchin and negative effects were observed on two sea urchin species embryos at concentrations of 300-3500 µg-BPA/L for *P. lividus* and 5 to 3500 µg/L for *A. lixula* [8, 9]. As a result of study of Arslan et al. dilutions of water and sediment samples were toxic for two sea urchin species and showed that *A. lixula* was found to be more tolerant to contaminated sediment and water than *P. lividus* [51]. In conclusion, this investigation has pointed out a clear response of early life stages of *P. lividus* and *A. lixula* to different dilutions of contaminated water and sediment samples and sperm bioassays test of sea urchins may be good method to evaluate the toxicity of contaminated environmental samples.

Finally, the results of this report we indicate that the sea urchin species *P. lividus* and *A. lixula* are not equally sensitive to different types of chemicals and complex mixtures and so can be used in Eco toxicological studies.

Conclusion

The present review and previous studies showed that a number of agents have been tested with sea urchin species bioassay including physical agents and chemicals such as inorganics, organics, pharmaceutical drugs and industrial and domestic effluents [6]. Beside that water quality bioassays by sea urchin embryos have been used to monitor the biological effects of contamination in marine environment. This bioassay may provide reliable information on several key-events such as: c, mitotic activity, larval differentiation [52].

These types of studies are important in predicting the toxic effects of pollution on living organisms. Besides that, the suitability of carrying out fertilization and larval development tests at different sea urchin species in risk assessment procedure.

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