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Review Article

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Sea Urchin Bioassays in Toxicity Testing: II. Sediment Evaluation

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Abstract

Bioassays on sea urchin early life stages have been used extensively in evaluating the pollution impact on seawater, coastal sediments, and other matrices as soil, freshwater sediment, and industry effluents. Here we review the literature in this field to determine whether testing whole sediment *vs.* pore water or elutriates by sea urchin bioassays provides a better estimation of actual risk. The present review of our results and from independent groups suggests that testing whole sediment as opposed to other substrates is better suitable, especially when a topographic evaluation of sediment toxicity is required, such as in enclosed bays or in lagoons. Unrestricted to marine sediment, the available methods in testing whole sediment provide an opportunity to test inland, freshwater or terrestrial materials, useful when answering complex mixture questions common to various monitoring and research programs, or for environmental assessment evaluations, or for remediation/ mitigation planning exercises.

Keywords

Sea urchin; Sediment; Pore water; Elutriate; Effluent; Soil

Introduction

Among environmentally occurring complex mixtures, marine sediments have been the focus of extensive research and monitoring efforts when concerned with ecosystem health status of coastal waters following pollutant releases and dredging operations [1,2]. Sea urchin bioassays are a recognized and broadly utilized component of our toolbox when assessing sediment contaminants and impacts to altered health status. After the pioneering report by Kobayashi in 1971 [3] focused on the evaluation of water quality in Seto Bay (Japan), sediment evaluations were recognized as being important when assessing the adverse effects of marine pollution on resident biota. The methodologies were further developed, standardized and regulated by several environmental protection agencies [4-11] due to their importance in answering important ecotoxicity questions.

The present review is aimed at providing a survey of the current body of literature on the use of bioassays on sea urchin early life stages in sediment toxicity testing, along with a critical evaluation of the methods utilized in sediment toxicity evaluations. The background literature and prospective use of non-marine substrates in answering similar questions are also outlined.

Methods in Sediment Toxicity Testing

Evaluating sediment quality is a complex procedure commonly ascribed to the so-called "triad" encompassing (i.) analytical determination of pollutants in sediment and resultant bioaccumulation in sediment-dwelling biota; (ii.) evaluation of biodiversity and population density of said biota in sediment and (iii.) *in vitro*, *in vivo*, or mesocosm toxicity bioassays in some selected bio indicator benthic biota [1,2,4-6,12].

Among bioassays in sediment toxicity testing, three sediment preparation methods have been utilized, i.e. by testing pore water (PW), elutriates (EL), or whole sediment (WS). For particular purposes, other substrates may be utilized, such as spiked sediment or overlying water.

Pore water, also termed interstitial water, is the water occupying space between sediment or soil particles [2] and is obtained by centrifugation or filtration of WS. Its presence and potential utilization is conditioned by sediment/soil granulometry due to its limited availability in sandy substrates.

Elutriate is the water extracted from shaking of WS with water (usually in ratio 1:4). The mixture is allowed to settle and the liquid phase is centrifuged and/or filtered [2,13]. The EL test was designed for evaluating the potential effects of water-soluble constituents of dredged material [14].

Whole sediment may be tested with or without minimal manipulation, such as freezing and thawing while maintaining both water and solid phase of the original sediment sample [2].

Following the recommendations of most environmental protection agencies [4-11], a frequently adopted practice in sediment toxicity testing includes a combination of both EL (and PW) and of WS by using different test organisms. For example, amphipods or polychaetes are commonly suggested when testing WS, while sea urchin and/or oyster embryos are recommended in EL or PW tests [15-19].

Thus, most of the available literature on sediment toxicity testing in sea urchin early life stages relies on EL or PW testing. As shown in Table 1, sea urchin embryo and sperm bioassays have been (and currently are) performed in an extensive number of investigations on sediment toxicity by means of EL or PW preparations [19-43]. It should be noted that a number of these reports showed PW-induced fading results [26-28], or openly recognized inconsistencies between the results of contaminant analyses and the outcomes of sea urchin bioassays [41-43].

The use of WS in toxicity evaluations has been applied in a number of studies of sea urchin embryo and sperm bioassays on marine and freshwater sediments, and on soil samples [44-55], as presented in Table 2. Most of this literature has been reported by our group [44,47-55] through active research projects of Toulon Bay (France) in 1990 [44] and later in a series of coastal sites in Europe under two European Commission-supported projects (BIOMAR I and BIOMAR II Projects, #ENV5V-CT94-0550 and #ENV4-CT96-0300) [47], and finally after more recent studies of sediments in Mytilene

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Table 1: Reports on sediment toxicity testing in sea urchin bioassays: use of pore water or elutriates.

Sediment extracts	Tested sediment sites	Other test organisms	References
Porewater	Galveston Bay, Texas	Amphipods	[19]
	St. Lawrence Estuary		[20]
	Lavaca Bay, Texas		[21]
	Spiked sandy marine sediment	Amphipods	[22]
	Sydney Harbor	Amphipods and Microtox	[23]
	Tampa Bay, Florida	Amphipods; polychaetes; seagrass; Microtox/Mutatox	[24]
	Corpus Christi Bay, Texas		[25]
	Puget Sound (Washington, USA)		[26]
	Venice Lagoon, Italy [27]	*Mytilus galloprovincialis; Crassostrea gigas*	[27]
	Portmán Bay (Spain)	Porewater and sediment–water interface	[28]
	Puget Sound (Washington, USA)		[29]
Elutriates	Venice Lagoon, Italy	*Corophium volutator; Crassostra virginica*	[30,31]
	22 different elutriates	Elutriate: rotifers (*Brachionus plicatilis*) WS: amphipods (*Corophium volutator*); polychaetes (*Arenicola marina*)	[32,33]
	Paranaguá Estuarine System, Brazil	Sediment-water interface vs. elutriate	[34]
	Ulla river (Galicia, Spain)	*Isochrysis galbana; Mytilus galloprovincialis; Venerupis pullastra; Siriella armata*	[35]
	Rio San Pedro (Spain)	*V. fischeri* (Microtox); amphipods	[36]
	various ports (Spain)		[37]
	Ceará River (Brazil)	Sediment-water interface vs. elutriate	[38]
	ports and harbors in Adriatic Sea (Italy)		[39]
	Bay of Cádiz (Spain)		[40]
	Vigo, Bilbao and Pasajes harbors (Spain)		[41]
	Mar Piccolo (Taranto, Italy)		[42]
	12 selected (estuarine, coastal, offshore) sites		[43]

Table 2: Reports on sediment toxicity testing in sea urchin bioassays: use of whole sediment or of non-marine

Substrates	Tested sites	Other test organisms	References
Whole marine sediment	Toulon Bay (France)		[44]
	Gironde Estuary (France)	*Crassostrea gigas; Tigriopus brevicornis*	[45]
	San Diego and San Francisco Bays (USA)		[46]
	Several European sites		[47]
	Izmir Bay (Turkey)		[48]
	Izmir Bay; Mytilene Harbor (Aegean Sea)		[49], [50]
Freshwater sediment	Two rivers in Campania (Italy)		[51] [52]
	Cd(II)-spiked river sediment (Italy)		[53]
Other substrates	Bauxite manufacturing by-products		[54]
	Bauxite residue-polluted soil		[55]

Harbor (Greece) and Izmir Bay (Turkey) [48-50]. Other groups also actively reported on WS toxicity testing and provided evidence that this approach yielded different results, and often supported the notion that WS was more toxic than elutriates by nearly two orders of magnitude [45]. Furthermore, toxicity was consistently found to be greater in embryos exposed to the sediment-water interface (SWI) rather than to intact (homogenized) sediment samples [46]. Unconfined to marine sediment, toxicity testing using sea urchin bioassays was conducted on other substrates, such as freshwater, brackish sediment, and soil samples, utilizing the same methods as in WS toxicity testing [51-55].

Evaluating sediment toxicity of water phase (PW/EL) or WS through sea urchin bioassays

Comparing strengths and weaknesses of sediment toxicity testing in sea urchin bioassays by means of water phase (PW/EL) or WS substrates involves both qualitative and quantitative remarks.

WS testing: As summarized in Figure 1, a major strength ("+") in testing WS toxicity consists of observing both the effects of water and solid phases at environmentally relevant concentrations, such as 0.5 to 1% in embryo-larval cultures. The contact of cleaving (pre-hatch) embryos on the bottom of culture wells warrants the transfer, if any, of toxic sediment components from solid phase to embryos.

A study by Romaña et al. [44] reported on the impacts of a wastewater treatment plant on sediment from Toulon Bay (France). The results showed superimposable topographic patterns of WS toxicity in sea urchin bioassays *vs.* chemical analyses of contaminants in this sediment as a function of particulate deposition from an urban wastewater outlet. Another study evaluated WS toxicity offshore the Sarno River estuary in the Bay of Naples (Italy) [47]. As shown in Figure 2, beyond the most toxic sediment close to river mouth ("Ø"), a steady decrease of WS toxicity was observed up to 1km offshore. This was followed by a significant toxicity peak at 1.5 km offshore, then declining at further distances from the mouth of the river [47].

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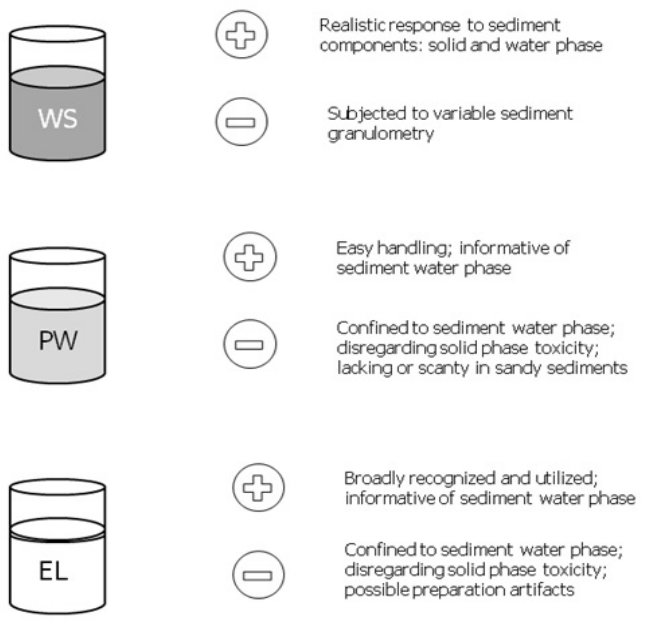

Figure 1: Outline of advantages (+) and disadvantages (-) in sediment toxicity testing by using whole sediment (WS) *vs.* pore water (PW) *vs.* elutriate (EL).

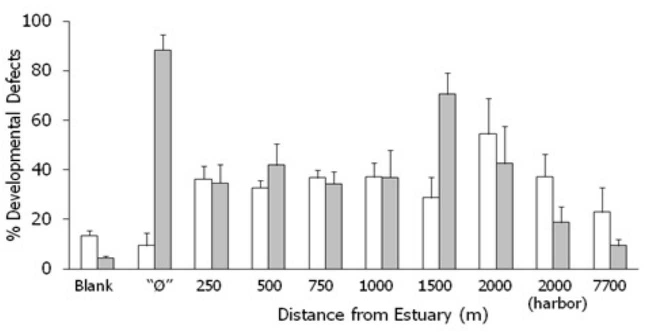

Figure 2: Sediment toxicity offshore the Sarno River estuary (Naples Bay, Italy) from river mouth ("Ø") to 7.7 km offshore showing a bimodal trend with two maxima at "Ø" and 1.5 km offshore [47], reproduced with Elsevier permission]. This trend was consistent with the data reported by Romaña et al. [44] in Toulon Bay (France).

A series of studies of sediment toxicity in several European coastal sites [47] allowed obtaining topographic grading of WS toxicity. Sediment collected in Kiel Fjord and offshore Warnemünde (Germany) allowed us to detect WS toxicity in two series of sampling sites, in terms of embryotoxicity, spermiotoxocity, offspring damage and of alterations in redox endpoints (reactive oxygen species production and glutathione levels) [47]. By comparing centrifuged WS pellet *vs*. supernatant, distinct toxicity patterns were detected according to sampling sites (Table 3). These data suggested that sediment toxicity evaluations should combine both solid and water phases, as present in WS.

A main limitation ("-") in WS toxicity testing relies on the granulometry sediment variable, that is recognized as exercising a bias on the effective concentrations of sediment granules (Figure 1). Historically, this granulometry bias was the ground for proposing water phase (PW/EL) sediment toxicity testing [1,2,4,13-15].

PW testing: Utilizing PW in sediment toxicity testing allows for easy sample preparation and quick testing. This procedure is also broadly recommended and applied in several studies; a number of those studies tested PW with sea urchin bioassays, combined with WS sediment testing in other bioassay models, such as amphipods [19-24,26, 27,31,32,37]. Two limitations affect the informative scope of PW toxicity testing, since this procedure disregards the role of sediment solid phase, while the amount of extractable PW strongly depends on sediment granulometry and composition.

EL testing: Derived from PW sediment toxicity testing, EL was designed as a means to extract potentially harmful sediment components by suspending sediments and filtering the aqueous product [5,6,13-15]. By far the most widely applied procedure in sediment toxicity testing, EL is the matrix utilized in the majority of published reports, as well as the recommended procedure by a number of national environmental protection agencies [4-14,25,28-30,33-36,38-43]. In spite of its broad-ranging recognition, however, EL testing also suffers from failure of testing solid phase sediment components. Moreover, one may argue that the complex procedure in EL preparation may underestimate actual risk as some particle- (or carbon-)-bound hydrophobic contaminants could be removed by the centrifugation/filtration steps, resulting in an environmental mixture that is not representative of actual sediment complex mixture toxicity. Not surprisingly, Geffard et al. [45] tested WS, unfiltered EL, and filtered EL in sea urchin and oyster embryos, and found that WS was more effective than EL by two orders of magnitude. In another study, Costa et al. [42] investigated pollution status and toxicity of sediment in a highly polluted bay in southern Italy, and found that EL toxicity testing in sea urchin embryos failed to corroborate the analytical data of heavy pollution status and WS testing conducted in other organisms. By referring to their unexpected results of sea urchin EL toxicity testing, the authors concluded that «considering the high levels of sediment contamination highlighted from chemical analysis, an unexpected very low toxic effect was observed, even considering the sub lethal end-point (larval swimming speed alteration)» [42].

Use of WS toxicity testing in freshwater and terrestrial substrates: Unconfined to marine coastal sediment, sea urchin bioassays can be utilized as a means to evaluate the adverse effects of other environmentally-contaminated or spiked substrates, including freshwater sediment or soil. Sediment toxicity testing by means of WS is commonly performed in crustacean models [56-58]. The use of WS testing procedures in a number of marine and freshwater substrates is compatible with the sample concentration (0.5 to 1%) in test cultures,

Table 3: Comparative embryo toxicity of sediment pellet (SP) *vs.* pore water (PW) from Kiel sampling sites. *Spaerechinus granularis*, five replicates.

#Site/Treatment Schedule	R	P1	P2
Blank	3.5 ± 1.0	3.3 ± 0.6	3.3 ± 0.8
SP			
K1	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
K2	15.5 ± 2.1	6.7 ± 1.6	40.7 ± 7.2
K3	24.5 ± 1.8	3.2 ± 1.4	63.0 ± 5.1
K4	48.3 ± 10.7	4.7 ± 2.0	40.8 ± 12.5
PW			
K1	6.8 ± 1.7	6.5 ± 1.1	4.5 ± 0.9
K2	26.7 ± 17.0	7.5 ± 3.9	65.8 ± 18.5
K3	27.5 ± 13.7	10.7 ± 5 .1	61.8 ± 17.6
K4	10.8 ± 4.7	13.7 ± 10.6	3.5 ± 1.8

Abbreviations: R= % Retarded larvae; P1= % Malformed larvae; P2= % Pre-larval arrest.

not affecting salinity or other variables. By considering the effects of solid sediment component, and the lack of movement of cleaving, pre-hatch sea urchin embryos, then one may assess the realistic use of WS testing in sea urchin early embryogenesis.

A study by Pagano et al. [51] reported on the effects of WS collected in the highly polluted Sarno River in Campania, Italy [46] and contrasted to sediment samples collected from a relatively unpolluted river. The results showed consistent WS toxicity data aligned with the results of sediment chemical analyses of a set of inorganic and organic pollutants [51,52].

Soil, dust and sludge collected from four bauxite manufacturing plants (in Italy, France, Greece and Turkey) were tested by sea urchin bioassays providing evidence for developmental and reproductive toxicity [54,55]. Soil samples (0.03-0.5% w/v) from Portovesme (Italy) displayed a significant association between toxicity and dose-related seawater release of Zn, Pb, and Mn. Seawater extraction of a toxic dust sample (G20) from the Gardanne factory (France) showed increasing seawater release of Al, Fe, and Mn. The G20 sample, at the 0.5% w/v level, affected both developing sea urchin embryos and sperm (inducing offspring damage). Soil samples around the Gardanne factory showed the highest frequency of toxic soil sites eastward from the factory, concomitant with prevailing winds [55]. These studies warranted further investigations on bauxite residues as a potential subject of environmental concern and WS toxicity testing coupled to sea urchin bioassays allows for these determinations. Further studies of polluted soil are currently planned, by combining more refined chemical analyses with a multi-test investigation.

Conclusions and Perspectives

A critical re-appraisal on the use of sea urchin bioassays in WS toxicity testing should prompt overcoming the current practice of testing only the water phase, either as PW or EL. On the other hand, low concentrations such as 0.3 to 1% of WS may allow for the detection of early biological effects in sea urchins, including fertilization success, embryogenesis, offspring quality and redox endpoints. By considering the early phases of sea urchin embryogenesis, the contact exposure of pre-hatching embryos with sediment pellets-or soil-should make this exposure both feasible and predictive of harmful effects on post-hatching larval differentiation.

The available literature dating back 25 years has provided evidence for superimposable topographic patterns in pollutant levels, effluent particle outfall, and informed the distribution of toxic sites requiring more monitoring and research efforts. Moreover, a body of evidence from independent groups [45,46,56-60] points to the key-role of solid phase in evaluating sediment toxicity. Notwithstanding the current recognition from environmental agencies in PW and EL testing, the available information should at least suggest that sediment toxicity testing should not neglect WS evaluations when running sea urchin bioassays.

A final, yet not minor consideration should be devoted to testing other, non-marine substrates by means of sea urchin bioassays, as a fast and practical model in toxicity testing. This should prompt further evaluations of other complex mixtures as soil or industrial sludge. Thus, the informative scope of sea urchin bioassays may thrive well beyond the scope of marine coastal pollution studies.

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