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Marine Invertebrates as Bioindicators of Heavy Metal Pollution

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Abstract

Atmosphere, earth and water compose the environment. The presence of heavy metals in the environment has grown because of their large employment in some industrial and agricultural activities. Although these metals are terrestrial products, they flow into the sea through effluents and sewage or are directly discharged from industries placed on the seawater front. It should be considered that metals concentrations vary widely according to different seawater latitudes and depths and can be strongly influenced by fresh water discharges from heavily polluted rivers. In this review recent studies on heavy metal pollution in marine ecosystems and their organisms will be presented. Metal speciation, bioaccumulation in biota, as well as abiotic and biotic factors affecting their bioavailability will be reviewed. Moreover, the use of bioindicator organisms for the biomonitoring of heavy metal toxicity and their ecological effects will be defined. Many marine invertebrate species fulfill the following criteria: Sensitivity to a wide range of chemicals (especially to heavy metals), cost-effectiveness for repeatable tests, readily interpretable biological consequences of pollution. Among the most important marine invertebrates used as bioindicators, the sea urchin embryo is one of the most suitable, especially to assess metal/heavy metal pollution.

Keywords

Pollution, Heavy Metals, Bioidicators, Marine Invertebrates, Sea Urchin Embryos

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1. Introduction

1.1. Heavy Metal and Marine Pollution

The environment is composed of the atmosphere, earth and water. More than 100,000 chemicals are released into the global environment every year as a consequence of their production, use and disposal. Chemical sub-

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stances or contaminants discharged into the environment may be of natural origin (e.g., erosion, volcanism), or of anthropogenic origin (e.g., combustion of fossil fuels, leachate from landfill sites, run-off from agricultural land, mining residues). Atmospheric metal pollution is responsible for most of the dissolved Cd, Cu, Fe, Zn, Ni and As in the oceans. The presence in the environment of heavy metals has grown because of its large employment in some industrial and agricultural activities.

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations in plants, animals and humans [1]. Approximately 30 metals and metalloids are potentially toxic to humans. These elements affect cells and living organisms in various ways; some heavy metals have essential functions and are toxic only in an overdose, whereas others are xenobiotic and highly toxic [2] [3]. Also, some metals which are essential in some organisms are not in the other in which they have a toxic effect (borderline).

Usually, metals and metalloids, relatively to living organisms, can be separated in three class: essentials, non-essentials and borderline class. Some of these are displayed in **Table 1**.

Heavy metals, not biodegradable and persistent in the environment for long periods, cause serious eco-toxicological problems. Furthermore, some toxic metals may mimic essential metals and thereby gain access to important molecular targets. To a small extent they enter into the organisms via food, drinking water and air and are bio-persistent pollutants that accumulate at the top of food chain [4].

Heavy metals are dangerous because they tend to bioaccumulate. The extent of bioaccumulation of metals is dependent on the total amount, the bioavailability of each metal in the environmental medium and the route of uptake, storage and excretion mechanisms. Compounds accumulate in animals and in plants any time they are taken up and stored faster than they are broken down (metabolized) or excreted [5].

Living organisms naturally exposed to high metal concentrations follow various mechanisms to counter potential toxicity. To detoxify the metals, they reduce their intake, enhance their excretion and/or sequestration. Metals can be sequestered through metal binding proteins, such as metallothioneins, in cellular vesicles and granules [6]. Moreover, some compounds of heavy metals are known to be stress agents and promote different defense strategies such as the synthesis of HSPs (heat shock proteins), apoptosis and autophagy, in a dose/time-dependent manner.

Although heavy metals are terrestrially produced (industrial and agricultural activities), they flow into the sea through effluents and sewage or are directly discharged on the sea waterfront. In many seas surface waters often have low discharge or renewal rates, hence pollutant contamination from industries have a high negative impact on the physico-chemical and biological quality of the water.

Information about heavy metals in marine organisms, both at the whole body and at tissue levels, mostly concerns mollusks and crustaceans, but significant evidences occur also for polychaetes, coelenterates and echinoderms. Aquatic organisms, such as mussels, can regulate internal metal levels more effectively that oysters, although both species have similar feeding preferences [7]. Certain species, as fish, polychaete and bivalve mollusks are also able to regulate the concentrations of essential metals in their tissues [8].

1.2. Marine Invertebrates as Bioindicators

In the sea, when evaporation exceeds the contribution of rivers, the concentration of salts and pollutants is subjected to increase, causing effects on organisms, biodiversity and human health.

Aquatic invertebrates are known to accumulate high levels of heavy metals in their tissues and yet survive in polluted environments [9] [10]. Often metals penetrate the cells via transport mechanisms normally used for other purposes and are irreversibly accumulated in cells where they interact with cellular components and mo-

Table 1. Classes of metals and metalloids relatively to living organisms.

Types of heavy metal	Heavy Metals
Essentials	Calcium (Ca), Magnesium (Mg), Manganese (Mn), Potassium (K), Sodium (Na), Strontium (Sr), Zinc (Zn), Iron (Fe), Copper (Cu)
Non-essentials	Cadmium (Cd), Mercury (Hg), Silver (Ag) Tallium (Ti), Lead (Pb)
Borderline	Chromium (Cr), Cobalt (Co) Nickel (Ni), Arsenic (As), Vanadium (V), Tin (Sn)

lecular targets. Their toxicity has been associated with blockage of oxidative phosphorylation, glutathione depletion and inhibition of antioxidant enzymatic activity, production of ROS (reactive oxygen species), DNA damage and inhibition of relevant repair mechanisms, protein misfolding disorders [11] [12].

Invertebrates occupy a key position as intermediate consumers in the pelagic as well benthonic food chains of aquatic ecosystems. Then, aquatic organisms may represent excellent bioindicators of the marine water quality. The main purpose of monitoring the concentrations of heavy metals in biota is to determine the toxicological risk faced by marine organisms and even by humans through the ingestion of contaminated edible species.

Many studies have been carried out to assess the status of chemical pollutants in marine ecosystems using different bioindicator organisms, for evaluating possible risks on human health.

To select the proper bioindicator organisms, the chemical effects were studied on adults, but, at present, they displayed two problems: 1) high efficient excretion systems for toxicants; 2) the difficulty to carefully determine which chemical pollutant is producing the observed effect, since the organisms come in contact with several contaminants. Considering these aspects, it is essential to find bioindicators that effectively reflects the health state of the sea and accurate markers of pollution. Some macroscopic markers are not always accurate because may be influenced by numerous parameters, instead, cellular/molecular markers, seem to be more reliable because are governed by specific pathways. Although it has long been highlighted the problem of marine contaminants, scientific research needs to be further developed in order to obtain a complete profile of the chemical effects on living beings, including the molecular and the cellular aspects that are governed by specific pathways and may have a key role in the definition of environmental pollution.

Embryos and larvae of marine invertebrates seem to be a suitable indicator. At present, few studies have been designed on cellular/molecular processes in developing organisms, in order to establish a toxicological profile induced by chemical pollutants.

2. The Effects of Heavy Metals on Marine Invertebrates

2.1. Mercury

Mercury (Hg) is a heavy, silvery-white metal. There are three chemical forms of Hg: elemental (Hg without any additional atoms attached to it), organic, and inorganic. These forms are interconvertible, and all can produce systemic toxicity [13].

Hg may occur naturally in the environment or from anthropogenic sources like mining, fossil fuels combustion, incineration, emission from smelters, fungicides and catalyst activities. Most Hg is volatilized and return to the atmosphere, but the greater part of this metal introduced into the coastal sea precipitates, because of the very low solubility products of its compounds.

It also accumulates in the sediment, which represents the principal sink. Most of inorganic and organic Hg in aquatic environment appears to be bound to particles, colloids and high molecular weight organic matter [14]. In sediments, due to bacterial activity, inorganic Hg may also be converted into methylmercury, the most toxic chemical species which may cause the permanent harm to the central nervous system, such as behavioral disorders and deficiencies in the immune system and development [15]. In this form methylmercury dissolves in the water column, becoming readily bioavailable; then it bioaccumulates and biomagnifies up into marine food chains leading to elevated concentrations especially in predatory organisms. Therefore, the consumption of marine products represents a non-negligible exposure pathway to Hg and, thereby, a risk for human health.

Many studies were carried out on the toxicity of Hg on marine invertebrates. For example, in the clam *Ruditapes philippinarum*, one of the most important sentinel organisms in "Mussel Watch Program" launched in China and therefore used as a bioindicator in marine and coastal ecotoxicology; metabolomics effects were detect to analyze the toxicity induced by Hg exposure in three pedigrees [16].

High level of Hg were detect in crustacean *Ligia italic*, a bioindicator of Hg pollution of marine rocky coasts. In this case Hg bioaccumulation resulted in remarkable ultrastructural alterations of two cellular types in the epithelium of the hepatopancreas [17]. Sublethal effects of Hg on cellular immune and biochemical responses were determined incrustacean *Scylla serrata*. In particular, changes in immune-associated parameters including, total haemocyte count, lysosomal membrane stability, phenoloxidase, superoxide generation and phagocytosis were observed after exposure to different Hg concentrations [18].

The role of multidrug efflux transport in the differential accumulation of inorganic (HgCl) and organic (CH_3HgCl) Hg in sea urchin (*Strongylocentrotus purpuratus*) embryos was examined. In particular the inhibition of specific molecular transporters increases intracellular accumulation of inorganic Hg but had no effect on accumulation of organic Hg. Similarly, pharmacological inhibition of metal conjugating enzymes by ligands significantly increases this antimitotic potency of inorganic mercury, but had no effect on the potency of organic Hg. These results point to a specific elimination of inorganic Hg conjugates as a cellular basis for differences in the accumulation and potency of the two major forms of Hg found in marine environments [19].

Another proposed bioindicator for Hg contamination and the risk to humans is the oyster *Saccostrea cucullata*. Results obtained in this organism, related to the toxicity induced by Hg, report that the soft tissues of oysters could be a good indicator of Hg in the aquatic system [20]. A proteomic approach for identifying gonad differential proteins was performed in the oyster (*Crassostrea angulata*) following food-chain contamination with HgCl. Hg discharged into the environmental waters can generally be bioaccumulated, transformed and transmitted by living organisms, thus resulting in the formation of Hg-toxicity. Among the analyzed molecules (14 - 3 - 3 protein, GTP binding protein, arginine kinase and 71 kDa heat shock connate protein) were considered to be suitable biomarkers of environmental Hg contamination [21].

The possibility of having suitable bioindicators of Hg pollution is of fundamental importance and the aforementioned organisms seem well suited for this role.

2.2. Cadmium

Cadmium (Cd), commonly detected in aquatic and terrestrial environment, is a heavy metal released both from natural sources and anthropogenic activities (e.g. pigments, nickel-cadmium batteries, smelting and refining of metals and many other sources). Then, the presence in the environment of this metal has increased because of its large utilization in some industrial and agricultural activities. Cd is a highly toxic environmental pollutant and potent cell poison that causes different types of damage including cell death. A pollutant of worldwide concern, in fact Cd has been included in the list of those chemical substances considered to be potentially dangerous at the global level. The toxicity associated to Cd is amplified in organisms as a consequence of the metal’s long biological half-life (15 - 30 years).This metal is highly dangerous not only because it easily penetrates the cells, via transport mechanisms normally used for other purposes, but also because it is eliminated very slowly, as it is not prone to bacterial detoxification [22].

Since Cd is a non-essential metal which is not physiologically present in organisms, it is irreversibly accumulated into cells, interacting with cellular components and molecular targets. The mechanisms by which Cd interacts with cellular components are probably various, though still poorly understood. It was suggested that Cd could enter cells via divalent ion transporters, such as zinc transporters [23]. Other experimental evidences suggested that the metal crosses the plasma membrane as divalent ions, exerting an agonistic role against calcium ionic channels [24]. In addition, salinity, temperature and calcium concentration may also influence Cd toxicity [25].

Although Cd toxicity is well proved, its effects are controversial, since some authors indicated that Cd can kill cells after a prolonged exposure, while some others emphasized its carcinogenic properties both in animals and humans, even at low concentrations [26]. Numerous pathologies, as teratogenesis and carcinogenesis, due to cytotoxic concentrations of the ion, have been described both in invertebrates and in higher organisms [27].

Accumulated evidence has also shown that Cd increased not only cellular ROS levels, but also lipid peroxidation and alteration in glutathione (GSH) levels in various cell types, suggesting that Cd-induced apoptosis may be connected with oxidative stress [28]. In invertebrates it up-regulates the expression of antioxidant enzymes, metallothioneins and heat shock proteins (HSPs) and down-regulates the expression of digestive enzymes, esterases and phospholipase A2. Cd also interferes with tissue organization, immune responses and cell cycles by inducing apoptosis [29].

This metal is a contaminant that is readily accumulated by aquatic animals and can be toxic to those who live in both fresh and salt water. For many aquatic predators, Cd comes largely from food and the ease with which these animals assimilate Cd from their prey depends in part upon the form in which this metal is bound in prey cells. It is shown that predators assimilate Cd located in the cytosol of prey cells more readily than when Cd is associated with insoluble prey components [30]. The factors controlling metal transfer between prey and predator are important for predicting trends in metal concentrations along aquatic food chains as well as the likelihood of toxic effects on animals at upper trophic levels. Specifically, high bioconcentration of this heavy metal has been found in aqueous organisms: invertebrates, such as sponges, mollusks, crustaceans, echinoderms and vertebrates, as fish. Sponges are particularly vulnerable to waterborne metals because they are able to process large amounts of water. Significant differences in Cd levels were found among sponges collected from differently impacted sites [31]. Cd, as other heavy metals, causes changes in cell morphology and affects cell aggregation in *Scopalina lophyropoda*, by enhancing pseudopodia/filopodia formation which promotes cell movement [32]. There is evidence of Cd accumulation in the digestive and excretory organs of some benthic organisms. Mussel gills and digestive gland of *Mytilus galloprovincialis* are the two main target tissues for heavy metal accumulation, which can alter the physiology of respiration and feeding processes [33]. Very high concentrations of the metal were found in the digestive gland of a few species of Antarctic molluscs [34], suggesting again that food is the primary pathway for Cd bio-accumulation and that the digestive gland plays a major role in the subsequent storage and detoxification. High concentrations of Cd were found in the digestive gland and kidney of mussel *Crenomytilus grayanus* [35]. In the renal tissue of Antarctic bivalve *Laternula elliptica* the high levels of Cd and its bio-accumulation can be a probable advantage for environmental adaptation in the Antarctic marine environment [36]. Cd cations were revealed in the hepatopancreas of *Mytilus edulis* and body wall of echinoderms such as *Asterias rubens*. Also sea urchins represent a widely used experimental model to test Cd accumulation, given their high sensitivity to chemical and physical environmental changes. In addition, their peculiar position in the marine trophic chain, where pelagic larvae are part of the diet of several planctonic and benthonic organisms, increases its interest (see below).

Sea urchin is a classical model to study the effects of pollutants in embryo-larval and adult life stages. This species is considered as a suitable bioindicator to monitor the marine environment. Data on cellular/molecular defence strategies, triggered after Cd-exposure, were obtained in *Paracentrotus lividus* embryos (apoptosis, autophagy, expression of metallothioneins and HSPs) but a lot of markers need to be tested [37] [38].

2.3. Arsenic

Arsenic (As) is one of the most important global environmental pollutants and is a persistent bioaccumulative carcinogen [39]. It is a toxic metalloid that exists in two oxidative states: a trivalent form and a pentavalent form, in the form of arsenous acid (H_3AsO_3) and its salts, and arsenic acid (H_3AsO_5) and its salts, respectively. It is rarely found as a free element in the natural environment, but arsenic compounds occur in air, water, soil, and all living tissues. The most important anthropogenic arsenic sources are the smelting of Cu, Ni, Pb and Zn ores and the burning of fossil fuels in households and power plants. Another source of As contamination was the use of arsenical fungicides, insecticides, herbicides, algicides, wood preservatives, and growth stimulants for plants and animals. However, human activities contribute little to the As increase of the open ocean, but may be important in estuaries and coastal waters receiving arsenic-contaminated drainage from the land.

As is considered to be an essential oligoelement for various animals, but many arsenic compounds are highly toxic [40]. Despite their known toxicity, especially organic ones, they are integral components of nutrients of many organisms in which they may play potentially important biological roles, while their utility is still uncertain in humans (in small doses they seem to improve the activity of certain neurotransmitters in the central nervous system). Arsenic presents strong carcinogenic properties, depending on oxidation state, chemical species, cell type, concentrations and time exposure, can induce apoptosis.

As may be absorbed by ingestion, inhalation, or through permeation of skin or mucous membranes. The mechanisms of arsenic toxicity differ greatly among chemical species, although all appear to cause similar signs of poisoning.

High levels of arsenicals were found in marine organisms comprising algae, crustaceans, bivalves, fish and mammals [41]. In general, concentrations of organic arsenicals in biota, except for gastropods, were directly proportional to the total concentration of As and its distribution and bioaccumulation were strongly correlated with salinity. It was demonstrated that arsenicals are mainly localized intheintestine of mullet and marsh clam [42]. Wu and colleagues have quantified the total arsenic content in 22 species of marine organisms collected from eight cities in Shandong, China. Their results suggest that benthonic organisms, such as bivalves, having a closer relationship with sediments, can accumulate more As then pelagic ones, while fishes accumulate As eating bivalves [43]. Already in 1982, Pagano and others [44] had demonstrated the highly toxic effects of As on the development of the embryos of sea urchin *Paracentrotus lividus*. Gaion and colleagues [45] recently investigated the toxicological effects of two As species (arsenate and dimethyl-arsinate) on the development of these embryos. Their results demonstrate that the biological damage depends both on the different arsenic compounds used and on the observed larval stage. These results confirm that *P. lividus* embryos and larvae are excellent bioindicators for different contaminants.

2.4. Chromium

Chromium (Cr) is a lustrous, brittle, hard metal. Its color is silver-gray and it can be highly polished. It does not tarnish in air, when heated it burns and forms the green chromic oxide. Cr is unstable in oxygen, it immediately produces a thin oxide layer that is impermeable to oxygen and protects the metal below.

This metal is a highly toxic trace metal presenting various degrees of risk for coastal ecosystems. Coastal Cr pollution is due mainly to dumping untreated or poorly treated industrial residues; Cr is usually found in its trivalent (III) and hexavalent (VI) forms. Cr (VI) is 30 times more toxic than Cr (III) and can be mutagenic and carcinogenic [46]. Different marine invertebrates were used to study the toxicity of Cr.

For example *Petrolisthes laevigatus* (crab) was used in a standard toxicity bioassay (semi-static, chronic) to evaluate EDTA as a chelating agent for reducing trivalent and hexavalent Cr toxicity. In particular the survival decreased linearly with the increase of Cr concentrations and dropped significantly at 40 mg/L Cr (VI) and 80 mg/L Cr (III). No significant differences were observed with Cr (III) + EDTA as compared with untreated controls. Cr (VI) toxicity was greater than of Cr (III), with low individual survival rates [47].

Others effects of sublethal, environmentally relevant concentrations of Cr (VI) was tested in the gills of *Mytilus galloprovincialis*. Cr (VI) increased the activities of GST (GSH-transferase), GSR (GSH-related enzymes) and total glutathione content at different exposure times and at different metal concentrations, suggesting Cr (VI) detoxication/reduction at the site of metal entry. Cr (VI) exposure also increased the activity of glycolytic enzymes, indicating modulation of carbohydrate metabolism. Significant changes in the transcription of different genes were observed. In particular, the mRNA level for the 5-HTR was increased, whereas both decreases and increases were observed for GST-π, MT10, MT20 and HSP70 mRNAs, showing sex- and concentration-related differences. The results demonstrate that Cr (VI) significantly affected functional and molecular parameters in mussel gills, and indicate that this tissue represents the major target of exposure to environmentally relevant concentrations of the metal [48].

Others *in vitro* effects of Cr (VI) on immune parameters of the marine bivalve *Mytilus galloprovincialis* were evaluated using environmentally relevant concentrations of Cr (VI). Hemocyte incubation with different concentrations of Cr (VI) (0.1 - 1 - 10 - 100 μM) showed a stimulation of extracellular superoxide production and nitrite accumulation. Results indicate that exposure to non-toxic concentrations of Cr (VI) can modulate functional and molecular immune parameters in this invertebrate [49].

The oxidation of DNA and lipid were analyzed in the marine mussel (*Mytilus edulis*) in response to exposure to Cr (VI). DNA strand breakage in gill cells (analyzed by the comet assay) was elevated, indicating that this heavy metal is dangerous for the genome [50].

In *Nereis succinea* (polychaete), experiments were performed exposing the adults to different metals, e.g. Cr (VI). The model showed that >97% of the body burden of these metals is accumulated through ingested sediment, indicating how these aquatic animals acquire metals from the environment, predominantly from their diet [51].

Increase in total Cr (VI) tissue content and lysosomal membranes destabilization were observed in *Mytilus galloprovincialis* digestive gland treated with high concentration of this heavy metal (100 $\mu g \cdot L^{-1}$). The results demonstrated that exposure to Cr (VI) at low concentration did not result in strong toxicity or oxidative stress in mussel digestive gland. On the other hand, authors suggest that low concentrations of the metal can exert pleiotropic effects on mussel physiology, from the modulation of lipid and carbohydrate metabolism, to effects on the expression of estrogen-responsive genes [52].

A suitable test organism for assessing the bioavailability of sediment-bound metals should accumulate metals (such as Cr). So, the sipunculan worm *Phascolosoma arcuatum* was proposed and its coelomic fluid was used for this purpose. This organism indiscriminately ingestes sediment particles and has a very low uptake rate of dissolved metals, appearing to be a good bioindicators. Since the amount of Cr was found to be similar both in the coelomic fluid and in somatic tissues, the measurement of metal concentrations in the coelomic-fluid can provide a rapid estimation of metal bioavailability in marine sediments [53].

Toxicity of different metals, in particular Cr, on early developmental stages of *Ciona intestinalis* was observed and a potential application in marine water quality assessment was proposed. In particular, rapid tests on sperm viability, effects on embryonic development and effects on attachment were proposed [54].

Cr is used in different industry sectors and it is very toxic for the environment; the identification of suitable bioindicators related to this heavy metal is very important to safeguard the environment and the human health.

2.5. Thallium

Thallium (Tl) is a non-essential, malleable and rare heavy metal that is highly toxic to plants, animals and humans and is one of the emergent pollutant. The average concentration of Tl in the Earth's crust is 490 $\mu g \cdot Kg^{-1}$ [55].

Some minerals such as lorandite and crooksite can contain up to 60% Tl [56]. Soil erosion, forest fires, and volcanic activity are the predominant means by which metals such as Tl are naturally mobilized into the aquatic environment [57].

Dissolved Tl can be found in two oxidation states, Tl (I) and Tl (III). Although Tl (I) is predicted to be more thermodynamically stable than Tl (III), photo oxidation reactions and microbial activity, combined with the formation of stable hydroxocomplexes, contribute to the persistence of Tl (III) in surface waters [58].

There is limited information available on Tl concentrations in marine invertebrates. Tl concentrations have been measured in only about half a dozen types of invertebrates (Crustacea: Amphipoda and Cladocera) and in an equal number of fish species (Cyprinidae, Perciformes and Salmonidae).

The ease with which elements such as Tl are transferred from one trophic level to the next is represented by their assimilation efficiency [57].

In Clams and Mussels data on the accumulation of Tl were reported. These molluscs accumulate certain trace elements from the water and may be used as indicators of pollution. In the Clam *Mya arenaria* and the mussel *Mytilus edulis*, an experimental-treatment with Tl was carried out. In both organisms the accumulation of this metal in their tissue was reported, indicating that Tl enters the body and gradually accumulates in it [58].

Other studies on the effect of Tl in marine invertebrates were carried out in deposit-feeding invertebrates (*Hediste diversicolor*, *Arenicola marina* and *Scrobicularia plana*). Although in smaller amounts if compared to others heavy metals, the accumulation of Tl in the body of these organisms was showed [59]. However, considering Tl is a highly toxic metal whose biogeochemical behaviour in the marine environment is poorly understood it is recommended to better study its effects on marine species and find out specific bioindicators in order to monitoring the marine environment.

2.6. Lead

Lead (Pb) is a bluish-white lustrous heavy metal. If ingested, Pb is poisonous to animals and humans, damaging the nervous system and causing different disorders. Pb compounds exist mainly in two main oxidation states, +2 and +4 [60].

Pb occurs naturally in the environment. However, most Pb concentrations that are found in the environment are the result of human activities. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this Pb will fall back on earth when it is raining.

Pb accumulates in the bodies of water and soil organisms and it is a bio-persistent pollutant that accumulates at the top of the food chain. The Pb-induced toxicity to marine invertebrates varies with species and their life stage. The concentration of Pb (and others heavy metal) was monitored and estimated in crab and shrimp. In these species different concentrations, depending on the geographical site analyzed, were found, indicating that these organisms can be considered biomarkers of Pb pollution [61].

A characterization of the cytosolic distribution of Pb was carried out in the digestive gland of the marine mussel *Mytilus galloprovincialis*. Pb was eluted in high molecular weight biomolecule range, but in elevated cytosolic Pb concentrations; significant amount of Pb was eluted in low molecular weight biomolecules. These results report the suitability of the distribution of selected metals among different cytosolic ligands as potential indicator for metal exposure [62].

Sea urchin embryos (*Paracentrotus lividus*), *in vitro* treated with Pb, a reduction of HSP70 synthesis was observed. Metal treatment caused an irregular morphology both at gastrula and, especially, at pluteus stages [63].

Others sublethal mechanisms of Pb toxicity were tested in the purple sea urchin (*Strongylocentrotus purpuratus*) during early development. In this specie Pb accumulation could be greatly observed, associated with a reduction of body calcium accumulation [64].

Biochemical and histological toxic effects induced by environmentally relevant Pb concentrations, were found in green mussel (*Perna viridis*). Acute and chronic toxicity tests revealed toxic effects in a dose and time dependent manner. In this study, histological and biochemical enzymes, namely, catalase, reduced glutathione, glutathione S-transferase, and lipid peroxides, were correlated with chronic values and survival endpoints of *P. viridis* after chronic exposure to Pb. Significant differences were observed when mussels were exposed to increasing concentrations, if compared to controls. These studies suggest different potential approaches to establish the seawater quality criteria thought the use of marine organisms [65].

3. Sea Urchin Embryos as a Suitable Marine Bioindicator

Marine organisms are highly sensitive to several kinds of stressors, and able to activate different defense strategies. The sea urchin embryo is one of the most important marine invertebrates used as bioindicators of metal/heavy metal pollution and an important model organism in developmental biology. Recently it has been proposed as a suitable model for eco-toxicological and environmental studies aimed at the determination of the effects of chemical pollutants in the field or in laboratory experiments [66], as it continuously faces environmental, chemical, physical and biological stressors [67].

The sea urchin has been recently introduced in the list of alternative methods proposed by the European Union Reference Laboratory for alternatives to animal testing (EURL EVCAM), for the validation of methods which reduce, refine or replace (the 3Rs rule, 86/609/CEE) the use of animals for safety testing and efficacy/potency testing of chemicals, biologicals and vaccines.

Among sea urchins, *Paracentrotus lividus* is a very common species in the Mediterranean Sea. The high number of gametes, its external fertilization, the high developmental synchrony and embryo transparency make the sea urchin embryo a suitable model organism for cellular and developmental biology studies.

Sea urchins are an ancient group (at least 450 million years old) of the Echinoidea Class in the Phylum Echinodermata, with hundreds of species known in the world's oceans. Since about 1880, the eggs and sperms of sea urchins have been used for the study of fertilization, the metabolic activation of development and gene regulatory mechanisms governing embryogenesis. In addition, the genome of the purple sea urchin, *Strongylocentrotus purpuratus*, is known; its size is ~800 Mb [68]. This permitted to explore if parts of the vertebrate toolkit are also present in invertebrate deuterostomes, allowing ascertaining a lineage-specific evolution of various molecular networks.

This marine organism is sensitive to several aquatic contaminants and adopts several defense mechanism against any environmental chemical, physical and mechanical stress, in an attempt to preserve the developmental program. The sea urchin embryo represents a suitable model system to investigate the adaptive response of cells exposed to stress during development and differentiation [69] [70].

In embryos and larvae, contrary to the view that these are the most fragile stages of life, development is stable under real-world conditions and organisms are able to face environmental alterations thanks to high levels of cellular defences already present in the egg before fertilization. Later in development, adaptive responses to the environment either buffer stress or produce alternative developmental phenotypes [71].

Pollutants of anthropogenic origin, especially heavy metals, are of considerable interest for their ability to induce the activation of defense systems or interrupt the developmental program. Among heavy metal, Cd is one of the most considered stress agent studied in the sea urchin embryo and it induces different cellular effects such as apoptosis, autophagy and the synthesis of molecules of cellular protection.

During the development of sea urchin embryos and larvae, subacute/sublethal concentrations of Cd induce, morphological abnormalities, synthesis of specific stress proteins (HSPs), increased expression of metallothioneins, apoptosis and autophagy, in a dose/time-dependent manner [66].

It is important to emphasize that in some experiments Cd is employed as a toxic insult and it in no way constitutes an environmental stressor. On the other hand, it has been demonstrated that long-lasting exposure to Cd concentrations, similar to those found in moderately or highly polluted seawaters, causes severe developmental delays and abnormalities, showing that even very small amounts of Cd, if accumulated in cells, produce significant cytotoxic effects [72] [73].

Autophagy has been recently reported in sea urchin embryo *P. lividus*, both during physiological development and in response to stress [38]. Several experimental approaches have been used to detect autophagy, like: identification of autophagolysosomes, staining with acidotropic dyes such as neutral red and acridine orange (AO); immunodetection of LC3-II (an autophagic marker) Western blot and immunofluorescence analyses.

In particular AO is a metachromatic dye, emitting green fluorescence in monomeric form, and red fluorescence in bi/oligomeric form, related to the protonation of autophagolysosomes [74].

Results obtained by in vivo AO fluorescence assay and confocal laser scanning microscopy (CLSM) demonstrated that autophagy is a molecular process present in sea urchin embryos at higher level after Cd treatment and at a basal level during physiological development. Specifically, the experiments revealed a higher level of autophagolysosomes for embryos treated for 38 hours with 100 µM $CdCl_2$, compared with controls. Embryos treated for 38 h, after AO vital staining, exhibited a considerable number of red dots located around nuclei (**Figure 1**).

The high level of autophagy a specific developmental stages seems to be critical and it canreaches high levels in some extreme cases, although it is a physiological mechanism of recycling. This indicates that autophagy is a very plastic process, essential for the survival of embryonic cells. Sea urchin embryos continuously exposed to Cd provide a suitable model to study the role of autophagy inresponses to Cd-stress [75].

Considering that embryos of this marine invertebrate exposed to Cd activate the autophagic process in a dose-time dependent manner and selectively in response to a given stressor, the detection of this molecular process can be an useful marker of heavy metal pollution [76].

This topic is relevant since autophagy is a mechanism of self-eating, responsible for degradation and recycling of catabolites.

4. Conclusions

One of the most important goals of toxicological studies is to determine whether heavy metal pollution causes adverse effects on organisms. Although concentrations of priority metals in the seawater are regularly monitored worldwide, great effort is being made towards the application of biomarkers that indicate an early response in selected target organisms that finally provide evidence of the exposure to the chemical pollutants and may indicate a toxic effect. Especially effects based on the response at molecular and cellular levels represent the earliest warning signals of an environmental disturbance. Biomonitoring of heavy metals and effect studied on natural populations of organisms must take into account the pollution-induced tolerance in the communities that are exposed to particular pollutants for a long time (several generations).

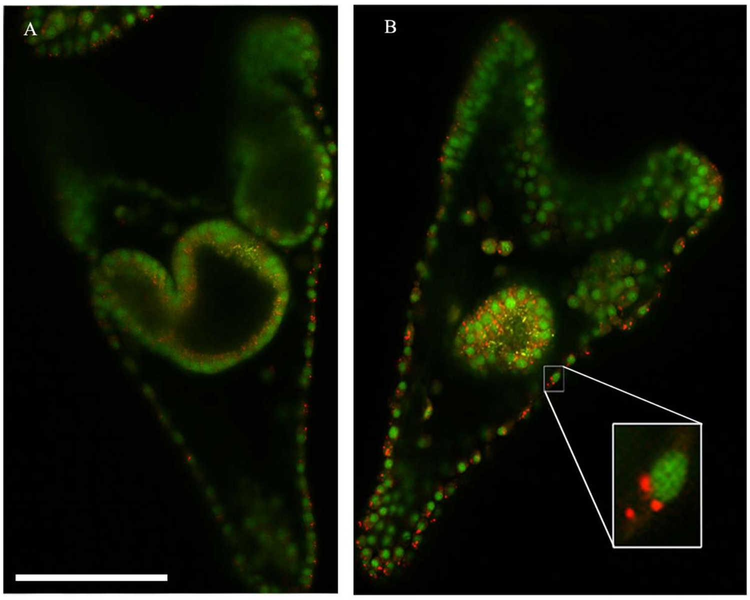

Figure 1. AO vital staining on whole-mount embryos. The images, of representative embryos, were captured by CLSM. Control embryo after 38 h of growth (A); Cd-treated embryo for 38 h (B). The box shows an enlargement of a section of (B). Bar = 50 µm.

The effects of heavy metals on marine invertebrates have been studied, examining accumulation, embryonic development perturbation, expression of metallothioneins, stress protein induction, apoptosis and others pathways.

Most marine invertebrates accumulate heavy metals mainly from seawater and/or diet. Mollusks, crustaceans and other marine invertebrates are known to accumulate high levels of heavy metals in their tissues and yet survive in polluted environments; thus, their peculiar position in the marine trophic chain, where pelagic larvae are part of the diet of several planktonic and benthic organisms (bio-magnification), increases the interest of many researchers. Trace essential/borderlinemetals are of environmental interest both as limiting nutrients (e.g. Fe, Zn, Mn, Cu, Co, and Ni), playing important roles in metal-requiring and metal-activated enzyme systems, and as toxicants when present at high concentrations. On the contrary, non-essential heavy metals (e.g. Cd, Hg, Ag and Pb) are toxic for living organisms even at low concentrations.

Cyto-protective response against stress induced by metals can vary greatly among different tissues and may result in their differential sensitivity to metal exposure, especially for non-essential metals. Many studies have shown that, in adult organisms, metal accumulation was particularly marked in specific tissues. In all examined species, heavy metals accumulated in a dose/time-dependent manner.

Considering various sources of marine pollution, monitoring the health state of the sea is essential to identify reliable markers and bioindicators highly sensitive to environmental changes.

Among the benthonic organisms, echinoderms represent a simple and significant model system to test how specific metals can simultaneously affect development and putative mechanisms of defense and/or cell death. However further studies are needed to investigate this topic, particularly in relation to the pollutants reported in this review.

The purpose of the summary of data presented here is not to review all of the work done over the years in the field by many authors, but rather to focus on a few arguments with the intent of re-examining some ideas and concepts.

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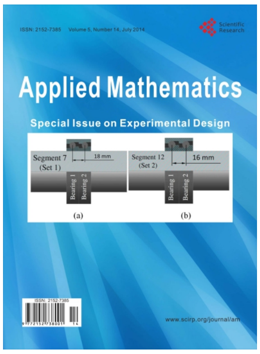
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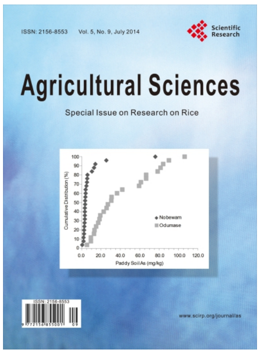
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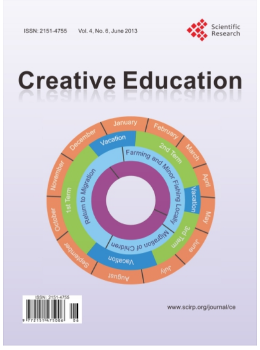
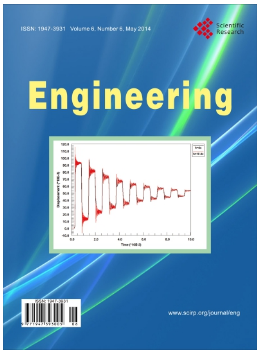
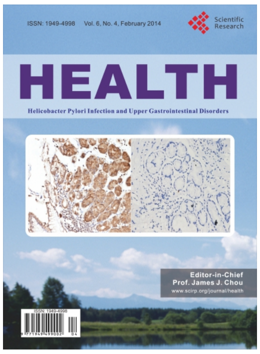

Creative Education


Engineering


Food and Nutrition Sciences


HEALTH
Helicobacter Pylori Infection and Upper Gastrointestinal Disorders
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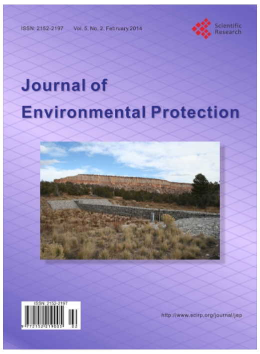
Journal of Cancer Therapy
Special Issue on Bone Tumor Research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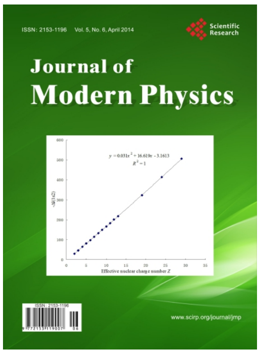
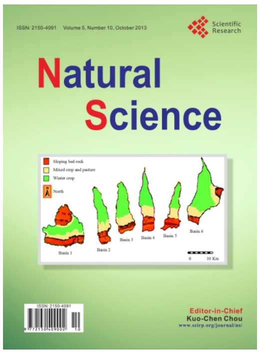

Journal of Modern Physics


Modern Economy


Natural Science
Editor-in-Chief
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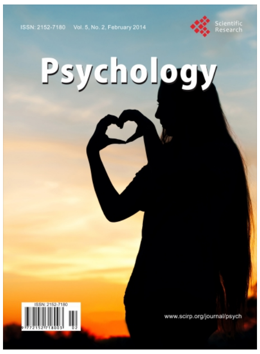
Psychology