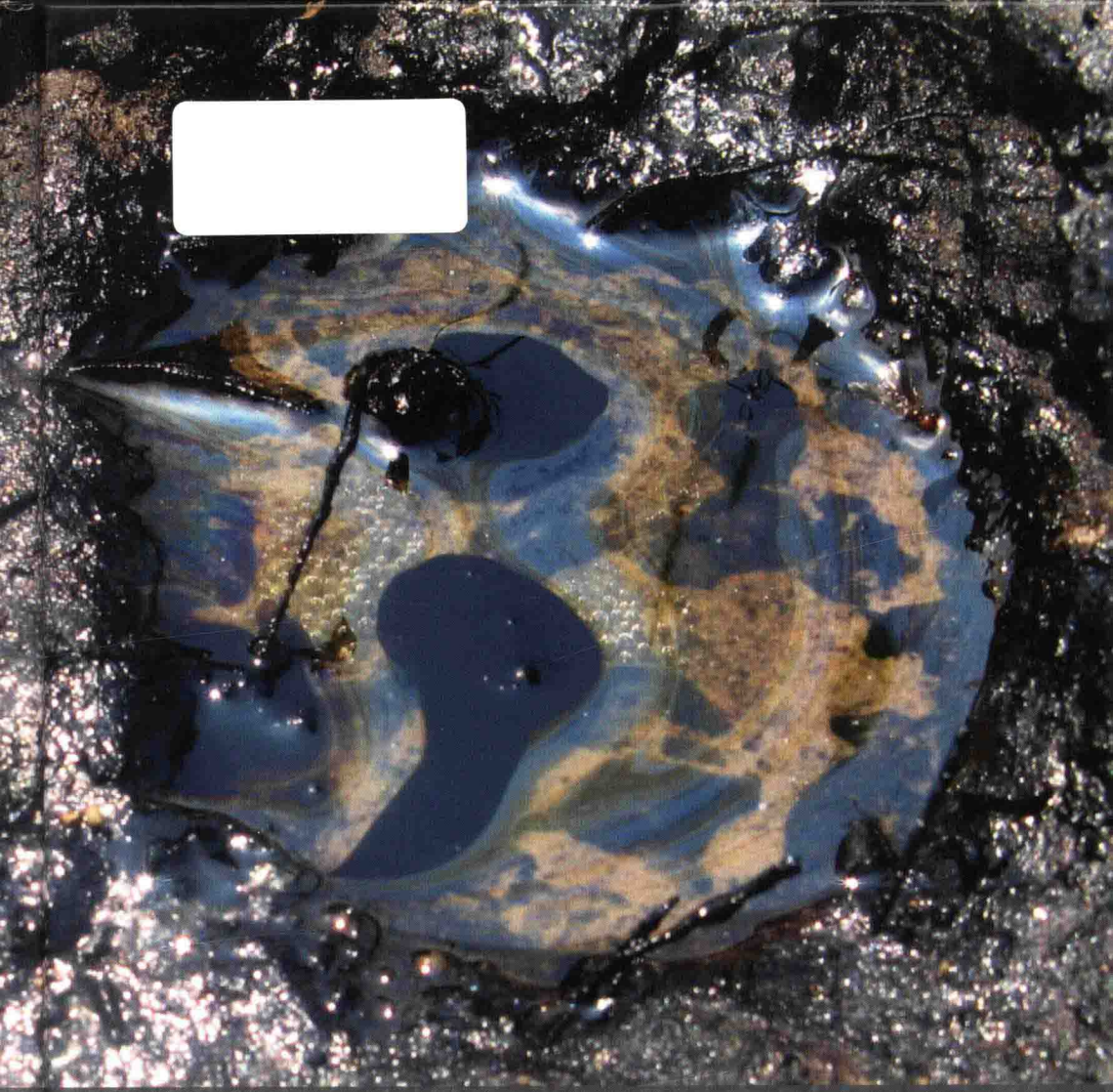

Environmental Contamination Handbook

Emma Layer

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Edited by **Emma Layer**

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Preface

This book has been an outcome of determined endeavour from a group of educationists in the field. The primary objective was to involve a broad spectrum of professionals from diverse cultural background involved in the field for developing new researches. The book not only targets students but also scholars pursuing higher research for further enhancement of the theoretical and practical applications of the subject.

Environmental contamination is a topic of great concern. Environment reduces hazards, while man amplifies them. This is not a supposition, but a gist of the conclusions made from studies of experts from all over the world. The last two centuries have seen an unsystematic expansion and overexploitation of natural resources by man leading to damage and destruction of our own environment. Environmental pollution is the effect of illogical use of resources at the incorrect place and at the incorrect time. Environmental pollution has affected the lifestyle of people almost all over the world, and has decreased the span of life on earth. Today, we are forced to compromise with such environmental circumstances, which nobody had predicted would need to be tackled with for the survival of humanity and other life forms. This book will help the readers find out the underlying crisis and help monitor their solutions through various methods and approaches.

It was an honour to edit such a profound book and also a challenging task to compile and examine all the relevant data for accuracy and originality. I wish to acknowledge the efforts of the contributors for submitting such brilliant and diverse chapters in the field and for endlessly working for the completion of the book. Last, but not the least; I thank my family for being a constant source of support in all my research endeavours.

Editor

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List of Contributors

Part 1

Climate Change, Plants and Heavy Metal Contamination

1

Manganese: A New Emerging Contaminant in the Environment

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1. Introduction

The environment is composed of the atmosphere, earth and water. According to the World Health Organization, more than 100,000 chemicals are released into the global ambient every year as a consequence of their production, use and disposal. The fate of a chemical substance depends on its chemical application and physical-chemical properties, in combination with the characteristics of the environment where it is released. Chemical substances or contaminants discharged into the environment may be "natural" or "man-made". One of the most misunderstood concepts regarding contamination is the miss-interpretation of term "natural". A "natural" contaminant is one substance that can occur without human introduction. For example, trace metals, such as iron, zinc, manganese, copper, cobalt and nickel, can be considered naturally-occurring contaminants. Generally, these metals are found in the environment only in moderate amounts that do not cause health threats. However, "natural" contaminants can also have anthropogenic origins: in fact human activities often cause the release of a large amount of naturally-occurring minerals into the environment. Moreover, it is not the mere presence of a contaminant that makes it toxic, but its concentration. Paracelsus' famous aphorism "only dose makes the difference" has laid the groundwork for the development of the modern toxicology by recognizing the importance of the dose-response relationship.

In the last century, the massive production of manganese-containing compounds (metallurgic and chemical products, municipal wastewater discharges, sewage sludge, alloys, steel, iron, ceramics, fungicide products) has attracted the attention of scientists who investigated manganese as a potential emerging contaminant in the environment, and especially in the marine environment (CICAD 63, 2004). In humans, manganese excess is renowned for its role in neurotoxicity, associated with a characteristic syndrome called 'manganese madness' or 'Parkinson-like' diseases (Perl & Olanow, 2007). This neurodegenerative disorder is due to the accumulation of manganese inside intracellular compartments, such as the Golgi apparatus and mitochondria. In mammals, prenatal and postnatal exposure to manganese is associated with embryo-toxicity, fetal-toxicity, and decreased postnatal growth (Sanchez et al., 1993; Colomina et al., 1996).

In marine organisms some studied showed that an excessive amount of manganese causes toxicity, although the cause-effect evidence is not extensive.

In this chapter, we will provide: firstly the information available regarding the natural behaviour of manganese in the environment and its role in the living organisms, with particular emphasis on the marine environment. Secondly, we will discuss how and why the manganese contamination has become a global problem recently. Thirdly, we will cover some aspects regarding the adverse effects resulting from the exposure of whole organisms to high levels of manganese. Advantage of the notion that marine invertebrates express qualitatively similar types of induced damage to those found in higher organisms, we will focus our attention on the toxicity of manganese at different levels of organization: whole-organism, cellular and embryonic levels. In this review chapter we intend to promote the embryos and the immune cells of echinoderms as useful models to study manganese toxicity.

2. Manganese: Environmental aspects

Manganese is one of the most abundant and widely distributed metals in nature. In fact it is typically found in rocks, soils and waters. The Earth's crust consists of 0.1% of manganese.

As constituent of the soil, its concentrations range from 40 to 900 mg kg^{-1}. Pure manganese is a silver-stained metal; however, it does not occur in the environment in a pure form. Rather, it occurs in manganese-compounds, combined with other elements such as oxygen, sulphur, carbon, silicon and chlorine. These forms of manganese are solid and some of them can dissolve in water or be suspended in the air as small particles. The small dust particles in the air usually settle at the bottom within a few days, depending on their size, weight, density and weather conditions. Manganese can exist in 11 oxidation states, ranging from -3 to +7, but the most common ones are: +2 (e.g., $MnCl_2$) and +4 (e.g., MnO_2).

2.1 Manganese behaviour in the aquatic environment

Natural waters, such as lakes, streams, rivers and oceans, contain variable quantities of dissolved manganese, ranging from 10 to 10,000 µg l^{-1}. In water, most manganese compounds tend to attach to circulating particles or settle as sediment. Ocean spray, forest fires, vegetation, crustal rock and volcanic activity are the major natural atmospheric sources of manganese (CICAD 63, 2004).

Manganese exists in the aquatic environment in two main forms: Mn^{2+} and Mn^{4+}. Oscillation between these two forms occurs via oxidation and reduction reactions that may be abiotic or biotic (Schamphelaire et al., 2008). The interconversions between these forms is of particular importance to the aquatic chemistry of manganese, as Mn^{2+} forms are soluble whereas Mn^{4+} is present in insoluble oxides.

Since the late 1970s, the bacterially-catalyzed oxidation of manganese has been receiving increasing attention, because of its important role in geochemical cycles. Three well-studied and phylogenetically distinct manganese-oxidizing bacteria have been described: i) the β-Proteobacterium *Leptothrix discophora,* isolated from a swamp; ii) the γ-Proteobacterium *Pseudomonas putida,* which is a ubiquitous freshwater and soil bacterium; iii) the *Bacillus sp* spores, isolated from a near-shore manganese sediment (De Schamphelaire et al., 2007). A number of related metal-reducing micro-organisms have been identified and classified as the Geobacteraceae (Caccavo et al., 1994; Coates et al., 1995, 2001; Holmes et al., 2004; Vandieken et al., 2006). The biochemical pathways involved in Mn^{2+} oxidation have not yet

been completely elucidated. A general scheme of the manganese cycle occurring in a sediment-water system is showed in Figure 1. The main oxidant in natural water is dissolved oxygen.

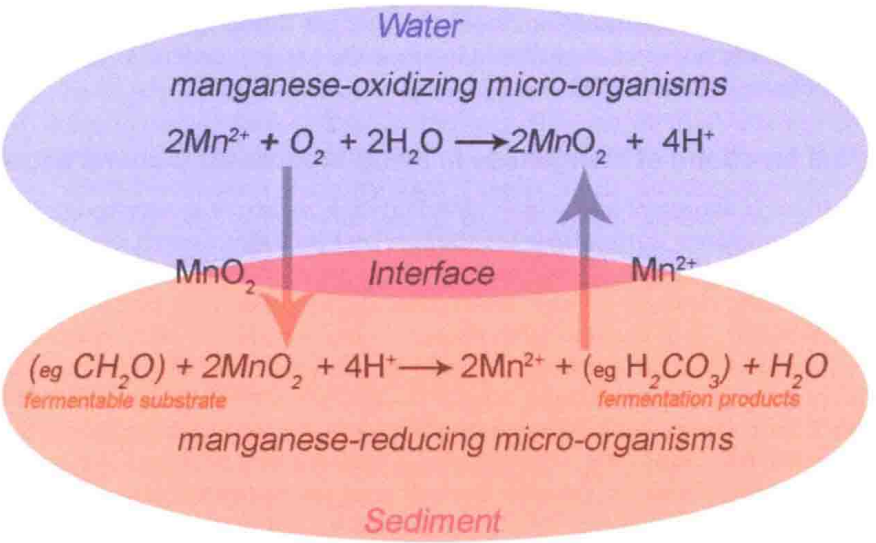

Fig. 1. A flux model for manganese interconversion. Manganese oxidation is performed in the oxic layer (water), while manganese reduction occurs in the anoxic layer (sediment). The oxic-anoxic boundary is located at the sediment-water interface. Note: all figures presented in this work are original.

The marine environmental chemistry of manganese is largely governed by pH, oxygen concentration of the solution and redox conditions. In fact, manganese oxidation increases with the decrease in acidity of the medium. The redox cycle of manganese in the oceans occurs at the oxic-anoxic boundary, which is often located at the sediment-water interface. Manganese oxides are present on the ocean floor as concretions, crusts and fine disseminations in sediments. It is well known, for example, that the soft bottom sediments of the oceans are particularly rich in manganese aggregates in the form of nodules (Bonatti & Nayudu, 1965; Wang et al., 2011).

Free manganese ions are released in the water by means of the photochemical and chemical reduction of manganese oxides coming from the organic matter (Sunda & Huntsman, 1998; De Schamphelaire et al., 2007). The process is initialised after the increase in temperature, the decrease in oxygen concentrations and the upward movement of the redox-cline (Balzer, 1982; Hunt, 1983). The transport of the dissolved manganese ions is governed by molecular diffusion in the water pores and it follows a manganese concentration gradient (the gradient decreases towards the oxic zone). In the marine environment, in absence of micro-organisms or mineral particles, manganese oxidation is a slow process (Wehrli et al., 1995). A reduced dissolved oxygen condition (called hypoxia) causes the rise of the ionic flux of manganese, which goes from the sediment to the overlying waters, where it reaches concentrations 1,000-folds higher than those normally occurring in seawater (up to 22 mg l^{-1}) (Trefry et al., 1984; Aller, 1994). Hypoxia in the marine environment can be natural or human-induced.

At present, costal hypoxia is increasing because of man-made alterations of coastal ecosystems and changes in oceanographic conditions due to global warming. In the deep ocean water, hypoxia is influenced mostly by the variations in the up-welling that is driven by the wind. Hypoxic areas are marine dead zones in the world's oceans which can happen for example in the fjords, coastlines or closed sea (such as Black, Baltic and Mediterranean Seas), where the water turnover, that should increase the oxygen content, is very slow or not present (Middelburg & Levin, 2009).

3. Biological functions of manganese in living organisms: General aspects

While manganese is abundant and widely distributed in nature, it is required only in trace amounts in the organisms during their life span, where it guides normal development and body function. In fact, it plays essential roles in many metabolic and non-metabolic regulatory functions, such as: i) bone mineralization; ii) connective tissue formation; iii) energetic metabolism; iv) enzyme activation; v) immunological and nervous system activities; vi) reproductive hormone regulation; vii) cellular defence; viii) amino acid, lipid, protein, and carbohydrate metabolisms; ix) glycosaminoglycans formation; x) blood clotting (ATSDR, 2008; Santamaria, 2008). Manganese works as a constituent of metallo-enzymes or as an enzyme activator. Examples of manganese-containing enzymes include: arginase, the cytosolic enzyme responsible for the urea formation; pyruvate carboxylase, the enzyme that catalyses the first step of the carbohydrate synthesis from pyruvate; and manganese-superoxide dismutase, the enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (Wedler, 1994; Crowley et al., 2000). In contrast to the relatively few manganese metallo-enzymes, there are a large number of manganese-activated enzymes, including the hydrolases, the kinases, the decarboxylases, the DNA and RNA polymerases and the transferases (Missiaen et al., 2004). Activation of these enzymes can occur either as a direct consequence of the binding of manganese to the proteins, which causes subsequent conformational changes, or by its binding to the substrate, as in the case of ATP. Mechanisms regulating manganese homeostasis in cells are largely unknown. Some studies indicate the importance of regulated intracellular trafficking of manganese transporters to balance its absorption and secretion. Multiple transporters mediate intracellular manganese uptake including: i) natural resistance-associated macrophage proteins (Nramp); ii) cation/H+ antiporter; iii) zinc-regulated transporter/iron-regulated transporter (ZRT/IRT1)-related proteins (ZIP); iv) transferrin receptors; v) various calcium-transport ATPases; vi) glutamate ionotropic receptors (Au et al., 2008). Some of these transporters are localized within specific intracellular compartments, but none of them are manganese-specific transporters. In yeast, under normal conditions, the intracellular manganese concentration is regulated by adjustments of surface levels of the Nramp transporter, which regulates its degradation by endocytosis and ubiquitin-mediated targeting to vacuoles (Culotta et al., 2005). In mammals, the Golgi-associated secretory pathway Ca^{2+}-ATPase (SPCA) is known to pump cytosolic manganese into the lumen of the Golgi complex, in order to be used by glycosylation pathway enzymes. However, SPCA role in manganese detoxification has not been well elucidated (Missiaen et al., 2004).

4. Manganese toxicity: Causes and concerns

Manganese is considered an emerging contaminant because it is a perceived or real threat to the human health and the environment. Manganese exposure occurs at different levels and

through a wide variety of industrial sources such as mining, alloy production, goods processing, iron-manganese operations, welding, agrochemical production and other anthropogenic activities. Manganese products can be discharged into the sea and become an unforeseen toxic metal in the marine environment. As manganese bioavailability increases, its uptake into living organisms occurs predominately through the water. The manganese rates of accumulation, as well as its elimination, are relatively fast-regulated processes. The exposure to high levels of manganese causes toxicity and decreases the fitness of the organisms (Roth, 2006). In humans, the neurological damage induced by excessive manganese exposure has been well documented for over a century (Cooper, 1837; Mena et al., 1967; Normandin & Hazel, 2002; Takeda, 2003). On the contrary, data on the effects of high manganese exposure in marine organisms are not well documented. In fact, although marine environment contains high natural concentrations of manganese, especially in the hypoxic zones, the potential danger to benthic and planktonic organisms has attracted the attention of scientists only recently.

4.1 Manganese toxicity in marine invertebrates

In all marine organisms manganese is accumulated into tissues; its amount reflects the concentrations of the bio-available manganese dissolved in sea water (Weinstein et al., 1992; Hansen & Bjerregaard, 1995; Baden & Eriksson, 2006). At the cellular level, manganese balance is proficiently managed by processes controlling cellular uptake, retention, and excretion (Roth, 2006), but these elaborate homeostatic mechanisms are altered under high levels of the available metal. Thus, it is important to consider that manganese dissolved in sea water is bio-concentrated significantly more at lower than at higher trophic levels (CICAD 63, 2004). The Bio Concentration Factor (BCF) correlates the concentration of a substance in animal tissues to the concentration of the same substance in the surrounding water. The reported BCF values range between: 100-600 for fish, 10,000-20,000 for marine and freshwater plants, 10,000-40,000 for invertebrates (ATSDR, 2008). In aquatic invertebrates manganese uptake significantly increases with temperature increase and salinity and with pH decrease. Dissolved oxygen has no significant effect (Baden et al., 1995).

Crustaceans and molluscs are the most manganese-sensitive invertebrates, followed by arthropods and echinoderms. The first studies on the effects of manganese in crustaceans (species *Homarus gammarus and H. vulgari*) were carried out by Bryan & Ward (1965).

High levels of manganese have been found in the haemolymph and body tissues of the lobster *Nephrops norvegicus* living in the SE Kattegat, Swedish west coast, as well as in lobsters living in the hypoxic areas near sludge dumping sites in the Firth of Clyde, Scotland (Baden & Neil, 1998). Exposure to high manganese impairs the lobster's antennular ficking activity, causing disorientation and inability to locate food (Krång & Rosenqvist, 2006). Likewise, unhealthy blue crabs, *Callinectes sapidus*, have been found in a manganese-contaminated area of North Carolina, USA (Gemperline et al., 1992; Weinstein et al., 1992). In general, internal tissues such as the intestine, nervous system, haemolymph and reproductive organs accumulate much more manganese than other tissues such as exoskeleton, but in the latter case, manganese elimination is a very slow process. In *Nephrops norvegicus*, manganese accumulation reached a plateau after 1.25 days of exposure in all tissues except for the mid-gut gland, which continued to accumulate manganese over time

(Baden et al., 1999). A similar accumulation pattern of manganese in soft tissues has also been described in mussels (Regoli et al., 1991). Specifically, in the species *Donacilla cornea,* manganese was rapidly accumulated; reaching a maximum after 3 days of exposure, and it was rapidly excreted (60% loss) after 3 days in clean sea water. Seasonal and sex differences in the manganese accumulation levels have been reported for both mussels (e.g. *Mytilus edulis* and *Mytilus californianus*) and oysters (e.g. *Crassostrea gigas* and *Crassostrea virginica*) (Nørum et al., 2005). For example, in the species *Mytilus edulis* the gonads of females accumulated manganese more than males. Manganese accumulation in the sea star *Asterias rubens* has shown linearity with time up to 23 days at low concentrations (0.1 mg l^{-1}), but its saturation kinetics were very fast at higher concentrations (Hansen & Bjerregaard, 1995). In fact, it was found that steady-state levels were reached in the coelomic fluids after only 5 days of exposure to 5.5 mg l^{-1} (Oweson et al., 2008).

Manganese excess may cause a Ca^{2+} pump dysfunction, affecting neuro-muscular transmission in benthic marine invertebrates (Hagiwara & Takahashi, 1967; Baden & Neil, 1998; Holmes et al., 1999). For example, in crustaceans, manganese acts as a competitive inhibitor of the calcium-regulated ion channels present in nerve and muscle membranes, thus inhibiting synaptic and neuromuscular transmission and muscle excitation (Hagiwara & Takahashi, 1967; Holmes et al., 1999).

Manganese affects the immune system of marine invertebrates in a species-specific manner.

In the immune system of *Nephrops norvegicus* (haemolymph), manganese is mainly found in the protein fraction that includes haemocyanin and immune cells (called haemocytes). Recent studies showed that high levels of manganese affect *Nephrops norvegicus* haemocytes causing: i) apoptosis-induced reduction of the number of circulating haemocytes ; ii) inhibition of their maturation to granular haemocytes; iii) inhibition of the recruitment of haematopoietic stem cells (Hernroth et al., 2004; Oweson et al., 2006). These immune suppressive effects were also found in *Mytilus edulis* (Oweson et al., 2009). In addition, manganese alters the immune system of sponges (*Geodia cydonium, Crella elegans* and *Chondrosia reniformis*) by inhibiting the activity of the 2', 5'-oligoadenylate synthetase (2-5A synthetase), an enzyme known to be involved in the functioning of the immune system of vertebrates (Saby et al., 2009).

Surprisingly, in contrast to what was recorded in crustaceans and molluscs, in echinoderms (*Asterias rubens)* manganese exposure stimulated haematopoiesis, thus causing an increase in the number of circulating immune cells (Oweson et al., 2008). Manganese effects on *Asterias rubens* immune system will be discussed in detail in the next sections.

4.2 How does manganese affect echinoderm immune cells?

Echinoderms play a key role in the maintenance of the integrity of the ecosystem where they live (Hereu et al., 2005) and are constantly exposed to pollutants deriving from different kinds of human activities (Bellas et al., 2008; Rosen et al., 2008). They are phylogenetically related to vertebrates and have a sophisticated and sensitive immune system. In echinoderms, immune cells (called coelomocytes) are a heterogeneous population of free moving cells found in all coelomic spaces, including the perivisceral coelomic cavities and the water-vascular system (reviewed in Matranga, 1996; Glinski & Jarosz, 2000; Smith et al., 2010). They are also present sparsely in the connective tissue (mesodermal stromal tissue)

and amongst tissues of various organs (Muñoz-Chápuli et al., 2005; Pinsino et al., 2007). Coelomocytes participate as immune cells in function similar to their vertebrate's immune system homologues. In fact, they are involved in: clot formation, phagocytosis, encapsulation and clearance of pathogens, as well as oxygen transport. The coelomic fluid in which the immunocytes or coelomocytes reside and move is a key factor governing the immunological capabilities of echinoderms, as it contains essential trophic and activating factors (for a review see Matranga et al., 2005; Smith et al., 2010). Four different morphotypes have been described in the asteroid *Asterias rubens*, with the phagocytes as the most abundant type, accounting for approximately 95% of the total population (Pinsino et al., 2007).

As previously reported, the accumulation of manganese into the coelomic fluid of exposed sea stars (*Asterias rubens)* induces the proliferation of haematopoietic cells (Oweson et al., 2008). Specifically, by using the substitute nucleotide 5-bromo-2'-deoxyuridine (BrdU) for tracing cell division, and by recording the mitotic index after nuclei staining, authors found that manganese induced the proliferation of cells from a putative haematopoietic tissue, the coelomic epithelium. In addition, the haematopoietic tissue and coelomocytes showed stress response in terms of changes in HSP70 levels and protein carbonyls. Incubation with heat-killed FITC-labelled yeast cells (*Saccharomyces cerevisiae*) exhibited an inhibited phagocyte capacity of coelomocytes. Moreover, measurement of dehydrogenase activity, using MTS/PMS, revealed that manganese showed cytotoxic properties. Although manganese was revealed as stressful to the coelomocytes and affected their ability to phagocyte, the increased number of coelomocytes compensated these impairments. In summary, the authors concluded that the exposure of *Asterias rubens* to manganese impaired their immune response, but induced renewal of coelomocytes, assuring survival. Co-occurrence of manganese with hypoxic conditions does not inhibit the elevated production of coelomocytes, but probably affects the composition of the subpopulations of these immune cells since hypoxia, but not manganese, increased the mRNA expression of Runt, a transcription factor, assumed necessary for cell differentiation (Oweson et al., 2010).

5. Sea urchin embryonic development

To address this issue, at the beginning of this section we will describe the basic steps of the sea urchin development. Briefly, upon appropriate stimulation, millions of eggs and sperm are released into the sea water; after fertilization, the single-celled zygote is converted into a multi-cellular embryo through rapid and repeated mitotic cell divisions (cleavage). Founder cells of the three germ layers ecto- meso- and endoderm, are the basic units where regulatory information is localized during cleavage. In particular, β-catenin is required for the development of all endo-mesoderm territories, including the archenteron, the primary mesenchyme cells (PMCs) and the secondary mesenchyme cells (SMCs) (Logan et al., 1999). Cell fates are fully specified by the blastula-early gastrula stage of development, when cells have begun to express particular sets of territory-specific genes (Davidson et al., 1998). Although maternal determinants are required for founder cell specification during development, interactions between the PMCs and external cues derived from the ectoderm specify many phases of the skeleton formation (Armstrong et al., 1993; Ettensohn & Malinda, 1993; Guss & Ettensohn, 1997; Zito et al., 1998). The blastula stage is characterized by the presence of a large fluid-filled blastocoels, surrounded by a single layer of cells.

During gastrulation extensive cellular rearrangements occur which convert the hollow-spherical-blastula into a multi-layered gastrula. Changes in shape and differentiation of embryo structures lead to the formation of a pluteus, the first larval stage. Genus-specific spicule growth and patterning is completed at this stage, directed by the spatial-temporal regulated expression of bio-mineralization related genes (Zito et al., 2005; Matranga et al., 2011). Sea urchin development from the blastula to the pluteus stage is showed in figure 2.

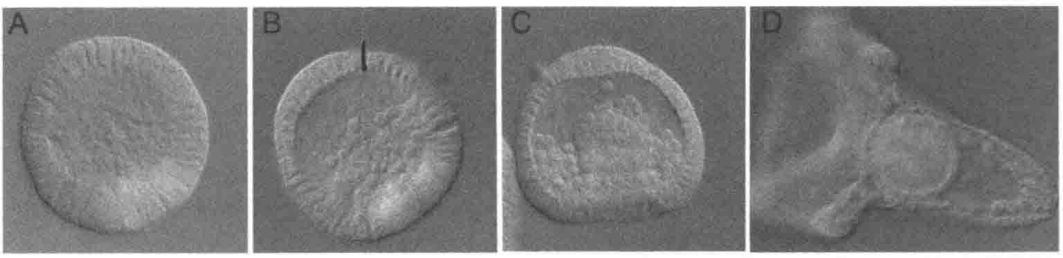

Fig. 2. Sea urchin development from the blastula to the pluteus stage. A) hatching blastula; B) mesenchyme blastula; C) middle gastrula; D) pluteus. Note: all figures presented in this work are original.

5.1 Sea urchin embryos as an *in vivo* model for the assessment of toxicity

The sea urchin is estimated to have 23,300 genes with representatives of nearly all vertebrate gene families (Sea Urchin Genome Sequencing Consortium, 2006). Since it has been demonstrated that the sea urchin genome shares at least 70% of the genes with the mankind, we shall consider how this provides an important tool kit to aid our understanding of eco-embryo- and geno-toxicological studies as well as for studies on embryonic development. Sea urchins are marine invertebrates with two life stages: i) an early and brief developmental stage (planktonic) and ii) a remarkably long-lived adult stage with life spans extending to over a century (epi-benthonic). Sea urchins are pivotal components of sub-tidal marine ecology (Hereu et al., 2005) and they are continuously exposed to environmental pressure, including changes in temperature, hypoxia, pathogens, UV radiation, free radicals, metals and toxicants. These marine invertebrates produce large numbers of susceptible, but not vulnerable, transparent embryos. The keys for their developmental success are the potent cellular mechanisms that provide them with protection, robustness and resistance, as well as the regulatory pathways that alter their developmental course in response to the conditions encountered (Hamdoun & Epel, 2007). The integrated network of genes, proteins and pathways that allow an organism to defend itself against chemical agents is known as the "chemical defensome" (Goldstone et al., 2006). In sea urchin embryos, many "defensome" genes are also expressed during their normal development as integral part of the developmental program, suggesting a dual function regulating both defence and development. In addition, genes involved in signal transduction often respond to environmental stress, activating alternative signalling pathways as a defence strategy for survival (Hamdoun & Epel, 2007). Thus, the sea urchin becomes an excellent candidate for the understanding of the two-fold function of genes/proteins and signalling pathways involved in both defence and regulation/preservation of development during environmental changes.