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The Environmental Effects of Sea Pollution on Microorganisms

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Abstract
Sea anemones from phylum cnidaria are benthic organisms and live on sea substrates, so water pollution can affect them easily. The marine environments are the target of contaminations caused by a complex mixture of metals and chemical materials from various anthropogenic sources. The pollution could have mortal effects on sea anemones. Although the anemones respond to increased metal exposure by closing their tentacles and changing the activities of the enzymes, CAT (catalse), GR (glutathione reductase), and CA (carbonic anhydrase), altered enzyme activity and tentacle retraction of sea anemones, as well as decreased zooxanthellae cell density could be observed responses over exposure. Metal depuration and physiological recovery are dependent on both the metal and the exposure concentration. Copper exposure can cause tentacle retraction and increase mucus production in both symbiotic and aposymbiotic anemones. Heavy metals especially copper can cause cellular damages in the level of nucleus and DNA. Some species of sea anemones accumulate heavy metals such as copper and are sensitive, as effects were detected at environmentally relevant copper concentrations. Likewise, they may be useful in biomonitoring copper polluted environments.

Keywords: Sea anemones; Contaminants; Antioxidant Enzymes; DNA Damage; Bioaccumulation; Symbiosis Relationship

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1. Introduction

Sea anemones are polyp like and solitary organisms which absorb dissolved organic matters as food or have symbiotic life with photosynthetic zooxanthellates. Being not much mobile make them feed on planktons [1]. Knowing about the reaction of these organisms against pollutions is important due to their little mobility and disability in escaping [2]. Despite their importance in the coastal environment worldwide, there are few studies related to metal accumulation in the tissues of cnidarians and even less in sea anemones [3,4]. Marine environment are contaminated via many different forms, such as solid waste (e.g., plastics), increased nutrient (e.g., nitrates and phosphates), toxic chemicals (e.g., organic compounds, DDT, PCB, metals, pharmaceuticals, gas), oil spills and sediment inputs due to human activities (e.g., industry, agriculture, on deforestation, sewage discharge, aquaculture), radioactivity, and discarded fishing nets. These contaminations change the biological, chemical and physical and characteristics of the oceans and coastal zones, and potentially threatens marine organism, ecosystems, and biodiversity and affects thus the quality and productivity of marine ecosystems. The final effect of pollution on marine resources and different organisms depend on the intensity (acute or chronic), form of pollution, and location of the contamination. Also there is an important point that some marine environments, ecosystems, and species are more sensitive than others to pollution [5].

As noted above, heavy metals are important source of contamination. The term "heavy metals" is defined for any metallic element that has a relatively high density and is toxic or poisonous even at low concentration [6]. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements [7]. Due to input of metal pollution from different ways, marine environments contain a complex mixture of metals. However, few studies have noted the effects of metal mixtures on sea anemones. Despite of this fact, anemones have been shown to be sensitive to metal exposure [4, 8-9].

Global climate changes, such as ocean acidification (OA) have intensified the effects of these local stressors. Anthropogenic activities like fossil fuel combustion, cement production and deforestation have significantly increased atmospheric Carbon dioxide (CO_2) levels [10, 11]. The ocean pH decreases by increasing of CO2 and it rises the hydrogen ion concentration in water [12].

Presence and absence of hydroxyl (OH^-) and carbonate ions ($CO3^{2-}$) change the fate of metals in the aquatic environments [13]. When the effects of both stressors (CO_2 and metals) combine, as well as the increased bioavailability of metals at lower pH levels, substantial biological effects might be at the physiological level, which may result in having repercussions for populations and even ecosystems [14-16].

Cu is a commonly used metal that can enter marine ecosystems through different ways such as sewage treatment discharge, industrial effluent, leaching of anti-fouling paints, and copper refineries [3, 17, 18], metal mining [19]. Marine cnidarians would be under oxidative stress by relatively low levels of Cu [20-22].

The sea anemone, *Aiptasia pallida*, is a symbiotic cnidarian, which is endemic to the southeastern United States and its habitat is commonly on hard substrates such as coral reefs and rocks [23][24]. The photosynthetic zooxanthellae symbiont earns protection, nitrogen, and a carbon dioxide source from *A. pallida* in exchange for energy [25]. Environmental stressors such as temperature change and contaminants may interrupt this relationship, potentially causing loss of the zooxanthellae, resulting in bleaching of *A. pallida* [26-27].

Some physiological effects have been well studied on marine cnidarians as a consequence of Cu exposure [4][22][28][29]. In particular, Cu has been shown to cause the generation of reactive oxygen species (ROS), which can lead to degradation of macro-molecules like proteins, lipids and DNA [30][31]. Several anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) [31] are used to combat the harmful effects of ROS. The activity of these enzymes as well as carbonic anhydrase (CA), responsible for acid/base balance, were altered in cnidarians exposed to Cu in previous studies [4, 20, 21, 32].

When an imbalance between the number of reactive oxygen species formed and the neutralization of these by antioxidant defenses is occurred, some type of damage and changes in cellular signals, it is said that the cell is under oxidative stress [33,35,36]. There is evidence in marine organisms that the toxicity of copper causes oxidative stress, an increase in antioxidants and DNA damage [37].

Carbonic anhydrase (CA) is an essential enzyme, catalyzes the interconversion of carbon dioxide to bicarbonate and is important in respiration and metabolism [38]. The metalloprotein and enzyme CA pivots around zinc as its catalytic redox center and is known to be sensitive to metal exposure. Metals have been shown to inhibit normal CA activity in fish, blue crabs, and recently corals and sea anemones [20, 39-41].

The sea anemone *A. pallida* are abundant in shallow temperate and tropic coastal environments. Also their sampling and compatibility to laboratory conditions is easy. These organisms reproduce quickly compared to other symbiotic cnidarians and their habitat is mostly near contaminated marine zones, so are good model organisms for toxicological studies [42]. This species has also been shown to be sensitive to several different metals, particularly Cu [4, 8, 9].

The induction of multiple xenobiotics can rises populational or individual resistance in contaminated waters. Therefore, evaluation of the level of xenobiotics induction in individuals could be used as a biomarker of capability in these organisms [43]. Of course, for better perception of the biological responses to stressors, both genetic and cellular responses should be integrated, and physiological responses should be analyzed from an ecotoxicological perspective [44, 45].

An inhibition in the extrusion mechanism of contaminants might result in roxidative stress, and can then generate genetic damage, which is often irreversible. In the relevant literature, one may find research involving copper, oxidative stress and DNA damage [46]; copper and xenobiotics in coral [47], lead and xenobiotics in bivalves [48]; however, finding researches using metals in invertebrates, showing oxidative stress, and multiple xenobiotic expression linked to DNA damage are not common.

Sea anemones are benthic marine organisms with less mobility that escape or immigration could not be a good method of dealing with pollution. Therefore physiological, biological and genetic responses and changes would be signs of alternations by different contaminants. The structure and the cellular mechanisms of *Bunodosoma caissarum* enables it to support extreme conditions, such as air exposure and fluctuations in salinity. This species has different mechanisms of adaptation for coping with stress situations, including mucus secretion, the presence of warts and the development of a protective dome shape [49].

2. Marine pollution on enzymatic changes:

Changes in the activity of antioxidant enzymes is one of the physiological effects of metal exposure. Carbonic anhydrase (CA) activity could be an important biomonitoring tool for assessing cnidarian health. Although some research has been conducted to elucidate the effects of individual metal exposure to sea anemones [4, 8, 9], little is known about the impact of metal mixtures.

When sufficient light is provided photosynthetic zooxanthellae can generate reactive oxygen species (ROS), which can cause cellular and molecular damage, such as oxidation of phospholipid membranes, denaturation of proteins, and alteration of nucleic acids [50-52].The cell membrane, is one of the centers of reactive oxygen species (ROS) activity. In biological systems, Cellular damage results basically from ROS attack on macromolecules such as sugars, DNA, proteins and lipids [34, 53, 54]. One way cells can avoid ROS generation and the resulting oxidative stress would be through the extrusion of the contaminants.

When abiotic stress increases, electron transport mechanisms (i.e., photosystems and mitochondrial transport chains) become less efficient. In turn, free electrons are more likely to interact with diatomic molecular oxygen ultimately resulting in a series of reduced reactive oxygen species (ROS). Both *Exaiptaisa pallida* and its symbiotic zooxanthellae have evolved mechanisms to protect against the damaging effects of ROS [51][52]. Due to photosynthesis, symbiotic anemones are subjected to very high levels of oxygen during the day, and at night they experience a hypoxic internal environment [55]. This continuous transition between extreme oxygen environments can result in the increased production of ROS [55][56]. ROS can denature proteins, mutate DNA, and cause lipid peroxidation [51]. To neutralize the potentially harmful effects of ROS, organisms produce enzymes such as superoxide dismutase (SOD) and catalase (CAT).

SOD catalyzes the conversion of superoxide anion into hydrogen peroxide and oxygen; whereas CAT catalyzes the conversion of hydrogen peroxide (H_2O_2) into water and oxygen [57]. H_2O_2 can be induced by a variety of factors including metal exposure [58]. In the presence of metal ions such as Cu, H_2O_2 can be quickly converted to hydroxyl radicals in a process known as the Fenton reaction [59]. Zn is one of other heavy metals can also cause oxidative stress and hydroxyl radical formation via different mechanisms.

Through another enzymatic pathway, glutathione peroxidase (GPX) can also catalyze the conversion of H_2O_2 to water, using monomeric glutathione as the reducing agent [60-63]. A subsequent reaction reduces the glutathione using NADPH and glutathione reductase (GR) as the catalyst, so that it may be recycled for the previous reaction [60-63]. Glutathione itself can also function in metal detoxification by binding to and removing metals from the organism via conjugation reactions [60].

Cellular destruction on the level of DNA damages by pollutants:

Copper, even at low concentrations and even within the limit allowed by Brazilian law, causes stress in anemone cells. This metal exhibits cytotoxicity, leading cells to activate cellular defense mechanisms to some extent (in lower concentration). This defense is not sufficiently effective to prevent an increase of reactive oxygen species and genetic damage, however, when it is not activated, (in the highest concentration) the cytotoxic and oxidative genetic damage, are even greater [2].

On the work of Schwarz and Collaborators (2013) it was seen that there is an increase in DNA damage in cells exposed to copper, with a clear increase in the percentage of genetic material in the tail. Copper has the potential to cause DNA damage, especially in relation to dispersion of the genetic material (larger DNA% than tail size, itself), which seems to show simple breaks in DNA chains [64]. It is noteworthy that the percentage of DNA present in the tail seems to be, among the parameters analyzed at Comet assay, the most sensitive, as it was the analysis that managed to show damage in 6 h of exposure, even in the copper concentration 7.8 lg/L. The occurrence of xenobiotic contaminants, such as metal ions, in the marine environment is highly harmful to the biological integrity as well as the function of marine organisms. Many of these pollutants are chemicals capable of causing damage to DNA, usually through oxidative stress. Benzo[a]pyrene, for example, increases the amount of ROS which leads to formation, directly or indirectly, of DNA adducts, resulting in genetic chain breaks [65-66]. Bopp and Collaborators (2008), in addition to investigating the formation of reactive oxygen species in gill cells exposed to copper, also performed comet assays, to analyze

DNA integrity in cells [67]. Generation of ROS"s are essential factors for the DNA damage, with the reactive oxygen species performing a crucial role in the process of DNA degradation, causing direct damage through oxidation of DNA molecule, or indirect, causing damage to lipids and proteins, subsequently leading to genotoxic damage [46,67-71].

Color and symbiotic relationship disorders by contaminants:

Due to close vicinity of *Aiptasia pallida* to anthropogenic inputs, may be a bioindicator of copper pollution. Biomonitoring organisms can indicate the quality of the environment they inhabit. These organisms can be used to understand the temporal and geographical variations in the bioavailability of contaminants [72]. The color of a healthy *A. pallida* is golden-brown in color due to their zooxanthellae (dinoflagellate algae of the genus Symbiodinium), which live in symbiosis with *A. pallida* [73]. In recent years, pulse-amplitude modulated (PAM) fluorometry has been used to assess the effects of pollutants on seaweed, algae, and zooxanthellae [74-76]. The PAM fluorometer can be used to distinguish between photon energy captured by a chlorophyll-a pigment molecule used to drive photosynthesis, and energy emitted as fluorescence or converted to heat [77]. In this symbiotic relationship *A. pallida*, provides the zooxanthellae with nutrients (nitrogenous waste and CO2) and protection. In return, zooxanthellae provide the anemone with photosynthate via carbon fixation pathways [77]. When this host–symbiont relationship becomes challenged by any number of environmental stressors, loss of the symbiont "bleaching" and color loosing may occur [78]. Bleaching of sea anemones may reduce tissue metal concentrations, and has been demonstrated in studies as a consequence of copper exposure [4, 21].

Corals and anemones both have been studied after copper exposure and tissue copper accumulation [8, 9, 79]. The pattern of copper accumulation in the tissues of symbiotic cnidarians may be affected by the presence of the symbiont Copper accumulation in the symbiotic sea anemone, *Anemonia viridis*, was not directly reflective of the copper concentrations in the environment, suggesting that detoxification and excretion mechanisms were used to combat increasing copper concentrations [79]. Harland and Nganro (1990) also suggested that greater extent copper is accumulated in the zooxanthellae comparison to host tissue and in a final detoxification attempt the anemone release their symbiotic algae to reduce internal copper concentrations [80]. The concept of zooxanthellae expulsion as a mechanism of metal detoxification has been suggested by several researchers, since zooxanthellae have been found to accumulate heavy metals to a larger extent and be more tolerant than their symbiotic hosts [81,82].

Bioaccumulation as a response to pollution:

The sea anemones *Bunodosoma cangicum* and *Bunodosoma caissarum* are commonly Brazilian endemic species found in the intertidal area of the Southeast regions of Brazil [49, 83]. Organisms from this species can bioaccumulate heavy metals from the environment [84]. *Bunodosoma caissarum* is capable of combining Hg and other metals such as cadmium, copper and zinc in its tissues [85].

In two microcosms with and without sea anemone *B. caissarum* different results were achieved in the study of Rizzini Ansari et al. (2015). In both microcosms, methylmercury (MeHg) formation was observed. A higher level of MeHg was detected in the seawater from the microcosm without *B. caissarum*. The reason for this might be that under these conditions the microorganisms were allowed to grow and to contribute to MeHg production without the Hg bioaccumulation interference of *B. caissarum*. Another possibility is that *B. caissarum* readily incorporated part of the MeHg produced. Mercury was probably less available for methylation in microcosms with *B. caissarum* because of bioaccumulation and due to the higher concentrations of suspended particulate matter that could form complexes with Hg. This experiment demonstrated the formation of MeHg in the seawater of microcosms without *B. caissarum*. However, we should note that in the microcosms containing *B. caissarum* this experiment does not permit a conclusion as to whether MeHg was formed in the specimens themselves, in the seawater, or in the mucus secretions and then accumulated by *B. caissarum* [85].

3. Conclusion

The copper represents an adversity to the anemones cells, being cytotoxic and genotoxic. Metal accumulation in the tissues of sea anemones are a good indicator of metal exposure at different testable concentrations. Understanding how sea anemones and their symbionts are affected by metal exposures in the laboratory may allow better understanding about the responses of symbiotic cnidarians in metal polluted aquatic environments. The Symbiont loss, as might be observed by decreased algal cell density and visible paling, might be aided in reducing the tissue metal burden of the anemones.

Additionally metal accumulation patterns in the anemones, marked differences were observed in responses of antioxidant enzymes to a mixed metal exposure regime as compared to individual metal exposure. The activity of CAT, for example, increased after exposure to Cu alone [4], and decreased as a consequence of mixed metal exposure, which may also be a consequence of the intial reduction in zooxanthellae cell density

These organisms could be susceptible to metal pollution in the environment, particularly along the coastline surrounding heavily populated areas with substantial anthropogenic inputs. Symbiotic sea anemone upregulates CAT activity to combat the damaging effects of hydrogen peroxide. SOD activity significantly decreases during the highest copper exposure. SOD is substantially reduced in aposymbiotic *A. pallida*, suggesting that the zooxanthellae are associated with the oxidative stress response. *A. pallida* may be a useful bioindicator species in laboratory studies and potentially in metal polluted environments. Sea anemones may face to Reduced zooxanthellae electron transport, Reduced carbonic anhydrase activity and Reduced growth when they exposure to contaminants.

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