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Effect of Heavy Metals on the Biofilm Formed by Microorganisms from Impacted Aquatic Environments

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Abstract

The aquatic environment is highly complex and diverse, consisting of several types of ecosystems that are dynamic products of complex interactions between biotic and abiotic components. Changes in the physical and chemical properties of these ecosystems can significantly affect the balance of life forms present, especially in their microbiota. Among the main pollutants present in these environments are heavy metals. Several studies demonstrate the effects of these minerals on the structure and function of microbial communities, which may develop adaptation mechanisms for survival and permanence in these sites. In addition, the resistance to heavy metals may contribute to the evolution of resistance genes to the different types of antimicrobials due to the increase of the selective pressure in the environment, becoming a public health problem. One of the adaptive mechanisms present in bacteria from impacted environments that has been frequently investigated is the formation of biofilms. Recent studies have reported significant changes in the structure and amount of biofilm formed in the presence of different metals, and consequently, an increase in the tolerance to these pollutants and antimicrobials. This review will discuss the effects of some metals on bacterial biofilms and their consequences for the marine environment.

Keywords: chemical pollution, toxicity, mechanisms of adaptation, metals, antimicrobials

1. Introduction

The aquatic environment is highly complex and diverse, comprising various types of ecosystems that are dynamic products of complex interactions between biological and abiotic components. Changes in physical properties and ecosystems may affect the balance of life forms present there [1, 2].

In recent decades, these ecosystems have been significantly altered due to multiple environmental impacts from the release of large amounts of effluent without adequate prior treatment, resulting in the scarcity of existing natural resources [3, 4]. Among the main pollutants that generate negative impacts on life

forms are heavy metals. The presence of these contaminants may cause changes in the structure and function of microbial communities [5], which can develop various resistance mechanisms that enable their survival [6]. In addition, heavy metal resistance may contribute to the evolution of resistance genes to different types of antimicrobials due to increased selective pressure in the environment [7].

Adaptability as well as metabolic and physiological differences are essential characteristics for microorganisms to remain in these locations. One of the adaptive mechanisms present in bacteria that has been frequently investigated is biofilm formation [8]. Biofilms are structures composed mainly of microbial cells and a matrix formed by a cluster of extracellular polymeric substances (EPSs) [9]. Biofilm-grown cells have some distinct properties from planktonic cells, one of which is increased resistance to antimicrobials and heavy metals [10]. In this review, we propose to report the latest findings on the survival strategies of microorganisms in impacted aquatic environments, more precisely on the influence of heavy metals on biofilm formation.

2. Microorganisms in contaminated aquatic environments

Water is an indispensable natural resource for the survival of man and other living beings [11, 12]. According to Raucci and Polette [13], 97% of the planet's water is found in the oceans, and of the remaining 3%, only 0.3% is available for human consumption and is stored in springs, lakes, rivers, and groundwater.

According to the United Nations (UN), access to water supply and sanitation is a human right and vital to the dignity and health of all people. However, there are still about 1.1 billion people without access to clean water and 2.4 billion people without access to basic sanitation services [14].

The decline in water quality has become one of the most serious problems worldwide, a fact that has been intensified by the increase in population and the absence of public policies aimed at the preservation of water resources. According to the World Health Organization—WHO [15], approximately half of the world's developing population will be affected by diseases that are directly related to poor-quality water and/or lack of adequate or even no sanitation.

Contamination of natural waters represents one of the main risks to public health, a fact that is directly related to the discharge of untreated domestic, hospital, and industrial effluents, which cause contamination of aquatic bodies by pathogenic microorganisms such as bacteria, viruses, protozoa, and helminth eggs [16].

Among the bacteria can be highlighted those belonging to the Enterobacteriaceae family, represented by species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* and *Providencia rettgeri*. Most of these species are commonly found in the intestinal tract of humans and animals, and their presence in aquatic environments indicates fecal contamination [4, 17].

Another problem found in the aquatic environment is the contamination by resistant bacteria from humans and animals exposed to antimicrobials [18, 19], as well as the disposal of antimicrobial waste from domestic and hospital effluents. Water is not only a means of spreading resistant microorganisms, but also the pathway through which resistance genes are introduced into the ecosystem, altering the environmental microbiota [20].

Studies have shown bacterial resistance in various aquatic environments including rivers and coastal areas, domestic sewage, hospital sewage, sediment, surface water, lakes, oceans, and drinking water [4, 21–24].

Among the main pollutants found in this environment, we highlight the heavy metals that when introduced into the environment can cause changes in

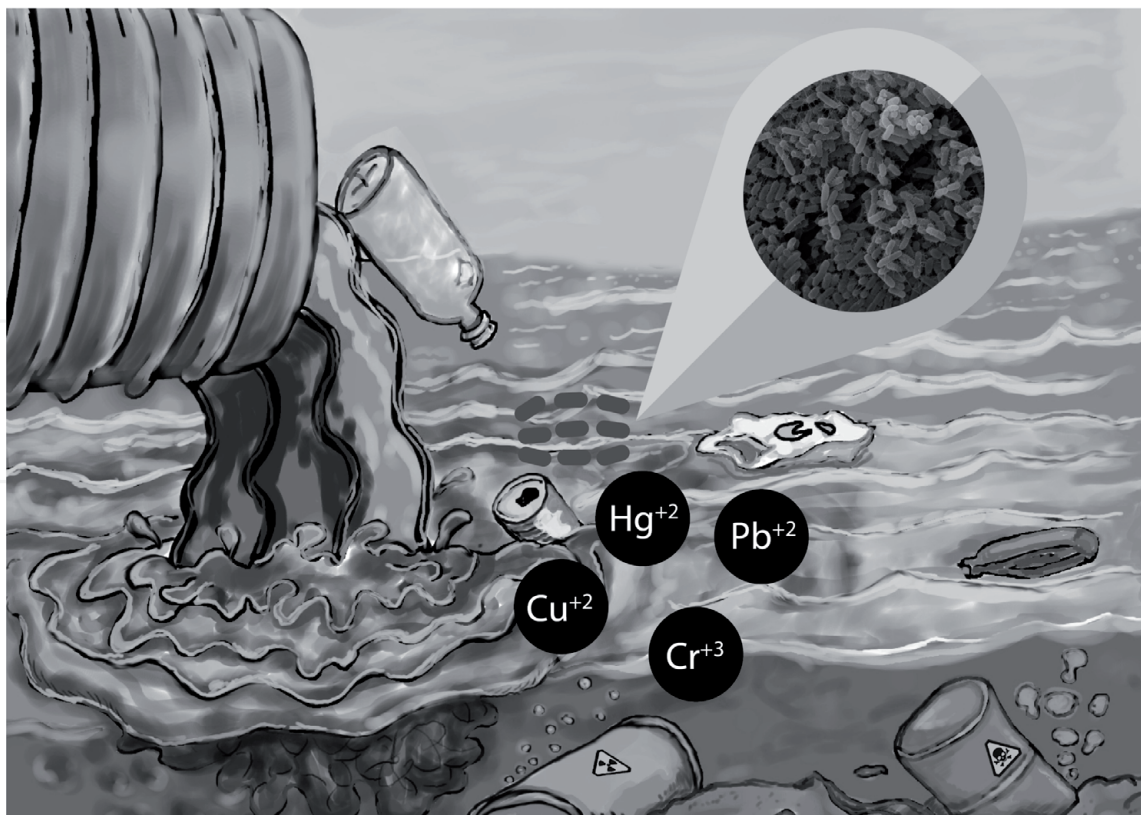


Figure 1.
Impacted aquatic environment and survival strategy of the present microorganisms.

the structure and function of microbial communities [25]. Aquatic systems may be introduced as a result of natural processes such as weathering, erosion, and volcanic eruptions [26]. However, in recent decades, the increase in urbanization and industrialization has contributed to the large increase of these environmental contaminants worldwide [27].

Thus, microorganisms have been developing various resistance mechanisms that allow their survival [6]. Among the various mechanisms, intra and extra-cellular, are bioaccumulation [28], biosorption [29], biomineralization and precipitation [30, 31], oxidation and enzymatic reduction of the metal to the less toxic form [32], production of siderophores [33], and biofilm formation [34]. **Figure 1** shows an impacted aquatic environment and a survival strategy for the microorganisms present there.

3. General characteristics of heavy metals

The term “heavy metals” is used to identify a group of chemical elements that have atomic density greater than 5 g cm^{-3} or have atomic number greater than 20 [35]. Some of these elements, such as sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), zinc (Zn), and copper (Cu), are essential microelements for various life forms, as they are necessary for the functioning of some metabolic pathways [36]. However, the excess or lack of these elements can lead to disturbances in organisms, and in extreme cases, even death [37]. Other elements such as mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) are highly toxic even when present in low concentrations, and account for most health problems due to environmental pollution [38].

Heavy metals participate in the global ecobiological cycle, derived from numerous sources and are dynamically transported through the atmosphere, soil, and

water; also, because they are not biodegradable, they can remain in the environment for long periods [39].

Among the various metals, mercury, cadmium, and lead stand out for being associated with contamination of the aquatic environment, which can cause problems of poisoning to man and other organisms. These elements are capable of reacting with molecules and ligands present in cell membranes, conferring them with the properties of bioaccumulation, food chain biomagnification, persistence in the environment, and metabolic disturbances of living beings [40].

4. Effects of heavy metals on biofilm

Biofilm is a porous and complex structure formed by one or more species of microorganisms, organized in several layers irreversibly adhered to a biotic or abiotic surface and enclosed in a matrix composed of extracellular polymeric substances (EPS) [9].

They are formed dynamically and gradually, involving several stages. The first is reversible bacterial adhesion that can occur on biotic surfaces mediated by molecular interactions or abiotic surfaces through physicochemical interactions. The second is irreversible adhesion, where the adhesion process is consolidated through the production of EPS. After the establishment and maturation of the protective matrix in the irreversible phase, the cycle ends with the rupture of the biofilm and the release of bacterial cells (**Figure 1**) [9, 41].

Bacteria in the form of free (planktonic) cells are not often found in nature; most of them live in communities or attached to various biotic or abiotic surfaces, such as clinical and industrial equipment. Several factors may contribute to bacterial adhesion such as flagella, fimbriae, adhesin, and polymers, as well as adhesion forces such as electrostatic and hydrophobic attraction, van der Waals interactions, hydrogen bridges, and covalent bond [10].

Biofilm formation is an effective strategy for microbial survival and persistence under stress conditions, such as in the presence of antimicrobials and heavy metals [42]. The biofilm structure may be associated with a protective mechanism that allows the bacteria to survive and persist in environments with high metal concentrations [43]. Studies have shown that subinhibitory heavy metal concentrations can induce biofilm formation [44, 45], like lead [46], cadmium [47], and nickel [48] among others.

Giovanella et al. [46] evidenced the increase in formation by an isolate of *Pseudomonas* sp. in the presence of mercury (Hg^{2+}). Similarly, Araújo et al. [49] verified an increase in biofilm formation in *Klebsiella pneumoniae* isolates obtained from an impacted urban stream. However, other studies show that depending on the metal and its concentration, biofilm formation may be reduced [50, 51]. These differences may be related to the fact that the effects of metals depend on their concentration and speciation [47, 51, 52], growth conditions, and especially the bacterial isolate that is being exposed [53, 54].

Recent studies have shown that metals can affect various stages in biofilm formation and development [55]. Metals can impact cell surface adhesion and/or cell-to-cell aggregation process, promoting biofilm formation and, consequently, its resistance. Harrison et al. [56] verified that the increase in cadmium concentration induces cell adhesion and biofilm formation in *Rhizobium alamii* YAS34. Subinhibitory concentrations of manganese (Mn) and zinc (Zn) affected cell aggregation in *Xylella fastidiosa* isolates. Mn increased the process of biofilm formation in this bacterium, while Zn impaired this process probably by reducing cell adhesion on the surface [50, 57]. Perrin et al. [48] observed that some isolates of *Escherichia coli* K-12 formed biofilm

in response to subinhibitory nickel (Ni) concentrations and that cells embedded in the biofilm were less affected by metal exposure than planktonic cells. These studies show that bacterial cells exposed to metals generally respond by inducing adhesion processes, and consequently, biofilm formation and maintenance [55].

In addition to changes in cell adhesion, exposure to heavy metals may cause structural changes in the biofilm extracellular polymeric substance (EPS) matrix. Araújo et al. [49] verified by scanning microscopy, the increase of EPS in *K. pneumoniae* biofilms formed when exposed to subinhibitory mercury concentrations (Hg^{2+}). Sheng et al. [58] also demonstrated that heavy metals stimulate EPS production in *Rhodopseudomonas acidophila*. Schue et al. [59] observed in *R. alarii* isolates the formation of a more condensed biofilm in the presence of subinhibitory concentrations of Cd when compared to isolates not exposed to this metal. The increase of extracellular matrix in *Thiomonas* sp. subinhibitory concentrations of arsenic (III) possibly contributed to biofilm integrity and physiological heterogeneity of immobilized cell subpopulations [60].

In stabilized biofilm, the presence of metals impacts cells via passive processes by the influence of gene expression, resulting in mechanisms of resistance or tolerance to these pollutants [55]. Extracellular polymeric matrix (EPS) acts as a barrier to toxic metals, which can be sequestered, immobilized, mineralized, and precipitated, diminishing their effect on bacteria [61]. In *Pseudomonas putida* ATCC 33015, sugars present in the biofilm matrix exposed to chromium (Cr) probably facilitated the immobilization process of this metal [62]. The biomineralization process was described in *Cupriavidus metallidurans* CH34, which was able to form gold (Au) nanoparticles in biofilm through the reduction and precipitation mechanism of the toxic gold complex (Au III) [63].

5. Heavy metal resistance

Environmental contamination by heavy metals has been increasing in recent years, due to various anthropogenic activities. Heavy metals, because they are not biodegradable, have a tendency for biomagnification and bioaccumulation and are extremely toxic to various biological functions, causing serious impacts on the environment and human health [64].

Microorganisms present in contaminated environments have developed different resistance mechanisms to adapt to stress caused by heavy metals. The ability to survive under these extreme conditions depends on acquired biochemical and physiological attributes, as well as genetic adaptations [65].

Several studies suggest that metal contamination in the natural environment may play an important role in maintaining and proliferating antimicrobial resistance (**Table 1**) [67–69]. In the environment, selective pressure exerted by metals may select resistant isolates similar to antibiotics, since both resistance genes are often located on the same moving elements [70, 71].

Bacteria develop some mechanisms to neutralize mercury toxicity, the most common being enzymatic reduction of the highly toxic mercuric ion (Hg^{2+}) to the volatile and less toxic elemental mercury (Hg^0). This reduction is catalyzed by the cytosolic mercury reductase (MerA) enzyme encoded by a gene belonging to the operon mer. Studies have shown the frequent association between operon mer and antimicrobial resistance [66, 72]. Péres-Valdespino et al. [73] demonstrated that several clinical isolates of *Aeromonas* sp. that presented the merA gene were resistant to different antibiotics such as tetracycline, trimethoprim, nalixidic acid, and streptomycin. Araújo et al. [49] verified, when comparing isolates of *K. pneumoniae*, that the isolate that presented the merA gene was resistant to the highest number of antimicrobials and presented the minimum inhibitory concentration

Resistance mechanisms	Heavy metals	Antibiotics	References
Reduction in permeability	As, Cu, Zn, Mn, Co, Ag	Cip, Tet, Cholr, β -lactâmicos	[32, 74]
Drug and metal alteration	As, Hg	β -lactâmicos, Chlor	[75, 76]
Drug and metal outflow	Cu, Co, Zn, Cd, Ni, As	Tet, Chlor, β -lactâmicos	[77, 78]
Cell signaling change	Hg, Zn, Cu	Cip, β -lactâmicos, Trim, Rif	[79, 80]

Abbreviations: Cholr, chloramphenicol; Cip, ciprofloxacin; Rif, rifampicin; Tet, tetracycline; Trim, trimetropim. Adapted from Baker-Austin et al. [66].

Table 1.
Examples of characteristics and negative effects on metal and antibiotic resistance mechanisms.

(MIC) value up to four times higher than the others, suggesting a co-resistance mechanism for mercury and antimicrobials tested.

Martins et al. [81] observed that isolates of *P. aeruginosa*, obtained from a contaminated river in southeastern Brazil, had a conjugative plasmid with co-resistance to tetracycline and copper, reinforcing that resistance to antibiotics may be induced by selective pressure of heavy metals in the environment. Caille et al. [82] demonstrated that in *P. aeruginosa*, copper can induce imipenem resistance by the CopR-CopS two-component regulatory system mechanism. Ghosh et al. [83] verified resistance to ampicillin, arsenic, chromium, cadmium, and mercury in *Salmonella abortus equi* isolates and observed that after removal of the plasmids, isolates became sensitive to these compounds.

In order to corroborate the evidence of co-resistance of metals and antibiotics, some studies compared the resistance profiles of bacteria collected in contaminated and uncontaminated environments. Rasmussen and Sørensen [84] demonstrated an increase in the occurrence of conjugative plasmids at contaminated sites and found that the mercury and tetracycline resistance genes were located on the same plasmid. Mcarthur and Tuckfield [85] examined metal and antibiotic resistance profiles in contaminated and uncontaminated stream sediments and found that isolates obtained from the contaminated sediment were more resistant to kanamycin and streptomycin than the others.

Thus, not only the indiscriminate use of antibiotics but also environmental contamination by heavy metals can pose risks and harm to human health, as resistance genes can be transferred horizontally from environmental microorganisms to human diners [66].

6. Conclusions

Increased urbanization and industrialization have contributed to heavy metal contamination in aquatic ecosystems, modifying the structure and function of microbial communities. The ability of microorganisms to survive under stress conditions, such as in the presence of heavy metals, depends on structural and biochemical attributes, as well as physiological and/or genetic adaptations. The studies cited demonstrated that the presence of heavy metals influences at different stages of biofilm formation. Additionally, the correlation between resistance to metals and antimicrobials was demonstrated, showing the environmental impact that these contaminants can cause in aquatic environments.

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