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Chapter 4

Organic Matter Biodegradation by Bacterial Consortium under Metal Stress

Simone Pennafirme, Inaya Lima, José Bitencourt, Mirian Crapez and Ricardo Lopes

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http://dx.doi.org/10.5772/60980

Abstract

Organic matter biodegradation proceeds via multiple enzymatic reactions, involving different oxidants as well as a number of intermediate compounds. Microbial reworking of organic matter can result in a substantial microbial contribution to the total organic matter pool. The investigations of the mechanisms, which can alter the microbial metabolism in marine sediments, are essential for understanding diagenetic processes, especially at those environments with toxic metal concentrations. Metals can bind with cells components, affecting their functioning. Consequently, the organic matter oxidation in the cellular metabolism may be affected. By contrast, the carbon sources are discriminated between labile and refractory organic compounds. The labile portion of organic matter mainly consists of biopolymers and includes carbohydrates, lipids, and proteins. The aim of this chapter is to present the main results of 10 years of studies regarding the organic matter oxidation by bacterial consortia under toxic metal levels on a tropical estuarine environment surrounded in part by mangrove areas. As the main find, the chronic dominance of lipids and carbohydrates at estuaries and mangroves systems may change the bacterial trophic state from aerobic to anaerobic metabolism. This alteration may reflect on decreasing both bacterial efficiency of organic matter degradation and bacterial productivity. Further, when these systems show high levels of metals at the sediment, the metabolic efficiency is even lower because, although bacteria consortia is able to produce extracellular polymeric substances (EPS) as defense mechanism, multimetal contam-
ination may hinder bacterial organic matter oxidation through dehydrogenase activity inhibition.

**Keywords:** Organic matter, biopolymers, bacterial consortium, dehydrogenase, metals

## 1. Introduction

Bacteria are cosmopolitan unicellular microorganisms that show great metabolic capacity, allowing them to live in a wide range of environments [1,2]. Although bacteria size can range between 0.2 and 2.0 μm [2,3], their tiny body do not limit their main features such as rapid growth, metabolic versatility, genetic plasticity, and ability to rapidly adapt to environmental changes [1,2]. These features allow a wide earth colonization by prokaryotes, estimated of 4–6 × 1030 cells [4], influencing biomass production with an amount of 350–550 Pg carbon (1 Pg = 1015 g). Thus, they correspond to 60%–100% of all carbon produced by plants [4] and comprise 30%–50% of the particulate matter on sea [5]. Furthermore, they are the main producer in some estuarine regions [1] and show capacity to live in places with recalcitrant elements, like metals and industrial effluents [6,7].

Bacteria play an important role in recent diagenesis of organic matter and on nutrient recycling [8–11]. Originally, the organic matter content is naturally occurring as organic compounds such as carbohydrates, amino acids, polypeptides, pigments, phenolic substances, lipids, and other constituents of living organisms [12,13]. During the sedimentation process, only a small portion of the initial organic matter reaches the bottom sediments because pelagic organisms can easily take nutrients and food from organic matter in this process, being left refractory elements in higher proportion [12]. Some microorganism can act transforming highly resistant enzymatic hydrolysis elements (refractory elements) into polypeptides or fatty acids [14] along diagenesis [15–19].

Furthermore, bacteria can enhance organic matter quality, transforming refractory elements (e.g., cellulose) into organic nitrogen compounds (e.g., ammonia, nitrate, and proteins) and vitamins [20]. During diagenesis, bacteria play an important role on biogeochemical process of metals, by transforming inorganic to organic forms or by recycling them due to energy production [15–19]. In this sense, bacteria are important link between biogeochemical cycles and environment, by taking part in recycling nutrients that pass through the ecosystems, especially those rich in organic matter.

### 1.1. Biochemical pathways of microbial organic matter biodegradation

The benthic degradation of organic matter proceeds via multiple enzymatic reactions involving different organisms and oxidants as well as a number of intermediate compounds. Although microbial biomass is only a small fraction of total organic matter in sediments, the continuous processing of organic matter by benthic microbes, combined with turnover of
microbial biomass, results in a continuous flux of microbial detritus into the sediment organic matter pool. In this way, microbial reworking of organic matter can result in a substantial microbial contribution to the total organic matter pool and may also alter its composition and thereby change its long-term fate [21]. Furthermore, aerobic and anaerobic biogeochemical cycles driven by microbial processes in marine sediments are essential for understanding diagenetic processes [22,23]. These diagenetic mechanisms play a fundamental role on, among others, the recycling of inorganic carbon and nutrients, the flux of organic carbon, and ultimately the burial of organic carbon in the sedimentary record.

The microbial community exerts an important influence on the degradability of organic matter. Microbes can respond to chemical changes across millimeter distances in marine sediments, thus altering the subsurface chemistry of those sediments, and the chemical changes are transmitted through sedimentary layers [24]. In this sense, the degradation of the deposited material is thermodynamically and/or kinetically controlled by the different abilities of the physiological groups that compete for the common substrate. In cases where the molecular weight of the substrate is equal or higher than 600 Da, bacteria may release extracellular enzymes, such as esterases. These enzymes reduce the size of the molecules by hydrolysing ester bond, allowing the entrance of intracellular carbon source [25].

Organic matter oxidation is coupled to the sequential utilization of terminal electron acceptors, typically in the order of \( \text{O}_2 \), \( \text{NO}_3^- \), \( \text{Mn}(\text{VI}) \), \( \text{Fe}(\text{III}) \), and \( \text{SO}_4^{2-} \) followed by methanogenesis and/or fermentation. Depending on the degradation pathway, organic matter is directly oxidized to \( \text{CO}_2 \) partly oxidized to intermediate compounds, or reduced to \( \text{CH}_4 \) [20, 26, 27].

Marine sediments typically become anoxic because the bacteria within them consume oxygen as it diffuses down from the overlying water. This process establishes a series of sedimentary layers, within which a different kind of molecule (or ion) accepts electrons from oxygen and is consumed to form a chemically reduced product. In one of the lower layers, sulfate (\( \text{SO}_4^{2-} \)) is converted to highly toxic hydrogen sulfide (\( \text{H}_2\text{S} \)), which accumulates and poisons the environment for most oxygen-consuming microorganisms. The hydrogen sulfide can, in turn, be converted into sulfate or other oxidized forms of sulfur by bacteria that use inorganic material as an energy source [24]. The sulfate-reducing bacteria do not require low \( \text{H}_2 \) concentration for their growth, which gives them a thermodynamic advantage. Besides, in absence of sulfate, they can use fermentative or acetogenic pathways to produce energy. They are the main competitors of methanogenic bacteria for the electron donor, such organic matter and \( \text{H}_2 \), and they release hydrogen sulfide as an excretion, which is an important methanogenesis inhibitor. The methane formation is performed through chemosynthesis by methanogenic Archaea that fix \( \text{CO}_2 \) and excrete methane. The same microorganisms can reduce nitrate and iron oxides, with a preference of nitrate. The reduction of iron oxides, sulfate, and carbon dioxide is made by different physiological groups of bacteria with specific affinities for electron donors such as acetate and hydrogen. Thus, the reduction of iron oxides occurs preferentially to the reduction of sulfate and carbon dioxide [28].

Nitrogen is the fourth most common element found in cells and includes the microbially catalyzed processes of nitrogen fixation, ammonium oxidation, assimilatory and dissimilatory
nitrate reduction, ammonification, and ammonium assimilation. The biological nitrogen removal is achieved by nitrification followed by a denitrification process, i.e., (i) aerobic nitrification of \(\text{NH}_4^+\) by chemolithoautotrophic bacteria to \(\text{NO}_2^-\) or \(\text{NO}_3^-\) with \(\text{O}_2\) as the electron acceptor, and (ii) anoxic denitrification of \(\text{NO}_2^-\) or \(\text{NO}_3^-\) to gaseous \(\text{N}_2\) by heterotrophic microorganisms using organic matter as carbon and energy source [29].

Manganese acts as a catalyst that shapes chemical gradients in the oxygen-deficient zones because it exists in multiple oxidation states and is recycled rapidly between these states by bacterial processes. These transformations serve as an electron-transfer system for other chemical cycles. The manganese may play a dominant role as a catalyst in chemical cycles like \(\text{HS}/\text{O}_2\) and the reduction of nitrate by \(\text{Mn(II)}\), and the oxidation of ammonia by \(\text{MnO}_2\). These cycles are thermodynamically favorable in suboxic marine environments [30].

Dissimilatory iron-reducing bacteria are a group of microorganisms that can oxidize organic matter and reduce \(\text{Fe(III)}\) to \(\text{Fe(II)}\) under anoxic conditions. They include both facultative and obligate anaerobes and can be classified as fermentative, photosynthetic, organic acid-oxidizing, and hydrogen-oxidizing bacteria [31].

### 1.2. Bacterial mechanisms of metal resistance and tolerance

Microorganisms can be resistant or tolerant to metal stress. While the resistance is the ability of a bacteria or bacterial consortium to continue growing in the presence of multimetal combination in a toxic level, the tolerance is their ability to survive at these conditions [32]. Although some metals are essential elements for organisms, playing a fundamental role on cellular functioning, they can become toxic depending on their intracellular concentrations. Metals are important switched elements, and the exposition and natural selection over thousands of years have made the association between organism and metal so close that bacteria need these elements in their biological process such as on proteins formation, enzymes function, and energy production [33, 34]. However, on environments with high metal concentrations, it may bind with nucleic acids, proteins, and enzymes, among others cells components, affecting their functioning [35]. Consequently, the organic matter oxidation in the cellular metabolism may be affected. Once the metal ions concentration are elevated intracellularly, they may bind with enzymes such as the dehydrogenases (DHA), which act on the oxidation–reduction reactions in the electron transport system activities (ETSAs) [36].

In this sense, cells may show regulatory mechanisms that control metals intracellular levels, keeping it at the minimal inhibitory concentrations values. One of these mechanisms is the production of a matrix of exopolymeric polymeric substances. The bacteria are embedded in a biofilm or an extracellular organic matrix that consists extracellular polymeric substances (EPS) produced by them. Generally, the EPS are composed of carbohydrates, heteropolysaccharides, proteins, and nucleic acids. Due to their negatively charged carboxyl and phosphoryl functional groups, EPS can bind with extracellular metals [37,38]. Thus, the EPS reduce the bioavailability and activity of the ionizable metal forms, such as \(\text{Pb, Cd, Co, Ni, Zn, and Cu}\), being a survival strategic against toxic flux of metals [37–40].
Once within the EPS, bacteria may form a complex consortium, with intra- and interspecific species [1,15,41]. The community helps bacteria to resist against environmental stress due to connection of different physiological features of these members. Consequently, they spend less energy on the competition for space, nutrients, or defense [9,10,29,42]. Due to intercellular communication throughout biofilm, the consortium becomes complex and multicellular, favoring syntrophic life, where two or more individuals combine their metabolic capacity or one species lives off the products of another species [41]. In this context, the consortia formation may help bacteria cells to colonize substrates [15,43], affecting the cycling of both organic matter and metals [15,18,19,43]. The bacterial consortium is more resistant toward multimetals than the pure cultures [44].

2. Aim

This chapter is going to present a temporal series compilation of the main results of 10 years of studies regarding the organic matter oxidation by bacterial consortia under toxic metal levels on a tropical estuarine environment surrounded in part by mangrove areas.

3. Methods

3.1. Study case

The sample sites of the study case presented in this chapter are located at the Guanabara Bay, Rio de Janeiro State, Brazil, between 22° 40′–23° 00′ S and 043° 00′–043° 18′ W. This bay is a complex tropical estuarine environment and one of the largest bays along the Brazilian coastline with an area of approximately 384 km². In the past, each of the 55 rivers might create a single estuary, each one distinct from the other [45]. These estuaries originally made the estuarine system of Guanabara Bay ecologically diverse [46]. However, human and industrial occupation caused the loss of the bay’s diversity and its natural characteristics, as suggested by data collected in [45, 47–49]

The drainage basin of Guanabara Bay has an area of 4080 km², and it comprises 32 separate subwatersheds [50]. However, although 55 rivers carry 4,000,000 t year⁻¹ of solid material [45,47], only 6 rivers are responsible for near 85% of the 100 m³ s⁻¹ total mean annual freshwater input [47]. Besides, these few rivers carry tons of untreated sewage directly into the bay [46,48].

Not only sewage but also hazard pollutants are carried and deposited in Guanabara Bay. Along its drainage basin and its surroundings, there are more than 12,000 industries and two oil refineries. Also, the bay is the homeport of two naval bases, which release large amounts of organic and inorganic pollutants into the subwatersheds [51]. To better analyse and compare the main features, the Guanabara Bay was divided in this present chapter into four main areas (Figure 1).
3.2. Sampling

At Guanabara Bay, the sediment samples were obtained using an Eckman sampler for the mud and a VanVeen grab sampler for the sand. Another sampling was performed at Suruí mangrove, located at the Bay background, inside the Guapimirim Environmental Protection Area (EPA). This mangrove area is situated in Magé Municipality and shows an area ranging of 80,000–100,000 m² [13]. Four sediment samples were collected in triplicate, using PVC soil cores (4.5 × 30.0 cm), and sliced in sections. The study of Suruí and surrounding site is important to understanding the dynamics of organic matter and metals.

3.3. Analysis

Total organic matter was estimated in triplicates by the calcinations method. Approximately 50 g of wet samples were dried to a constant weight and calcinated. Organic matter was determined as the differences between sediment dry weight (60°C, 24 h) and weight of the residue after combustion (450°C, 4 h) [52–54].
Concentrations of different sources of carbons (carbohydrate, lipids, and proteins) were determined in separate triplicates using 1 g of sediment samples. All determinations were done by spectrophotometric methods. Carbohydrates were quantified using glucose as a standard [55,56]. For lipid analysis, samples were extracted with chloroform and methanol, and tripalmitin was used as the standard [57]. Proteins were determined with standard of bovine albumin, fraction V (Sigma) [58,59]. The sum of protein, carbohydrate, and lipid carbon equivalents was referred to as biopolymeric carbon [60].

The bioavailable organic carbon (%) was determined according to the equation:

\[
\text{Bioavailable organic carbon (\%)} = \left(\frac{\text{total biopolymeric carbon}}{\text{total biopolymers}} \times 100\right)
\]

The unavailable organic carbon (%) was determined according to the equation:

\[
\text{Unavailable organic carbon (\%)} = 100 - \text{Bioavailable organic carbon (\%)}
\]

Esterase enzyme activity (EST) was based on fluorogenic compounds, which are enzymatically transformed into fluorescent products that can be quantified by spectrophotometric assay to [61]. These enzymes act on biopolymers and transform them into low-molecular-weight organic carbon. Triplicates of 1 g of each sample were analyzed. The results were expressed in l μg fluorescein h\(^{-1}\) g\(^{-1}\) of sediment. Electron transport system activity (ETSA) was based on dehydrogenase enzyme activities measurements [62,63]. These enzymes provide equivalents for ATP synthesis in the electron transport systems. Triplicates of 1 g of each sample were used. Results from this assay were expressed in μL O\(_2\) h\(^{-1}\) g\(^{-1}\) of sediment. Metabolic bacterial activity on sediment such as aerobic, facultative anaerobic, denitrification, and sulfate reduction was measured using triplicates of 1 g of each sample on assay tube and specific selective liquid medium [64].

Bacterial cell was enumerated by epifluorescence microscopy (Axiosp 1, Zeiss, triple filter Texas Red—DAPI—fluorescein isothiocyanate, 1000× magnification) and using fluorochromes such fluorescein diacetate or acridine orange and irradiated with UV wave [65].

For metal analyses, the selective extraction analysis was used with both shaking and heating techniques [66], where 0.500 g of prepared sample was weighed into acid washed polypropylene tubes and blanks were prepared by taking each extractant, without the sample through all the preparation procedures prior to analysis. Water-soluble ions were extracted using a modified version of the [67] technique, where a smaller sample weight (0.5 g) was extracted with a lower volume of deionized water (2.5 ml), diluted to 10 ml and membrane filtered (0.2 μm), prior to analysis. This extraction protocol was modified to include the organic phase to ensure better oxidation of organic matter (Lefort aqua regia). An elemental analysis was carried out using a Perkin Elmer Model 3100 atomic absorption spectrometer.

In order to investigate the metal retention by bacterial biofilm, samples of resistant bacterial consortia biofilm from bioassays were analyzed through X-ray fluorescence microscopy (XRF).
The XRF is a nondestructive semiquantitative method that allows to identify as well as to estimate the concentrations of the elements present in a multielemental sample at once analysis. It is based on the evaluation of the energies and intensities (number of detectable X-rays per unity time) of the characteristic X-rays (fluorescent photons) emitted by the elements that constitute the sample. Once a sample is irradiated with X-rays, its constitutive elements can emit fluorescent photons afterward. The element identification is possible because each element emits fluorescent photons at a given energy. Further, the energy intensity of these fluorescent photons is related to the element concentration at the sample [68]. The bacteria consortia used on bioassays were isolated from metal contaminated mangrove sediments. The bacterial consortia resistant to zinc was incubated at 37°C for 15 days in a liquid medium (sea water, yeast extract (2 g L\(^{-1}\)) and urea (2 g L\(^{-1}\)) with 50 ppm of sulfate of zinc). The bacterial consortium resistant to cupper was cultivated at same condition but with 50 ppm of sulfate of cupper. As a control, other two cultures with respective consortia were prepared without metal. After 10 days of incubation, 5 ml of each culture was vacuum filtered through a membrane filter 0.22 μm, fixed on a support and positioned on a XZ table controlled by a microcomputer. These analyses were performed through synchrotron X-ray fluorescence microscopy (microXRF) at the Synchrotron Light National Laboratory (LNLS), São Paulo State, Brazil (HPGe detector, resolution of 150 eV at 5.9 keV, white beam, pixel size of 20 μm diameter, 45°/45° geometry). To confirm the biofilm formation, the membrane filters were analyzed through scanning electron microscopy (SEM; Jeol, JSM6460 model; LV, Japan; EDS: Noran System Six; 20 kV).

4. Results and discussion

Changes in the trophic state of the sediments can be more evident in terms of labile organic matter composition (proteins, carbohydrates, and lipids) than in terms of organic matter/organic carbon concentrations [60]. The labile organic matter is the carbon source for benthic organisms. The oxidation of organic matter, which is an overall estimate of metabolism (aerobic and anaerobic), can be obtained by measuring the activity of the dehydrogenase enzymes. However, generally, at polluted environments, the cell exposure to metal ions results in decrease or inhibition of dehydrogenase activity. Thus, heavy metals distribution within sediments results from diagenetic processes linked to organic decomposition [69].

In this context, in order to investigate the organic matter oxidation by benthic marine bacteria exposed to metal stress, the dehydrogenase activity, biopolymer concentrations, and cell number were tracked from 2003 to 2011 at Guanabara Bay (Tables 1 and 2). As the central result, at first glance, the ETSA was maintained at high levels, despite high metal concentrations, indicating the presence of tolerance or resistance mechanisms by bacterial consortia. Bioassays showed that EPS formation and metal retention by biofilm can be one of these strategies. However, afterward, both cell number and ETSA decreased along the years. Meanwhile, the input of organic matter and metals continues to rise. In addition, the anaerobic metabolism was characterized as the main process of organic matter degradation. Once the anaerobic metabolism is less efficient than aerobic, the observed decreased on ETSA may be a
consequence of the also observed decrease on the metabolic rate, which is at least one order of magnitude on the bacterial cell numbers. The chronic sewage discharge and the metal release in the Bay are contributing to changes on the bacterial community physiology and biomass, bringing serious consequences for energy production.

In 2003, at Boa Viagem Beach, located at the east margin of the entrance of Guanabara Bay (area 4), seasonal variations were observed on ETSA activity. During summer, bacteria reached $10^8$ cells, ETSA achieved highest level (7.48 $\mu$mol O$_2$ h$^{-1}$ g$^{-1}$), followed by the same pattern for organic matter (1.764 g g$^{-1}$ of sediment). The mean metal concentrations evaluated are presented in Table 1. At this point, although the metal concentrations were already higher than natural background, these levels were not sufficient to inhibit the dehydrogenase activity, and thus, the bacteria were able to continue oxidizing organic matter [54].

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Organic matter</th>
<th>ETSA (µmol O$_2$ h$^{-1}$ g$^{-1}$)</th>
<th>EST (µg of fluorescein h$^{-1}$ g$^{-1}$)</th>
<th>Pb (ppm)</th>
<th>Ni (ppm)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Cr (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Boa Viagem Beach</td>
<td>1.764 g g$^{-1}$ of sediment</td>
<td>7.48</td>
<td>28</td>
<td>23</td>
<td>8</td>
<td>24</td>
<td>16</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Jurujuba Sound</td>
<td>-</td>
<td>3.02-3.38</td>
<td>3.14-3.63</td>
<td>84</td>
<td>63</td>
<td>241</td>
<td>299</td>
<td>116</td>
<td>[70]</td>
</tr>
<tr>
<td>2005</td>
<td>Guanabara Bay</td>
<td>5.62 %</td>
<td>1.25-4.69</td>
<td>0.047-0.366</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[72,73]</td>
</tr>
</tbody>
</table>

Table 1. Bacterial metabolic activity and metal concentrations at Guanabara Bay, Rio de Janeiro State, Brazil.

Two years later, 15 samples were collected at Jurujuba Sound, located at east margin of Guanabara Bay (area 4), including Boa Viagem Beach. Jurujuba Sound is a harbor, and the high metal levels found over there were already expected. Both ETSA and EST evaluations were lower than the previous study at Boa Viagem (Table 1). Such results indicated that, despite the high levels of metals within sediments, bacterial DHA activity decreased but was not inhibited, and the microbial community may show some tolerance or resistance mechanism against metals [70].

In order to investigate the tolerance and resistance through metal retention by bacterial biofilm, bioassays and X-ray fluorescence analysis were performed, and results showed that even under elevated metal concentrations (Cu and Zn; 50 ppm), bacteria consortia isolated from polluted mangrove sediments were able to form biofilm, which, in its turns, was able to retain the metals (Figure 2) [71].

Once bacterial biofilm has the capability to adsorb metal through chemical interactions with its constituents [32], resistant bacteria consortia from metal contaminated mangroves are able to use this adaptation to bind metals and consequently to avoid, or at least to hinder, the metal negative effects on their metabolisms, allowing them to oxidize organic matter. Furthermore, the enzymatic analysis showed that the dehydrogenase activity was not affected in the presence of zinc (50 mg L$^{-1}$), and a dense biofilm with zinc superposing on it was observed.

http://dx.doi.org/10.5772/60980
By contrast, although the presence of copper hindered the activity of dehydrogenases during the bacteria growth's lag phase, the enzyme activity increased afterward, following the exponential growth phase, but with lower magnitude when compared with the control bioassay (without copper). The presence of biofilm was also observed with superposition of copper, however, in less amounts than zinc bioassays. Without a dense biofilm as a protection against metal encounter, dehydrogenases activity decreases. However, as biofilm grows, dehydrogenase activity increases at higher rates during exponential growth, similar to the event observed for zinc bioassay. In this sense, biofilm may be acting both as metal sequestering as protector of bacterial cells embedded in the exopolymeric matrix at mangrove polluted sediments [71].

In 2005, 30 sampling stations were distributed all over the Guanabara Bay, including sampling stations near to Guapimirim mangrove preservation area. The highest levels of organic matter were found in area 1, while the high population concentration was observed at area 2 close to the Guapimirim mangrove. Both EST and ETSA were evaluated at 1.25 to 4.69 μg of fluorescein h⁻¹ g⁻¹ and 0.047 μL O₂ h⁻¹ g⁻¹ to 0.366 μL g⁻¹ h⁻¹, respectively. The microbial community was characterized through the following anaerobic mechanisms: fermentation, denitrification, and sulfate reduction. The bacterial consortia formed by sulfate-reducing and sulfate-denitrifying bacteria sustain the benthic trophic food web in Guanabara Bay, mainly in the regions near to mangrove areas (2 and 4). The anaerobic bacterial metabolism, besides producing organic acids and sulfate and releasing nitrogen to the atmosphere, usually produces less intracellular energy than aerobic organisms, generating, on a macroscopic scale, a low carbon and nutrient cycle in the anoxic sediment [72,73].

Regarding the carbon sources available for microorganisms, the organic matter quality means discriminating between labile and refractory organic compounds [78]. The labile portion of organic matter mainly consists of biopolymers and includes carbohydrates, lipids, and proteins, which is available to feeding strategies and the distribution of benthic organisms [75,76].
In this context, in order to investigate the carbon sources present at the Guanabara Bay, studies performed in 2005 found highest values of organic matter and biopolymers (proteins, carbohydrates, and lipids) near the Guapimirim protected mangrove (area 2) (Figure 1, Table 2) [75,76].

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Organic matter (%)</th>
<th>Carbohydrate (mg.g(^{-1}))</th>
<th>Lipid (mg.g(^{-1}))</th>
<th>Protein (mg.g(^{-1}))</th>
<th>Biopolymeric carbon (mg.g(^{-1}))</th>
<th>Bioavailable carbon (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Guanabara Bay area 1 (sewage input)</td>
<td>5.83-7.06</td>
<td>0.83-1.5</td>
<td>0.6-1.7</td>
<td>0.04-0.06</td>
<td>0.84-1.70</td>
<td>56.8-62.3</td>
<td>[75,76]</td>
</tr>
<tr>
<td>2005</td>
<td>Guanabara Bay area 2 (Guapimirim mangrove)</td>
<td>0.59-6.68</td>
<td>0.210-1.20</td>
<td>0.07-0.50</td>
<td>0.02-0.11</td>
<td>0.19-0.80</td>
<td>46.6-50.3</td>
<td>[75,76]</td>
</tr>
<tr>
<td>2005</td>
<td>Guanabara Bay area 3</td>
<td>0.73-6.13</td>
<td>0.55-1.13</td>
<td>0.17-1.23</td>
<td>0.03-0.11</td>
<td>0.30-1.43</td>
<td>49.6-60.4</td>
<td>[75,76]</td>
</tr>
<tr>
<td>2005</td>
<td>Guanabara Bay area 4</td>
<td>2.10-6.2</td>
<td>0.61-1.11</td>
<td>0.33-0.62</td>
<td>0.02-0.06</td>
<td>0.52-1.00</td>
<td>52.10-52.9</td>
<td>[75,76]</td>
</tr>
<tr>
<td>2008</td>
<td>Guanabara Bay (Suruí mangrove)</td>
<td>1.3-4.0</td>
<td>0.3</td>
<td>0.40</td>
<td>0.13-0.27</td>
<td>-</td>
<td>31-75</td>
<td>[13]</td>
</tr>
<tr>
<td>2011</td>
<td>Guanabara Bay area 2 (Guapimirim mangrove)</td>
<td>-</td>
<td>0.12-12.20</td>
<td>0.04-12.78</td>
<td>0.02-0.89</td>
<td>-</td>
<td>31-75</td>
<td>[78]</td>
</tr>
</tbody>
</table>

Table 2. Biopolymer concentrations at Guanabara Bay, Rio de Janeiro State, Brazil.

In 2008, investigations at the mangrove located at northwest of Guanabara Bay (Suruí mangrove) showed similar characteristics from surrounding polluted area (Table 2). The organic matter varied from 1.3% to 4%, with the largest values top layers of cores and highest values for biopolymeric organic carbon (0.13–0.27 g g\(^{-1}\)). The higher biopolymer concentration was found in the upper layer of sediments. The carbohydrates showed relative highest level (0.3 g g\(^{-1}\)) near Guapimirim mangrove (site 2). Proteins spotted the second highest level with the highest concentration at site 2, with 0.40 g g\(^{-1}\). The microbial metabolism was anaerobic (fermentation, denitrification, and sulfate reduction), with high bacterial population on up sediment layer hydrolyzing organic matter biopolymers, with high esterase enzyme activity and less ETSA. Due these characteristics, this mangrove promotes the formation of pyrite and metal retention, which in it turns contributes decreasing the metal availability to microorganisms and therefore to the other organisms [13,77].

Most recently, in 2011, metal and biopolymers concentrations were evaluated at Guanabara Bay. Comparing with the earlier studies, biopolymers concentration increased in ten times at the bay. High biopolymers values were found again at area 2, near to Guapimirim mangrove. Proteins concentrations showed also lowest values (Table 2). Highest reactive metal concentrations were found associated with finest sediments, high organic content from sludge on areas 1 and 3, and near entrance of the bay, on Jurujuba Sound. The mean bioavailable carbon was evaluated on 53% ± 22%. High metal levels were found in areas with high organic matter content and fine sediments [78].

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http://dx.doi.org/10.5772/60980
The anthropogenic activities, interacting with the complexity of the natural environment, may undermine the distribution of nutrients [79], microbial physiology [54,72,78,80], physical parameters [81], and trace elements [82] in marine environments, as well as biological resistance to the pollution [83,84].

5. Conclusions

The chronic dominance of lipids and carbohydrates, mainly due to sewage discharge at estuaries and mangroves systems, may change the bacterial trophic state from aerobic to anaerobic metabolism. This alteration may reflect on decreasing both bacterial efficiency of organic matter degradation and the bacterial productivity. Although the high levels of metals at the sediment, bacteria consortia are able to produce EPS as defense mechanisms. However, although the use of defense mechanisms against metals, the multimetal contamination may still hinder bacterial organic matter oxidation. Thus, the decrease in organic matter biodegradation due to toxic metal levels summed to the less efficient anaerobic metabolisms contributes to degenerating the diversity of microbial benthic communities.

Acknowledgements

The authors would like to acknowledge CAPES-Ministry of Education/Brazil for scholarship, CNPq and LNLS—Ministry of Science and Technology/Brazil for research funding.

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Organic Matter Biodegradation by Bacterial Consortium under Metal Stress
http://dx.doi.org/10.5772/60980


