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Microbiological monitoring in two areas with different levels of conservation in the mangroves of an Ecological Station, Vitoria, ES

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ABSTRACT. Mangroves are classified as permanent preservation areas and regarded as natural nurseries. However, they have suffered several anthropogenic stresses, resulting in their decline. In the light of that, comes the importance of researching their environmental characteristics and revealing possible factors that have led to the degradation of this important ecosystem. The aim of this study was to evaluate the environmental quality of different areas in the mangroves of Ilha do Lameirão Ecological Station through microbiological analyzes of sediment and interstitial water along ten (10) sites, distributed in two areas with different conservation levels (Canal dos Escravos (CE) and Maria Ortiz (MO)) between 2010 and 2012. The microbiological analyzes revealed that MO region, in all seasons of the year, achieved total coliform and thermo-tolerant coliform values above those permitted by the CONAMA Resolution 357/05, fitting the Class 2 conservation standard. The presence of high levels of total and thermo-tolerant coliforms in MO is a strong indicator of impacts originated from the human population and, consequently, the decline of the mangrove itself and the health of human communities surrounding that area.

Keywords: coliforms, thermo-tolerant, mangroves, Vitoria bay.

Monitoramento microbiológico em duas áreas com diferentes níveis de conservação nos manguezais de uma Estação Ecológica, Vitória, ES

RESUMO. Os manguezais são classificados como áreas de preservação permanente e considerados como berçários naturais, no entanto sofrem várias tensões antropogênicas, resultando em seu declínio. À luz disso, evidencia-se a importância de pesquisar suas características ambientais e reveladoras de possíveis fatores que levaram à degradação desse importante ecossistema. O objetivo deste estudo é avaliar a qualidade ambiental das diferentes áreas em manguezais da Estação Ecológica Ilha do Lameirão por meio de análises microbiológicas de sedimentos e da água intersticial ao longo de dez locais, distribuídos em duas áreas com diferentes níveis de conservação (Canal dos Escravos (CE) e Maria Ortiz (MO)) entre 2010 e 2012. As análises microbiológicas revelaram que a região MO, em todas as estações do ano, obteve valores de coliformes termo-tolerantes e totais acima do permitido pela resolução CONAMA 357/05, Classe 2. A presença de níveis elevados de coliformes totais e termo-tolerantes em MO é um forte indicador dos impactos originados da população humana e, consequentemente, do declínio do próprio mangue e da saúde das comunidades humanas que cercam a área.

Palavras-chave: coliformes, termo-tolerantes, manguezais, baía de Vitória.

Introduction

The mangrove ecosystem is one of the world's most productive ecosystems found as a transition zone between the sea and river hosting a wide range of flora and fauna. The mangrove area is rich in organic matter influencing a positive growth of many microbes (Grisi & Gorlach-Lira, 2010). The mangroves from Espirito Santo State (ES) are distributed from the mouth of river Doce, in the state's far north, to Itabapoana river, on the border of Rio de Janeiro State (RJ), covering approximately 70 km² (Vale & Ferreira, 1998). In these areas some mangroves are well-preserved from anthropogenic influence, as in São Mateus river, while others have suffered more from anthropogenic impacts, as the example of the Victoria bay (Carmo, Almeida, Oliveira, & Zanotti-Xavier, 1998). Such disturbances are mainly due to the urbanization, road construction, landfills, dredging, pipelines placement, extraction of different primary resources and wastewater discharges. Regarding Physiographic Environmental Units, the State of Espírito Santo is within the VI unit, which extends from the Recôncavo Baiano (13° 00'S) to Cabo Frio (23° 00'S), whose mangroves are commonly found next to sandbank vegetation, with extensive distribution areas (Herz, 1991).

In the northwest of Vitoria there is a Municipal Ecological Station named Lameirão Island (EEMIL), where the mangrove covers approximately 92.66% of the Ecological Station, occupying an area of 891.83 hectares and harboring three mangrove plant species: *Rhizophora mangle* (red mangrove), *Laguncularia racemosa* (white mangrove) and *Avicennia schaueriana* (black mangrove or siriuba). However, despite being an Ecological Station, the anthropogenic influence is increasing on a large scale and the mangrove becomes more fragile. The main reason for that comes from landfills for construction of riverside homes, which increases the flow of sewage from houses to the estuary, as happens in Maria Ortiz (MO) region and areas that have high vulnerability to disturbance as in the Canal dos Escravos (CE).

Among the research needs and assessment of environmental quality for the Mangroves is the microbiological monitoring, that aims to obtain basic information that will help future discussions on the health of the mangrove ecosystem, in addition to possible adverse effects related to anthropogenic tensioners that can affect health of animals and humans alike.

Thus, the aim of this study was to evaluate the quality of environmental microbiological standards in different areas of the EEMIL mangrove, according to their vulnerability to anthropogenic influence (CE/ MO) by microbiological analysis.

Material and methods

Study area

Ten sites were chosen along the EEMIL, the first five (sites 1-5) were located in a highly impacted area, which receives a discharge of effluents from a sewage treatment plant, located around the MO neighborhood. The other 5 sites (6-10 sites) were located in an area with much less human presence, away from any effluent discharges (approximately 2.5 km away from MO), located in the CE in the city of Vitoria (Table 1) (Figure 1). In addition, our research was authorized by Vitoria City Hall, as stated in Case No. 4053546/2013 - Environmental Department (SMA), Espirito Santo State.

Table 1. Description of sampling sites for analysis of water quality in EEMIL, Vitoria, ES.

Points	Sampling sites	Coordinates geographical
1	Maria Ortiz (next to the sewage launching points)	20°15'07.02"S 40°17'38.44"W
2	Maria Ortiz	20°15'05.71"S 40°17'44.28"W
3	Maria Ortiz	20°15'08.84"S 40°17'50.71"W
4	Maria Ortiz	20°15'10.17"S 40°17'57.98"W
5	Maria Ortiz (next to the sewage launching points)	20°15'15.12"S 40°18'02.10"W
6	Canal dos Escravos (next to the Apicum)	20°14'35.59"S 40°19'13.89"W
7	Canal dos Escravos	20°14'40.69"S 40°19'15.44"W
8	Canal dos Escravos	20°14'45.48"S 40°19'16.44"W
9	Canal dos Escravos	20°14'50.68"S 40°19'17.55"W
10	Canal dos Escravos (mouth to the bay)	20°14'54.52"S 40°19'18.99"W

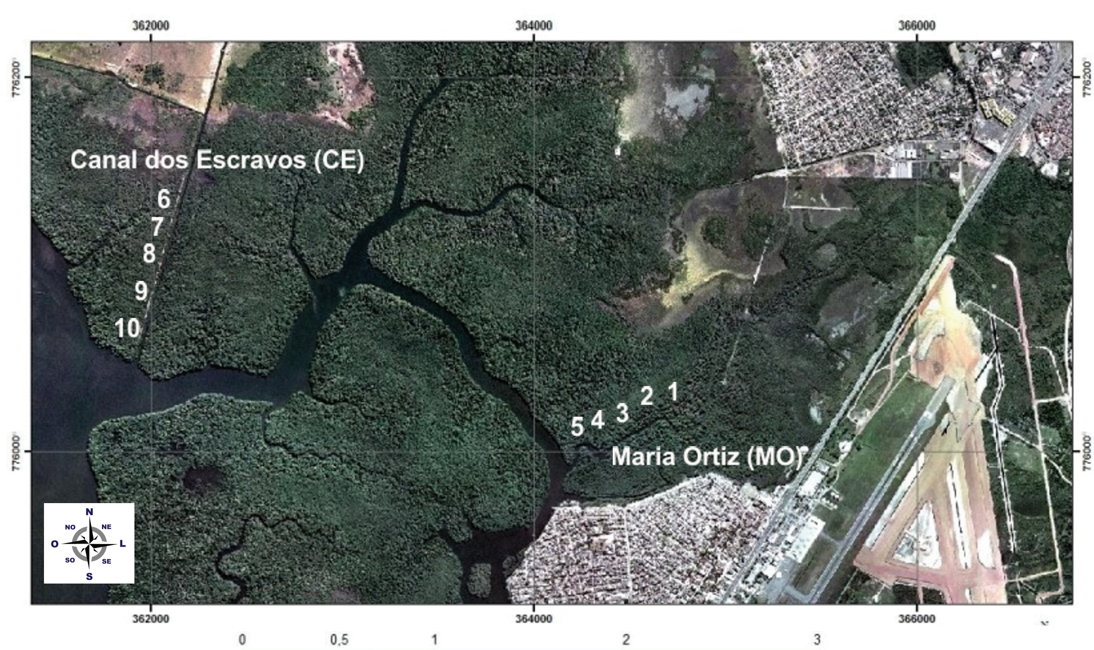

Figure 1. Location of collection points for microbiological data. Maria Ortiz (1-5 points) and Canal dos Escravos (6-10 points).

Sediment and water sampling

The sediment was collected with the help of a 'sampling core', placed in an aseptic container and stored in a Styrofoam compartment. It was also collected 100 mL of interstitial water using Falcon tubes and kept on a cooler box. Once collected, samples were taken to the Laboratory of Environmental Microbiology and Biotechnology (LMAB) at the *Universidade de Vila Velha* (UVV) and placed in the refrigerator to subsequent analysis.

The MPN mL^{-1} method (most probable number mL^{-1}) is based on the serial dilution technique (Apha, 1995), the samples were placed in autoclaved 'shots' tubes and inoculated in stock nutrient solution. Using the MPN mL^{-1} method, serial dilutions were made in saline-based water (water + NaCl - 0.85%). The first step was a presumptive test, where 10 mL and 10 g samples of water and soil, respectively, were aseptically removed and three successive dilutions were generated (0.1, 0.01 and 0.001 mL). The obtained dilutions were then inoculated into three tubes containing 10 mL of Sodium Lauryl Sulfate stock nutrient solution (SLT), in inverted *Durhan* tubes, which were subsequently incubated in a bacteriological oven at a temperature between 35 and 37°C for 24 hours.

In the second step, tubes that showed turbidity and gas formation in the SLT stock had an aliquot removed and inoculated in tubes containing 10 mL of 2% Brilliant green stock (BG), in inverted *Durhan* tubes. Another aliquot part was added to tubes containing 10 mL of *Escherichia coli* stock (EC), with *Durhan* tubes inverted. Tubes with bright green (BG) were then incubated in bacteriological oven with temperature between 35 and 37°C for 24 hours and the tubes with EC were placed in a water bath with temperature between 44.5 and 45°C for 24 hours. This second stage consisted in the confirmatory test, where gas production inside the *Durhan* marked them as positive. The results were analyzed by the Most Probable Number (MPN) table, as Morelli, Vieira, Reis, Rodrigues, and Fonteles-Filho (2003) with some adjustments.

Results and Discussion

Observing the values of all samples (Figures 2-5) it can be seen that the values of total and thermo-tolerant coliforms, both from water and sediment in the MO region and in all seasons at least at some point, obtained values above 1000 MPN mL^{-1}. Moreover, significant values ($p < 0.05$) of CE were found, after comparing the sampled sites, in any of the studied seasons. Only exception was in the sediment samples collected in autumn (Table 2).

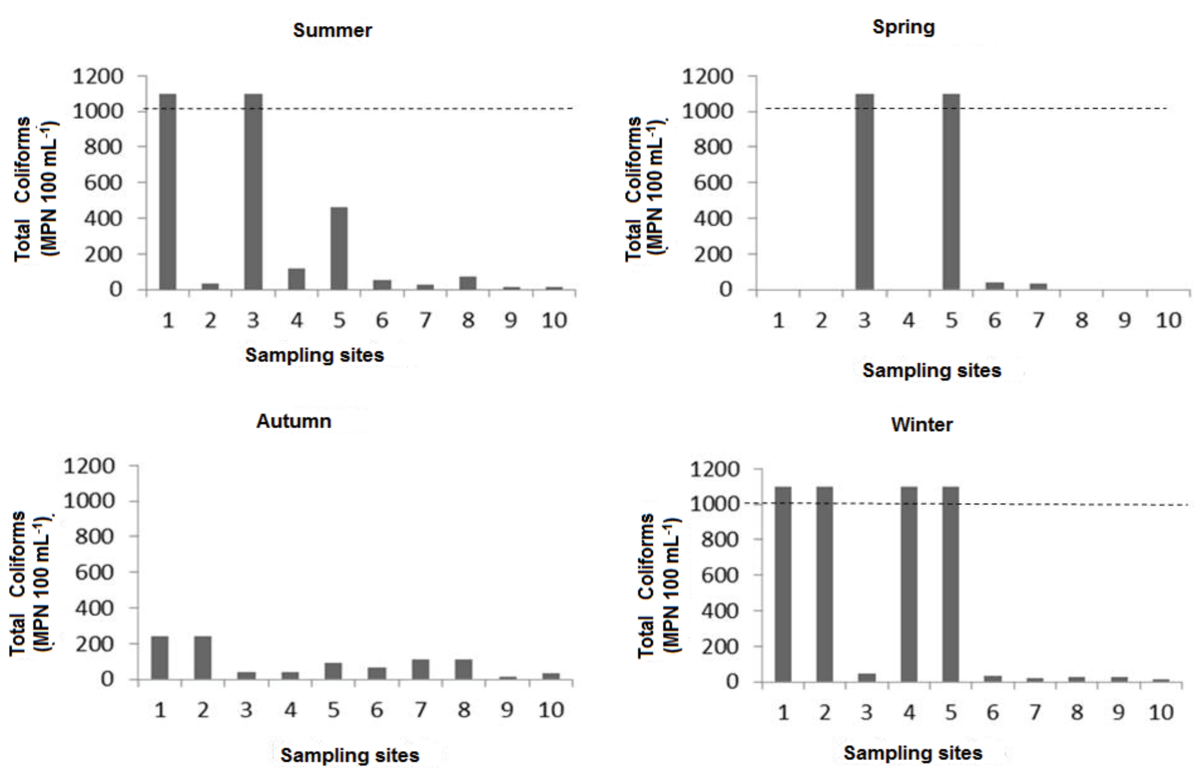

Figure 2. Most probable number (MPN) of total coliforms in 100 mL water at all sampling points (points 1-5) MO/(Points 6-10) CE , located at EEMIL, throughout the seasons.

Table 2 - Comparative *p* values[1] the Kruskal-Wallis test between points (1-5) of Maria Ortiz (MO) and (6-10) Canal dos Escravos (CE) in all seasons of the year (spring/summer/autumn/winter) for total and thermo-tolerant coliforms in water and sediment.

Season	Water (Total C.)[2]	Water (Thermo C.)[3]	Sediment (Total C.)[2]	Sediment (Thermo C.)[3]
Spring	0.053	0.059	0.022	0.011
Summer	0.028	0.001	0.098	0.054
Autumn	0.073	0.079	0.222	0.382
Winter	0.007	0.013	0.07	0.069

[1]Non-parametrical test (Kruskal-Wallis) (DF = 9); [2](Total C.) Totals Coliforms; [3](Thermo C.) Thermo-tolerant Coliforms.

In estuarine regions of the present study, physical and environmental conditions such as tides, self-depuration capacity, dispersion and dilution of pollutants, including microbiological pollutants in the water, became influencing factors in the results. The tidal regime considers the water body as whole in its dilution (Velz, 1984; Cunha, Cunha, Souza, & Pantoja, 2005). Thus, depending on the hydrodynamic characteristics, the plume of fecal coliforms disperses in the aquatic environment because, occurring the change in flow direction, the direction of the pollution plume is reversed and may or may not be brought back to its place of origin. Therefore, data collection close to the site of polluters is very important, as made in the sampled sites in MO region (all near some point of effluent discharge). The sample site number 1 was closer to the treated sewage discharge, so as a result, this site showed the presence of a coliform group containing bacteria (*Escherichia coli* – these bacteria are Gram negative, fermentative, facultative anaerobic, and is usually not pathogenic as fecal coliform, it does not cause disease. In the region there were groups containing pathogenic strains which are often found in environments rich in low quality organic matter (Miquelante & Kolm, 2011). The presence of high levels of coliforms in MO region can be related to anthropogenic activity. Several kinds of human activities involving the disposal of household waste are prevalent in coastal areas, leading to negative impacts on water quality (Edun & Efiuvwevwere, 2012). The bacterium *E. coli* is dominant in the sewage water, which can compete with local microflora. The presence of fecal bacteria such as *E. coli* suggests mainly sewage contamination in mangroves. The prevalence of *E. coli* in water bodies due to human activity has been previously reported. *E. coli* causes serious consequences (when pathogenic) such as: oscillating low fever, bloody diarrhea, stomach cramps, nausea, vomiting and mild fever in humans, while some complications can lead to renal failure, anemia, dehydration, spontaneous hemorrhage, organ failure and death (Poharkar, Kerkar, Doijad, & Barbuddhe, 2014).

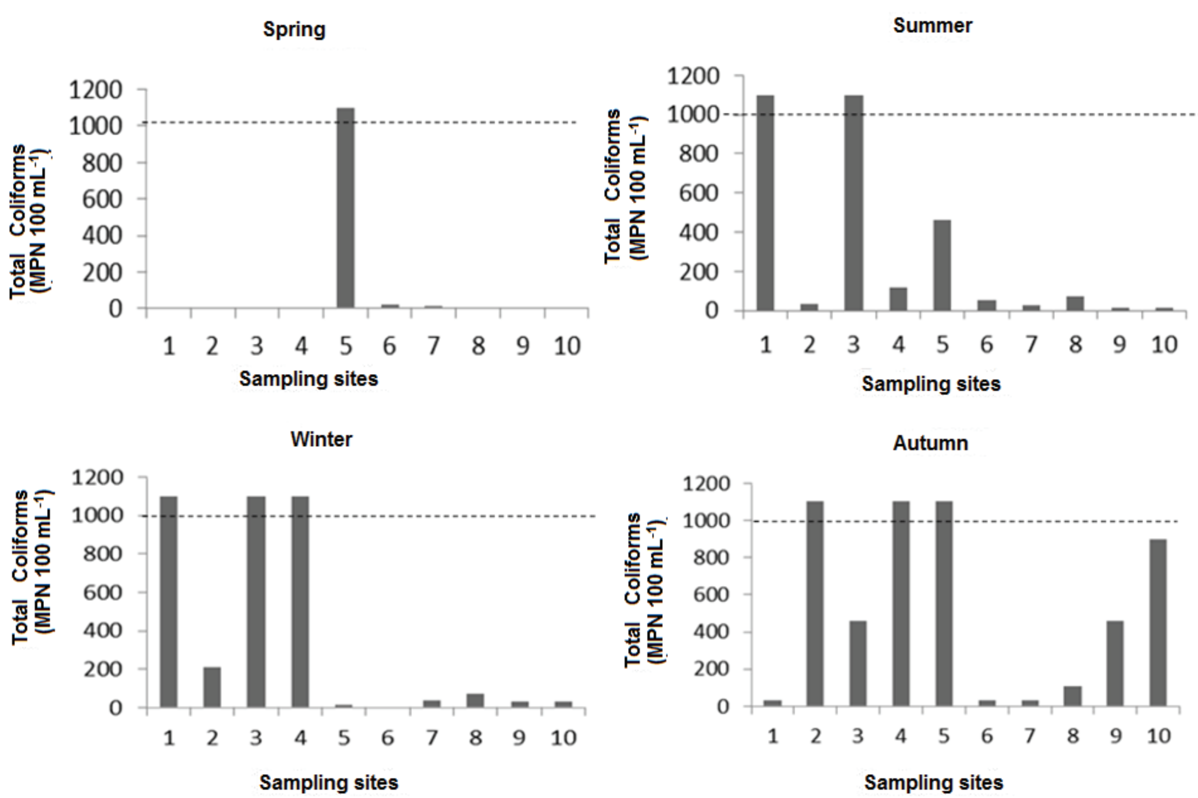

Figure 3. Most probable number (MPN) of total coliforms per 100 mL in sediment at all sampling points (points 1-5) MO/(points 6-10) CE, located at EEMIL, throughout the seasons.

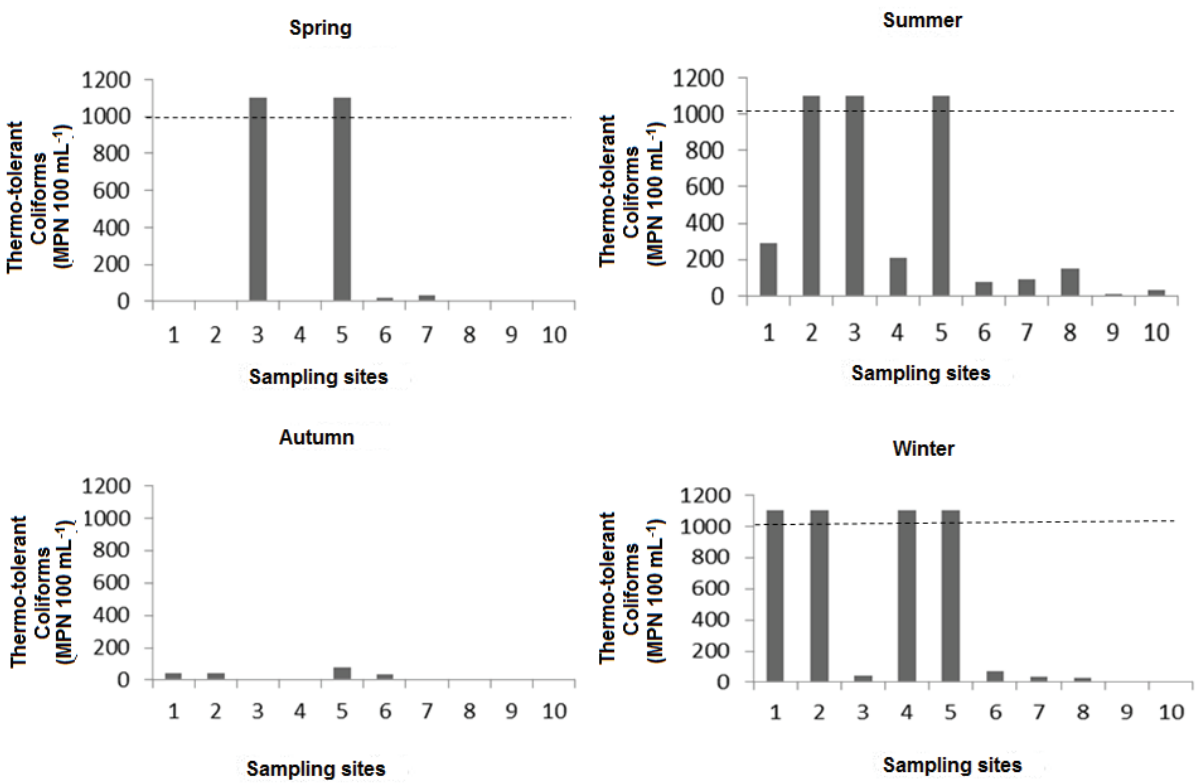

Figure 4. Most probable number (MPN) of thermos-tolerant coliforms per 100 mL water at all sampling points (points 1-5) MO/(points 6-10) CE, at EEMIL, throughout the seasons.

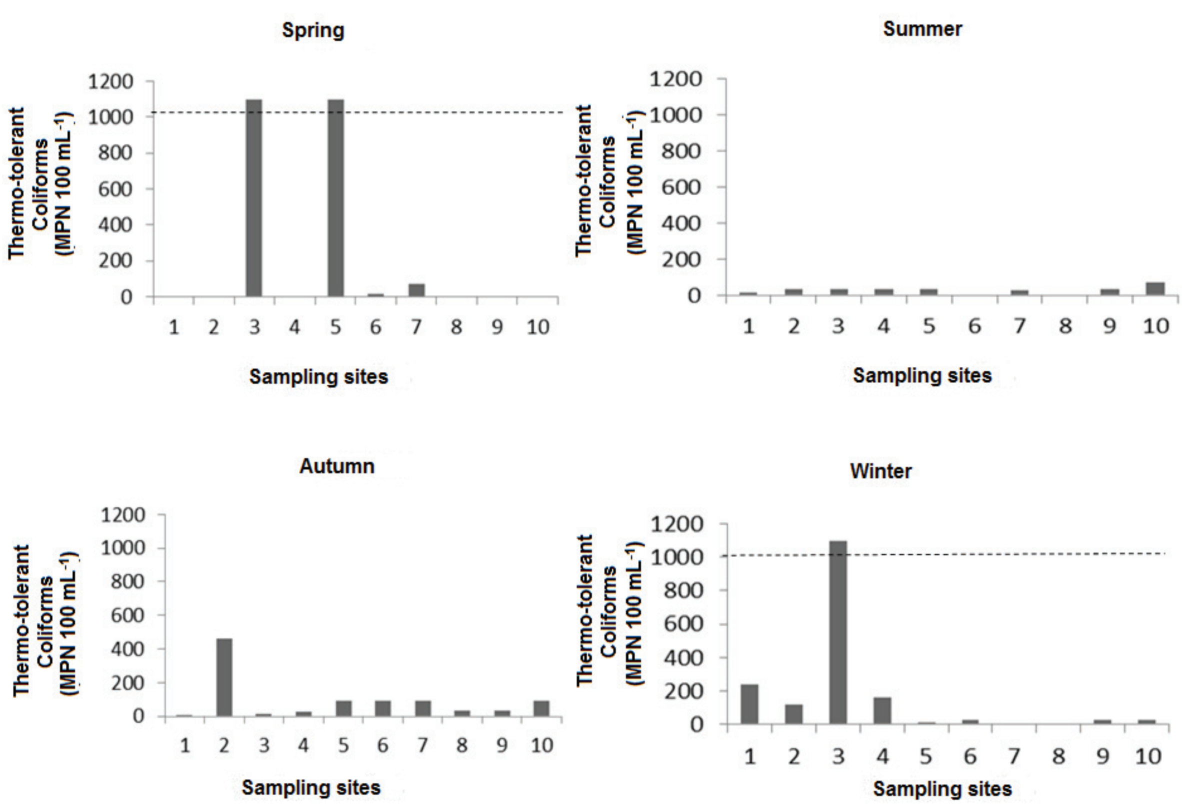

Figure 5. Most probable number (MPN) of thermo-tolerant coliforms per 100 mL in sediment at all sampling points (points 1-5) MO/(points 6-10) CE, at EEMIL, throughout the seasons.

Environmental biomonitoring is defined as a systematic analysis of environmental performance (or behavior) and is included in control of environmental quality standards. Among these analyzes there are physical, chemical and biological aspects, which reveals the behavior of organisms. Changing one of the characteristics can generate stress that, consequently, causes changes in other already installed systems (Rosenberg & Resh, 1993; Sládecek, 1973).

According to the National Council of environment (CONAMA) resolution 357/2005 (Brasil, 2005), the data from the MO area (sites 1-5) does not refer to the Class 1 maximum tolerated values for thermo-tolerant and total coliforms, as such, the sites fit in the Class 2 that accepts the limit of 2500 MPN 100 mL^{-1}. Among the limitations, Class 2 standards allow only amateur fishing and 'secondary contact' recreation. Such activities refer to those in which contact with water is sporadic or accidental and the possibility of ingesting water is small, as in fishing and navigation.

Regarding the sites from the CE area (sites 6-10), the values obtained fit into Class 1 standards (values should not exceeded 1,000 fecal coliforms per 100 mL of water). This class allows the use of water in primary contact recreation and protection of aquatic communities.

It is noteworthy that this contamination is a form of environmental degradation, though there is information of the complete sanitation of the city of Vitoria and halving the emission of sewage, marginal areas tend to be forgotten. Besides, there is no encouragement by the government or the water treatment companies to lessen the discharge of sewage. This is generating environmental degradation and it is negatively affecting the health of human populations that live nearby, decreasing their life expectation by exposing them to certain harmful effects. When combined with genetic factors, nutrition parameters and lifestyle, these effects may cause diseases and even death. Health is always linked to the environment, making it impossible to attain and maintain a healthful life when exposed to unfavorable, and even precarious, environmental context (Ayach, Guimarães, Cappi, & Ayach, 2012).

Other factors, however, affect the self-depuration capacity of the environment, such as water temperature, pH, excess of nutrients, sedimentation, organic extraction, adsorption (represented by total solids suspended and total solids dissolved) and water salinity (represented by the electrical conductivity) (Velz, 1984). Factors such as pH, salinity and temperature can influence the environmental quality of the areas studied in this work.

High levels of total coliforms and thermo-tolerant coliforms (both in water and in sediment) were mentioned in the MO area, so the microbiological approach is fundamentally relevant, once this method showed the presence of coliform arising from irregular sewage releases from the water treatment plant, whose consequences can be harmful to the environment, human health and longevity. In the environmental context, the discharge of sewage and pollutants in principle cannot affect adult trees (in small discharge rates) and at a certain point the mangrove forest may function as phyto-remediation (Yang et al., 2008; Cannicci et al., 2009). However, it should be considered that pollution may affect all the structural dynamics of the mangrove system in all chain levels, from seedlings to forest (Sun et al., 2015).

Conclusion

Mangrove is suffering from unsustainable decline, reducing the resilience, pressured by anthropogenic actions, increasing the vulnerability of these areas to a possible threat or close to extinction. Brazil has around 7% of the world's mangroves. It is the third country in mangrove extension in the world, but at least 35% of these forests have been destroyed by human aggressions, disrespecting their ecological and social importance.

Despite the fact that both sites (CE and MO) are not far apart, there is a possibility of the riverside population run health risks when in contact with contaminated water. There is also economic risk because many of them depend on fishing for survive, and that pressure can turn the Canal dos Escravos even more vulnerable once its forest may be suffering disturbances due to the imbalance caused by the changes of the environmental dynamics. This may be a subject for further studies.

After analyzing the monitoring, we conclude that the mangrove Maria Ortiz is suffering with the dumping of sewage in the bay. An environmental education action should be taken in order to help the population to stop releasing sewage in the bay and to establish a network to treat the sewage. Regarding the Canal dos Escravos, due to minimal contact with the population, it does not have anthropic pressure on the view of anthropogenic agents so the quality of sediment and water of this site are in good state of preservation.

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