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Characterization of Pseudomonas consortia isolated in sediments of the Estero Salado in the northern sector of Guayaquil

Caracterización de consorcios de pseudomonas aislados en sedimentos del Estero Salado en el sector norte de Guayaquil

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Abstract
There are many open research fronts to solve the pollution problem of the estuary of Estero Salado in the city of Guayaquil, carried out in-situ, where they aim to reduce hydrocarbon contamination through bioremediation methods, including bioaugmentation methods. of endogenous microorganisms, however, are not optimal study conditions, so we set out to characterize the capacity of these organisms to degrade hydrocarbons under controlled environments, where we proceeded to take a sample of sandy and rocky soil, both studied in triplicate, to later extract from them bacterial consortiums, increasing their population and determining by means of infrared spectrophotometry the changes produced in the concentrations of total petroleum hydrocarbons (TPH) before and after introducing the bio-increased consortia, presenting optimal results in the Attachment after the respective statistical analysis that show a reduction of 90% under controlled conditions the levels of TPH in the different treatments.

Keywords
Bioremediation, consortia, TPH, bioaumentacion, degradation.
Resumen
Existen muchos frentes de investigación abiertos para resolver la problemática de la contaminación del estuario del Estero Salado de la ciudad de Guayaquil, uno de estos frentes pretende la reducción de la contaminación por hidrocarburos mediante métodos de Biorremediación, entre estos se incluyen los métodos de bioaumentacion de microorganismos endógenos, sin embargo, las pruebas se realizan mediante condiciones no controladas (in situs), por ello trazamos como objetivo caracterizar la capacidad de organismos endógenos del estuario para degradar hidrocarburos bajo ambientes controlados, donde se procedió a tomar una muestra de suelo arenoso y rocoso, ambos estudiados por triplicado, para después extraer de ellas consorcios bacterianos, incrementando su población y determinando mediante la espectrofotometría de infrarrojos los cambios producidos en las concentraciones de hidrocarburos totales de petróleo (TPH) antes y después de introducir los consorcios bio-aumentados, presentando resultados óptimos en los tratamiento luego de los respectivo análisis estadístico que arrojan una reducción del 90% bajo condiciones controladas los niveles de TPH en los diferentes tratamientos.

Palabras Claves
Bioremediación, consorcios, TPH, bioaumentacion, degradación.

1. Introduction

Estero Salado is an estuary located in the eastern part of the city of Guayaquil, flowing into the homonymous gulf of the city, encompassing approximately 93 km in its course. This arm of the sea, together with certain low-lying areas of the Babahoyo and Daule rivers, constitutes a single ecosystem, which has been designated as the Inner Estuary of the Gulf of Guayaquil.

Since the issue of hydrocarbon pollution in the Estero Salado estuary was identified, various research groups have primarily focused on measuring contaminant levels [1] and on in-situ and laboratory-scale biodegradation processes [2].

Thanks to these investigations, we now know that the Miraflores Sector of the estuary has the highest levels of hydrocarbons [1]. Additionally, we know that among the endogenous microorganisms of the estuary, there is a wide variety of Pseudomonas, and among these microorganisms, some species may have the capacity to degrade hydrocarbons [2].

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There is a wide variety of methods that can be employed for this type of hydrocarbon contamination, ranging from placing sediments in areas considered safe, to incinerating waste or applying treatments with chemicals or living organisms [3]. Bioremediation is one of the best methods for cleaning up this type of waste, primarily because of its much lower operating costs, but also because it has been successfully used in such contaminations [4].

In some studies, marine strains capable of degrading hydrocarbons have been isolated, with particular emphasis on the strain Pseudomonas sp. F21, which is competent in degrading both linear saturated hydrocarbons (TPH) and aromatic rings (PAHs) found in light crude oil compounds [5].

Later, further analysis was conducted to deepen the understanding of the microbial biodegradation capacity of other types of petroleum, using the Pseudomonas sp. F21 strain [5].

The number of variables that can be manipulated to improve the efficiency of bioremediation methods is extensive and can be adapted to the conditions; sometimes nutrients are added, or certain types of microorganisms are inoculated into the soil (F6) [6]. Among these variables, the structure of the contaminant to be treated is paramount for all types of hydrocarbon remediation, as the degradation kinetics of aromatic compounds vary relative to aliphatic compounds; they have different speeds and reaction mechanisms. Additionally, some hydrocarbons contain halogen atoms that present strong toxicity to certain families within the Monera kingdom, inhibiting their growth [7].

Estero Salado is an iconic estuary in the city of Guayaquil, as well as being one of the most important aquatic environments in the country. Continuous pollution over the last half-century has caused severe changes in both the water and soil, resulting in the loss of its natural beauty. Numerous studies have been conducted to identify the causes of this pollution, measure contaminant levels, investigate its effects, and seek alternatives to remediate the damage caused[8].

Among the most recent studies on hydrocarbon levels in Estero Salado is the analysis of heavy metal and hydrocarbon concentrations in branch B of the estuary, conducted by Engineer Víctor Hugo Rivera Pizarro as a thesis for obtaining his chemical engineering degree. In his study, hydrocarbon levels were found to range between 800 and 1000 mg/Kg at six stations in branch B. An earlier study on the effects of hydrocarbon pollution on the macroinvertebrates of Estero Salado was conducted by Biologist Maritza Cárdenas Calle as a postgraduate thesis, identifying the Miraflores sector as the area in branch B with the highest levels, around 1124 mg/Kg, while in areas like Kennedy and Urdesa, levels ranged between 200 and 100 mg/Kg.

Regarding the causes, Noelia Vascones, in her thesis, points to the almost complete lack of compliance with environmental regulations by industries located in the northern part of branch B and the lack of more effective sanctions and control measures by state agencies and sectional governments as the main causes of the pollution. Despite this, the advances achieved in the southern sector of the estuary are also mentioned, specifically those that are part of the Guayaquil Ecológico project, including the relocation of families living along the banks through the Socio Vivienda plan in coordination with the MAE, the reduction of bad odors in branch A through super-oxygenation, and the reforestation of the banks in the Cisne II sector. It is noted that since 2007, the company VISOLIT S.A. has participated in and won all contracts for the maintenance of Estero Salado, with contracts in place until 2019.

1.1. Composition of Crude Oil

Crude oil is a black, highly viscous liquid composed of a wide variety of organic substances, potentially containing thousands of compounds, primarily hydrocarbons (50-98% of the composition) [9]. Hydrocarbons are used in the production of numerous products, making them one of the most important groups of environmental pollutants, not only due to their abundance but also because of their persistence in different ecosystems [10].

Most hydrocarbons are n-alkanes or linear-chain alkanes, also known as paraffins, while others are branched alkanes (in smaller quantities), naphthenes, and various amounts of aromatic hydrocarbons [11]. Crude oil has an elemental composition predominantly of carbon-hydrogen, with around 84-87% C and 11-14% H, and also contains 0-8% S, 0-4% O and N, and traces of metals such as nickel, vanadium, among others [12].

1.2. Composition by Hydrocarbon Families

For a more detailed study of hydrocarbons derived from petroleum, the compounds are usually grouped into families:

- Volatile paraffins (alkanes up to C10)
- Non-volatile paraffins (alkanes between C10-C40)
- Naphthenes (cycloalkanes)
- Olefins (alkenes)
- Aromatics (monoaromatics and polyaromatics)
- Resins and asphaltenes

1.3. Socio-environmental Issues Generated by the Oil Industry

Today, different countries depend on oil and its products; the physical structure and lifestyle of the peripheral agglomerations surrounding major cities are possible thanks to a relatively abundant and inexpensive supply of oil. However, in recent years, the global availability of this resource has declined, and its relative cost has increased. It is likely that, by the middle of the 21st century, oil will no longer be commonly used commercially [13].

Soil as a Habitat for Microorganisms

Soil refers to the outermost part of the Earth's crust, resulting from the weathering of underlying rocks and having characteristics distinctly different from those rocks. Soil can be considered a system of interaction between three well-defined phases: a solid phase, consisting of mineral and organic matter, a liquid phase, and a gaseous phase or soil atmosphere [14].

The type and composition of the mineral matter are determined by the characteristics of the subsurface rocks and the edaphic processes that have occurred in its formation. The inorganic portion is very important because of its influence on nutrient availability, aeration, water retention, etc. The organic matter originates from the activity of various living organisms in the soil, and its composition and quantity vary, mainly depending on the type of vegetation cover. The remainder of the soil volume is largely composed of porous spaces, which are in turn occupied by water and the gases that make up the soil atmosphere [15].

The porosity (amount and size of the pores) depends on the texture, which is determined by the amount of sand, silt, and clay, the structure, and the organic matter content. These factors, in turn, determine the movement and water retention capacity of the soil and the gaseous composition of its atmosphere. Typically, the soil atmosphere is enriched in carbon dioxide and depleted in oxygen, as a result of the aerobic respiration of plant roots, animals, and microorganisms. However, when anaerobic conditions occur (due to the accumulation of water in the soil pores), other gases such as nitrous oxide, nitrogen gas, and methane, which result from anaerobic bacterial respiratory activity, appear in the soil atmosphere. Both the water content and the composition of the soil atmosphere fluctuate widely [16].

This complex system that constitutes the soil, characteristically heterogeneous in space and time, harbors a great diversity of plant, animal, and microbial species. Soil is a highly suitable environment for the development of both eukaryotic microorganisms (algae, fungi, protozoa) and prokaryotic microorganisms (bacteria and archaea). Viruses and bacteriophages are also present. All of these organisms establish relationships among themselves in very varied and complex ways and also contribute to the soil's characteristics through their role in modifying the aforementioned solid, liquid, and gaseous phases. Microorganisms play very important roles in relation to processes such as pedogenesis, biogeochemical cycles of elements like carbon, nitrogen, oxygen, sulfur, phosphorus, iron, and other metals, plant fertility and protection against pathogens, degradation of xenobiotic compounds, etc. [17].

Soil organisms are not randomly distributed but follow spatial aggregation patterns at different scales (from nm to km) that overlap. This structuring is due to the effect caused by different control factors and is completely dynamic. Using techniques such as ultrathin soil section observation by electron microscopy, tomography, geostatistical analysis, and modeling, it has been demonstrated that the distribution of soil bacteria is highly structured, and this structuring is important for soil functionality. Bacteria organize into microcolonies composed of a few cells that can belong to different morphotypes. Factors such as the presence of roots, small aggregates, nutrients, and pores seem to govern the distribution of bacteria in microhabitats [18].

The complexity of soil as an ecosystem (including the microscopic level), along with the unique characteristics of microorganisms, such as their microscopic size and the challenges in differentiating them based on morphology, had led to a view of the soil microbial world as a 'black

box'—one whose function was known, but whose contents were not fully understood [19].

1.4. Factors Influencing Bioremediation

The concentration and composition of the microbial community and the rate of contaminant transformation are influenced by various factors:

- **Nutrient requirements:** Microbial metabolism is oriented toward the reproduction of organisms, and they require that chemical constituents be available for assimilation and synthesis. The primary nutrients required are phosphorus and nitrogen. Generally, there is a sufficient concentration of nutrients in the soil; however, if they are not within the normal range, additional amounts can be introduced into the medium. The normal C: N: P ratio depends on the treatment system being used, typically 100:10:1.
- **Soil pH:** ignificantly affects microbial activity. The growth of most microorganisms is optimal within a pH range of 6 to 8. Additionally, pH directly affects the solubility of phosphorus and the transport of heavy metals in the soil. Acidification or reduction of soil pH can be achieved by adding sulfur or sulfur compounds.
- **Moisture:** Microorganisms require minimum moisture conditions for growth. Water is part of the bacterial protoplasm and serves as a transport medium through which organic compounds and nutrients are mobilized into cells. Excess moisture will inhibit bacterial growth by reducing the oxygen concentration in the soil. The range varies depending on the technique

1.5. Biodegradation

Biodegradation is the result of the digestion, assimilation, and metabolism of an organic compound carried out by bacteria, fungi, protozoa, and other organisms [20].

Soil microorganisms transform both organic and inorganic compounds. Biodegradation processes include oxidation-reduction reactions, adsorption processes, ion exchange, and chelation reactions for complex formation, leading to metal fixation [21].

Many studies have shown that biodegradation by indigenous microbes can significantly contribute to the destruction of organic compounds.
Biodegradation is a beneficial natural process, not only because it allows the elimination of harmful compounds, preventing their concentration, but also because it is essential for the recycling of elements in the biosphere, allowing the restitution of essential elements for the formation and growth of organisms (carbohydrates, lipids, proteins). Decomposition can occur in the presence of oxygen (aerobic) or in its absence (anaerobic). The former is more complete and releases energy, carbon dioxide, and water; it is the most energy-efficient and is described as follows:

Aerobic Degradation

$$Substrate + O2 \qquad Biomass + CO2\,(g) + H2O$$

Aerobic biodegradation is the easiest reaction for terminal electron acceptance and is used for the biodegradation of petroleum hydrocarbons. This degradation process occurs in the presence of aerobic microbes. The final products of organic compound mineralization are carbon dioxide, water, and cell mass. Facultative anaerobic organisms can use oxygen when it is present or can switch to alternative electron acceptors.
Anaerobic processes are incomplete oxidations and release less energy, described as follows:

Anaerobic Degradation

$$Substrate + (NO3-, SO42-, Fe3+, Mn4+, CO2) + Biomass + CO2(g) + (N2(g), Mn2+, H2S(g), Fe2+, CH4(g))$$

Other electron acceptors, such as nitrate, sulfate, ferric, manganese ions, and so on, are used when oxygen is not available. Obligate anaerobic organisms become dominant in the absence of oxygen [22]. Degradation under anaerobic conditions can be relatively slow. The metabolic products of anaerobic biodegradation include simple organic acids, CO2, H2O, CH4, H2, N2, and cell mass [23].

In the city of Guayaquil, Estero Salado represents an iconic estuarine system of the metropolis, extending approximately 60 km from the Maritime Port to Posorja, forming part of the Gulf of Guayaquil.

This estuarine ecosystem provides a diversity of significant and valuable resources and ecological services for the sustenance of the metropolis of Guayaquil, hosting a wide variety of aquaculture enterprises, which are an important part of the country's non-oil economy, thereby supporting its high ecological and commercial value[20].

There are designated areas for the sustainable management of resources in Estero Salado, such as:

- Salado del Norte Protected Forest (47.23 hectares)
- Puerto Hondo Protected Forest (2,000 hectares)
- Manglares El Salado Wildlife Production Reserve (5,409 hectares).

However, as it is located in the most populous city in the country [A9], the estuary of Estero Salado is being adversely affected in various ways by different anthropogenic causes that occur in the city and its surroundings. Adding to this is the accelerated population growth in the city and the immediate expansion of the urban perimeter, which has led to illegal and unplanned urban settlements by the authorities, resulting in insufficient sewer systems, no wastewater treatment, and the disposal of solid waste directly into the estuary [24].

Of the wastewater with little or no treatment that continuously enters the various branches of the estuary, about 60% is from domestic use, and 40% is from industrial use [25].

At the same time, pollutants enter discontinuously through runoff from agricultural areas and quarrying activities in the surrounding areas of the metropolis, as well as hydrocarbon waste, leachates from domestic and industrial solid waste, through runoff from impermeable surfaces [26].

Estero Salado, being an arm of the sea and not receiving inflows from upstream tributaries or rivers, has waters with certain movement not predominantly directed toward the open sea; the body of water flows with the tide toward the sea but recovers with the ebb tide, bringing in wastewater with little or no treatment, entering the various branches of the estuary, about 60% from domestic use and 40% from industrial use [24]. Additionally, non-point pollutants enter through runoff from agricultural areas and quarrying activities in the city's periphery, as well as trash, leachates, and contaminants from runoff on impermeable surfaces (roofs, bridges, streets, sidewalks) [25].

This arm of the sea, not being connected to other upstream tributaries or rivers, and with waters that do not predominantly flow toward the open sea, experiences water displacement with the tide toward the sea, but then returns to its original state as the tide recedes. This process disrupts renewal and makes the self-purification of waters in the estuary of Estero Salado much more difficult, particularly in the more peripheral areas of the city of Guayaquil [27].

Furthermore, the intertidal zones show an immediate response to all these alterations in the ecosystems adjacent to the intertidal área [25].

This effect has influenced, in one way or another, the deterioration of the environmental, aesthetic, and productive quality of the estuary, negatively impacting:

- he economic activity in the area by hindering the exploitation of its fishing resources and deteriorating the water quality used by the shrimp farming industry, which draws water from nearby ecosystems [28].
- A large number of species that coexist in the estuary, gradually destroying this ecosystem[26].

2. Materials and Methods

The research was conducted from August 2018 to February 2019, during low tide periods, as verified in the tide tables from INOCAR.

According to the Municipality of Guayaquil, the sectors to be studied, based on environmental studies, are among the areas most affected by hydrocarbon pollution due to the discharge of domestic wastewater from nearby urban developments and industrial waste [29].

Fig. 1. hotograph of the Miraflores Sampling Area
Source: Own work

Soil samples were taken at depths between 20 and 35 cm, stored on aluminum trays, and left to dry at room temperature for 48 hours.

Three treatments were conducted with two replicates each, in two areas of Estero Salado in the city of Guayaquil. The samples were stored at room temperature for 48 hours [29].

2.1. Total Petroleum Hydrocarbons

Procedure

- Label the sample container to later determine the sample volume. If the sample was not acidified at the time of collection, add 5 ml of hydrochloric acid to the sample container.
- After mixing the sample, check the pH by touching the pH-sensitive paper to the lid to ensure the pH is 2 or lower.
- Add more acid if necessary.
- Pour the sample into a separatory funnel, adding 30 ml of fluorocarbon-113 to the sample container, and swirl to rinse the sides. Transfer the solvent to the separatory funnel, extracting by vigorously shaking for 2 minutes. Allow the layers to separate.
- Filter the solvent layer through a funnel containing filter paper moistened with solvent into a 100 ml volumetric flask.
- NOTE 1: An emulsion that does not dissipate can be broken by pouring approximately 1 g of sodium sulfate into the filter paper cone and slowly draining the emulsion through the salt. Additional 1 g portions may also be added to the cone as needed.
- Repeat the previous two steps twice more with 30 ml portions of fresh solvent, combining all solvent in the volumetric flask.
- Rinse the tip of the separatory funnel, filter paper, and funnel with a total of 5-10 ml of solvent, collecting the rinses in the flask. Dilute the extract to 100 ml. If the extract is known to contain more than 100 mg of non-hydrocarbon organic material, pipette an appropriate portion of the sample into a 100 ml volumetric flask and dilute to volume.
- Discard approximately 5-10 ml of solution from the volumetric flask. Add 3 g of silica gel and a stirring bar; cap the volumetric flask and stir the solution for a minimum of 5 minutes with a magnetic stirrer.
- Calibrate the instrument for the appropriate cells using a series of working standards. It is not necessary to add silica gel to the standards.
- Determine the absorbance directly for each solution at the maximum absorbance at approximately 2930 cm-1.
- Prepare a calibration curve of absorbance vs. mg of oil.
- Hydrocarbons per100 ml of solution.
- After the silica gel has settled in the sample extract, fill a clean cell with the solution and determine the extract's absorbance. If the absorbance exceeds 0.8, prepare an appropriate dilution.
- NOTE 2: The possibility that the absorption capacity of the silica gel has been exceeded can be tested at this point by adding another 3.0 g of silica gel to the extract and repeating the treatment and determination.
- Determine the concentration of petroleum hydrocarbons in the extract by comparing the response to the calibration curve [27].

2.2. Respirometry

From each of the samples, 100 g of soil sample was taken.

The samples were placed on an aluminum tray, and 25 ml of NaOH (0.1 N) was added to the surface of the tray and covered with a beaker. They were left at room temperature for 48 hours. Phenolphthalein was used as an indicator, and titration was performed with 0.1 N HCl.

3. Results

3.1. Experimental Results and Analysis of Results

pH

The hydrogen potential levels of the sediments obtained show slightly alkaline levels of around 8 before treatment, with the Miraflores station being the most alkaline. After treatment, pH levels decrease in all sediments, reaching neutral levels of 7 pH units and slightly acidic levels of 6.8 pH, with the greatest change occurring in Sample C of the Miraflores Sector, where it drops from 8.86 to 6.85, and the least in Sample C of the Zig Zag Bridge Sector.

Table 1.
pH Results

Sample	Before	After	Location
Miraflores	8,7	7	A
Miraflores	8,58	7,02	B
Miraflores	8,86	6,85	C
Zig Zag Bridge	8,08	6,85	A
Zig Zag Bridge	7,87	7	B
Zig Zag Bridge	7,55	6,24	C

Source: Own work

In Figure 2, we can better observe the change in pH levels for each treatment, noting that in both the Miraflores Sector and the Zig Zag Bridge Sector, the pH change tends to a value always very close to absolute neutrality.

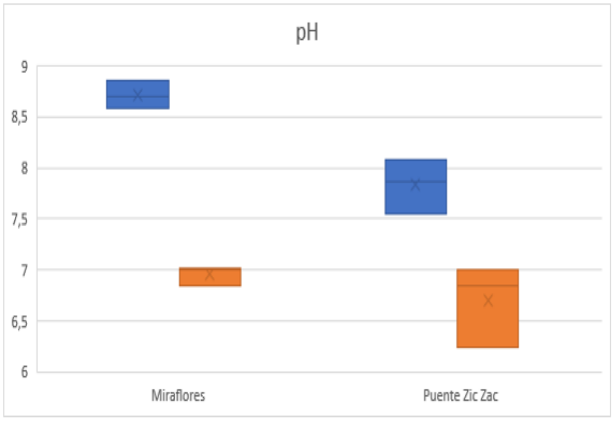

Fig. 2. pH Changes
Sources: Own work

It is observed that the pH reduction in the Miraflores Sector treatments is around 2, and in the Zigzag Bridge Sector, it is 1, with the average pH level decrease after treatment being around 1.44 with a standard deviation of 0.4.

All these statements will be verified using statistical models, either confirming or refuting each hypothesis that we can quantify.

3.2. Moisture

Using the result obtained by subtracting the weight of the container from the weight of the dry sample in the container, we calculate the moisture percentage:

$$\%Moisture = \frac{grams\ of\ wet\ sample - grams\ of\ dry\ sample}{grams\ of\ wet\ sample} \quad (1)$$

The results achieved are shown in the following Table 2:

Table 2.
Moisture results

	BEFORE		AFTER		
	Gr of dry sample	% Moisture	Gr of wet sample	% Moisture	Difference
A1	28	53,33	48	20	20
B1	14	76,66	40	33,33	26

C1	13	78,33	38	36,66	25
A2	25	58,33	46	23,33	21
B2	28	53,33	37	38,33	9
C2	34	43,33	36	40	2

Source: Own work

As we can observe in the following [28], the initial moisture percentages are higher in Miraflores (blue), where it reaches an average of 69.44%, while in the Zig Zag Sector, the average moisture is 51.66%.

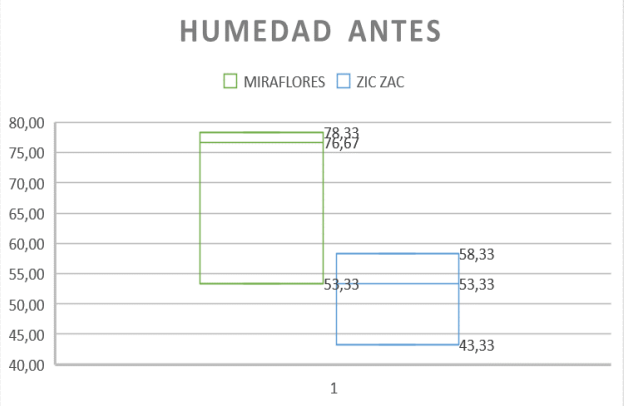

Fig. 3. Moisture Behavior (Before)
Source: Own work

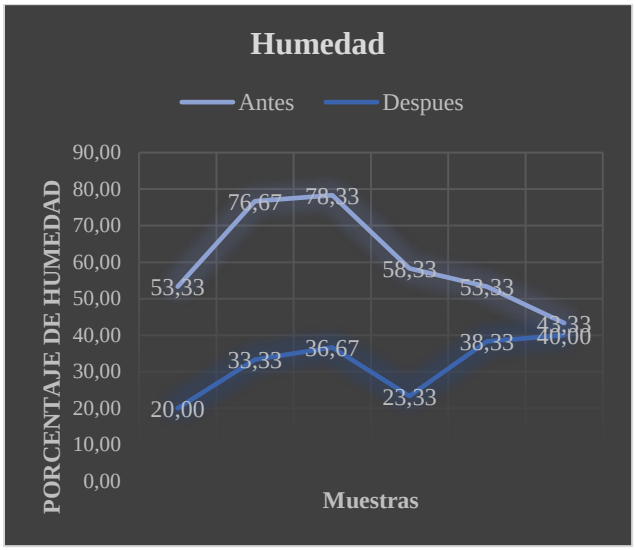

Fig. 4. Moisture Behavior (After)
Source: Own work

The deviation in moisture results before treatment is lower in the Zig Zag Bridge Sector..

Parameter	Total	Zig Zag	Miraflores
Average	60,555556	51,6666667	69,4444444
Deviation	14,010578	7,63762616	13,9774951

Source: Own worka

Given that these are sedimentary sludges found at the bottom of Estero Salado, it is understandable that water makes up around two-thirds of their composition.

The reduction in moisture may be due to the change in conditions the sample experienced from the estuary's shore to the laboratory or to the physicochemical changes that occur as a result of the increased population of bacterial consortia.

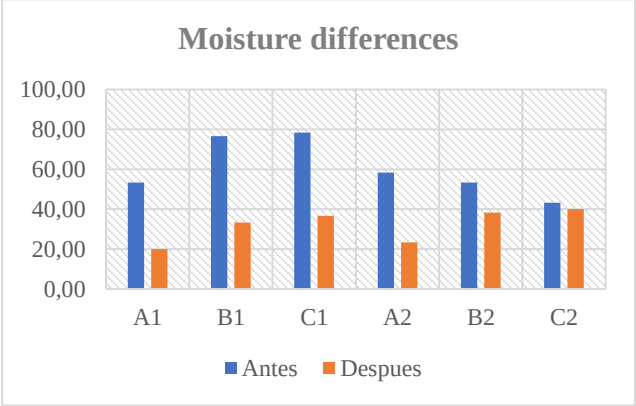

Fig. 5. Moisture differences
Source: Own work

3.3. CO2 Production

To determine the milliliters of 0.1 N Hydrochloric Acid needed to neutralize the solution, the following formula was used:

$$molCO_2 = \frac{(50ml - mlClH) * 0.1mol/l}{(1000\, ml/l)} \qquad (2)$$

Obtaining from it the moles of CO2 produced in each case, the following Table 3 shows the values obtained:

Table 3.
Values obtained for CO2

ml ClH	1	2	molC02	1	2
A	40	39	A	0,001	0,0011
B	43	42	B	0,0007	0,0008
C	42	40	C	0,0008	0,001

Source: Own work

Using the values obtained in moles of CO2, the parts per million of carbon lost in the samples were calculated, as shown in table 5

$$Ppm_{(C/sample)} = \frac{molCO_2 * 14g/mol * 1000mg/g}{Av} \quad (3)$$

Table 4.
Ppm of Carbon

Ppm C/sample	1	2
A	151,260504	166,386555
B	105,882353	121,008403
C	121,008403	151,260504

Source: Own work

PPM (Carbon) represents the values obtained in each case:

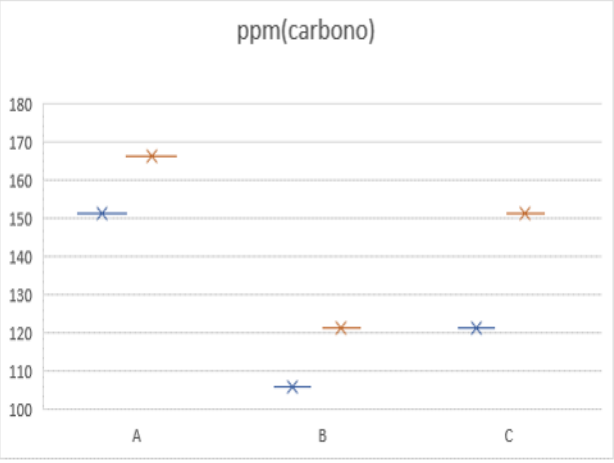

Fig. 6. Carbon Production Behavior
Source: Own Work

The average parts per million of carbon consumed by the bacteria over 15 days was 136.13 ppm Table 5, which gives a rate of approximately 9 ppm/day.

Table 5.
Averages of PPM of Carbon

Parameter	Total	Zigzag	Miraflores
Average	136,134454	146,218487	126,05042
Deviation	14,2609771	23,1054237	23,1054237

Source: Own Work

The data obtained from this experiment were compared with the results from the Total Petroleum Hydrocarbons concentration tests to determine whether the reduction in those concentrations is due to degradation carried out by the bacterial consortia used or if it is due to some other factor.

4. Conclusions

- The sludge subjected to treatment reduced their pH levels by 1 to 2 points, from slightly alkaline to slightly acidic and neutral without exception.
- The decrease in pH levels in both sectors is practically the same, and they have similar moisture levels, so it could be concluded that the physicochemical characteristics measured in both types of soil are identical.
- The sludge subjected to treatment reduced their moisture in varying proportions without exception, but the efficiency varies significantly, which leads us to conclude that the conditions differed between the different methods.
- In the CO2 tests, it was confirmed that carbon metabolism is similar in all cases, with an average of 9±0.63 ppm/day.
- The bacterial consortia metabolized the carbon from the hydrocarbons present in the sample, reducing TPH concentration levels and generating CO2 as waste.
- The reduction in TPH concentrations allows us to conclude that degradation of aliphatic hydrocarbons is occurring.
- The production of CO2 in the respirometry traps indicates that the bacteria are metabolizing the carbon they obtain from soil sources, including the TPH that was degraded.

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