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Toxicological Evaluation of the Sediments of the Juan Angola Channel (Cartagena) using *C. Elegans* as Biological Model

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Recently, the pollution in bodies of water near urban settlements has drawn the attention of government agencies. The city of Cartagena, being a coastal city, has multiple bodies of water that make up its ecosystems, both maritime and freshwater. One of these bodies of water is the Juan Angola channel, the focus of our research, which fulfills its function of flowing nutrients to the *Ciénaga de la Virgen*. However, due to the accumulation of pollutants and the neglect of the environmental authorities, the “Juan Angola” channel has become a dumping ground for pollution and a garbage dump for its surrounding neighborhoods, significantly affecting the living beings that reside in it, including the mangrove swamp. The “Juan Angola” channel is a water corridor that runs from the *Ciénaga de la Virgen* to the Cabrero Lagoon, Cartagena - Colombia. This work aimed to evaluate the toxicological effects using the nematode *Caenorhabditis elegans* in extracts of sediments of the channel. This work had quantitative experimental methodology by using the samples taken in the field trips, which were conditioned and analyzed by using the nematode *nematodes* through endpoints such as Lethality. The results showed a high case fatality rate of nematodes after exposing them to the extract of sediments.

1. Introduction

A high level of contamination, foul odors, and turbid water is qualitatively evident in the Juan Angola stream, which is why we seek to determine the level of toxicity of this body of water using *Caenorhabditis elegans* as a biological indicator. Water is a scarce natural resource, necessary for human life and for the maintenance of the environment, but due to accelerated human and economic growth and development and its excessive use, it has been used as a means of waste disposal and has suffered an alarming decline. During the last few years, tons of biologically active substances, synthesized for use in agriculture, industry, and medicine, have been discharged into the environment without taking into account the possible consequences on the ecosystem (Lina et al., 2010). Pollution in bodies of water near urban settlements has become a focus of attention for governmental agencies, especially in sectors where communities are more vulnerable.
The city of Cartagena de Indias, being a coastal city, has multiple water bodies that make up its ecosystems, both marine and freshwater. One of these bodies of water is the Juan Angola Channel which fulfills its function of breathing the *Ciénaga de la Virgen*; however, the Juan Angola Channel has become an environmentally affected water body due to the accumulation of pollutants and the lack of awareness from the community. This has affected the living beings in the surrounding neighborhoods and the mangrove. The Juan Angola Channel is a water corridor that runs from the *Ciénaga de la Virgen* to the El Cabrero Lagoon. Its waters surround the Rafael Núñez International Airport and different neighborhoods of the city such as San Francisco, La María, Canapote, Marbella, Crespito, and Crespo, among others. These neighborhoods, due to social aspects, contribute to the high rate of contamination that the channel contains. Informal human settlements, sewage dumping, and sedimentation have caused the environmental deterioration of the Juan Angola channel; the channel through which nutrients and oxygen flow from the city's two largest bodies of water (Pereira, 2020). In addition to this situation, there is evidence of mangrove overpopulation in some points of the Juan Angola Channel, specifically in the area parallel to the Cartagena airport, since at certain points of the channel the

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mangrove is growing excessively on both banks and is preventing sunlight from entering the water, thus increasing the turbidity of the water in those areas.
In the last 30 years the Juan Angola channel, located in the northern area of Cartagena de Indias, has been affected by several problems that have caused its environmental deterioration, mainly the landfills and invasions that have proliferated around its banks have caused a gradual degradation of its environmental offer. Due to its geographic location, the Juan Angola channel has attracted the attention of real estate developers, who have built large buildings with the complicity of local authorities. In addition, the dumping of sewage, lack of environmental education, and the inadequate disposal of solid waste represent a historical problem that has contributed to the recognition of elements that are decisive for the dimension of the contamination of the area. (Cabeza and Castaño, 2021). In the city of Cartagena, the entity responsible for the conservation of water bodies is the Environmental Public Establishment (EPA), which has presented projects for the recovery of the Juan Angola channel, being the most recent presented by the entity in May 2021, proposing a complete change in the infrastructure of the banks of the channel, improvement of the channel, control of human settlements and the recovery of the mangrove and fauna of the area.
This research was focused on the environmental evaluation of the water quality of the Juan Angola channel The natural processes for the formation of aquatic sediments are altered by human activities and are recognized as a reservoir for different chemical species, including heavy metals and organic compounds of a hydrophobic nature. A sediment has a great diversity of components such as clays, inorganic colloids such as iron and aluminum hydroxides, and soil organic matter, which consists of humic substances, and partially decomposed biomass. All these components present large areas of interaction that allow a wide variety of metals to be incorporated by different processes. Oxides and hydroxides of iron and aluminum adsorb trace metals much more than silicon oxides and hydroxides; that function as a support for organic matter. The sediments are formed by sand and gravel (2.00 - 0.20 mm), fine sand (0.20 - 0.02 mm), silt (0.02-0.002 mm), and clay with grain sizes less than 0.002 mm. The finer sediments, which generally contain higher concentrations of metals, accumulate in calm waters. These physicochemical phenomena, control the sedimentation processes and the associated mechanisms of different pollutants, depending on the pH, temperature, and dissolved oxygen conditions (Tejeda-Benitez et al., 2016).
There is abundant evidence about the accumulation of pollutants in the sediment of rivers, lakes, and bays. Due to their high capacity to accumulate persistent organic pollutants and metals, aquatic sediments are considered a source of long-term contamination for aquatic organisms. The study of sediments is an important step in the mapping of possible routes of exposure to various aquatic organisms, since contaminants in sediments may be bioavailable to the organisms inhabiting the sediments (Tejeda-Benitez et al., 2016).
When a body of water is contaminated by multiple sources and chemical compounds, the evaluation of a group of them does not provide sufficient information on its toxicological or environmental profile. For this reason, we use toxicological determinations in which the effect of the sediment on a whole organism is evaluated, in this case, *Caenorhanditis elegans*. This organism is a non-parasitic nematode which due to its many convenient features has become an important model in biology research because of several advantages: easy maintenance, short life cycle, and low cost, which is repeatedly used as a biological model in toxicology assays. The lethality assay is performed to determine the death rate derived from acute toxicity in a concentration-response curve basis. 10±1 young adults are transferred in microplates that contain different concentrations of the toxicant and negative control. The exposure is carried out at 20 °C during 24 h in the absence of food. Then, the number of live and dead worms is counted through visual inspection using a dissecting microscope (Zhuang et al., 2014; Williams and Dusenbery, 1990). Death is assumed when there is no movement during an observation period of 30 s (Choi, 2008).
The pollution present in the Juan Angola Channel directly affects the flow of nutrients and oxygen between Cartagena's largest aquifer ecosystems, the Bay of Cartagena and the *Ciénega de la Virgen*. In addition, this increases social problems and influences the life quality of vulnerable communities in the surrounding areas.

2. Methodology

2.1 Study area

The study area in this research was the Juan Angola Channel, from which sediment samples were taken at different points: E2(10°25'38.1 "N 75°30'47.5 "W) and E2(10°26'26.2 "N 75°30'49.6 "W) during the rainy (2021) and dry (2022) seasons. The points are represented geographically in Figure 1.

Figure 1. Sample points.

3. Materials, Equipment, and Reagents

The equipment and instruments used for the experimental development of the research are shown in Table 1.

Table 1 Equipment required for the experimental procedure.

Name of the equipment	Brand	Function/use
Analytical scale	Vibra HT	Determine the weight of the samples before submitting them to the lyophilization process.
Lyophilizer	--	Decrease moisture content in sediment samples.
Sifter	Edibon orto alresa Mod. Vibro	Select the particle size of sediment samples after passing them through the freeze-drying process.
Soxleth	–	
Incubator	–	Incubation and growth of *Caenorhabditis elegans.*

4. Experimental Procedure

Two field trips were during the rainy and dry seasons in which the sediment samples were collected from the Juan Angola channel for toxicological analysis. A general scheme of the process is shown in Figure 2.

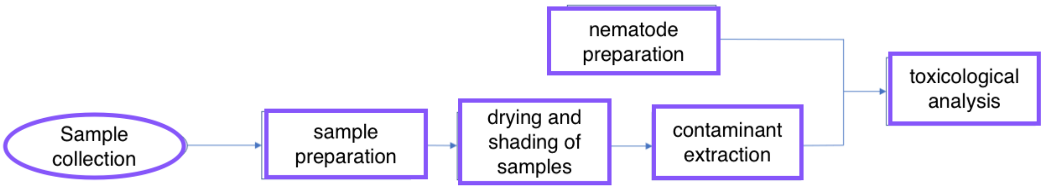

Figure 2 Experimental procedure.

4.1 Preparation of sediment samples

The samples taken were stored at temperature conditions of (-20 °C) in a refrigerator located in the facilities of the University of Cartagena, in order to preserve the samples (González et al., 2021). The samples were distributed in glass material, using four bottles per sampling point, having 24 bottles in total with 20g of content each (Tejeda et al., 2016).

4.2 Sample drying and sieving process

All samples were kept in polyethylene bags, stored in an icebox at 4 °C, and preserved at -20 °C. Sediment moisture was removed by freeze drying at -50 °C and -0.019 mbar. The dried sediments were crushed, sieved to <63 µm, and stored at -20 °C. The preparation process of sediments is shown in Figure 3 (González et al., 2021).

Figure 3. Preparation of sediments A. Extraction. B. Freeze drying. C. Dried sediment. D. Homogenization. E. Sieving. F. Sieved sediment.

4.3 Extraction of contaminants

Aqueous extracts were obtained as follows: about 30 g samples of dried sediment were mixed strongly with 30 mL of water until entirely wet sediment and then placed overnight at 4 °C (Anbalagan et al., 2012). Contaminants were extracted from the sediment samples by Soxhlet extraction (Santos, 2011) using distilled water as a solvent and using 20 g of the previously sieved sediments as solute, in a 1:1 solution.

4.4 Nematode preparation

The nematodes used were supplied by the microbiology laboratory of the University of Cartagena, the nematodes remained in Petri dishes in an incubator at 20°C (Torres and De Los Rios, 2022), which ensured the optimal state of the worms prior to analysis. Experiments were performed with the Bristol N2 wild-type strain. Strains were kept at 20 °C in Petri dishes with K agar, prepared with KCl, NaCl, agar, peptone, cholesterol, $CaCl_2$, and $MgSO_4$ seeded with Escherichia coli OP50 (Williams and Dusenbery, 1990). Worms were subjected to age-synchronization via bleach solution prepared with NaOH and HClO, which oxides any organism, but eggs are protected by their shell, and approximately 14 h after, eggs have hatched, and larvae are in the L1 stage. To perform the toxicological analysis with the nematode, 10 cell culture plates of 96 wells each were used, and approximately 10 nematodes were introduced in each well in larval stage L4 and exposed for 24 h to totally extract, and a 1:1 solution of extract in distilled water (Quijano, 2021).

4.5 Toxicological analysis

To perform the toxicological analysis with the nematode, 10 cell culture plates of 96 wells each were used, and approximately 10 nematodes were introduced in each well in larval stage L4 and exposed for 24 h to totally extract, and a 1:1 solution of extract in distilled water at 20 °C, with four replicates for each treatment (González et al., 2021). After 24 hours, using a stereoscope, the number of live individuals were counted and a comparison was made between each of the points analyzed, and the locomotion of the live organisms after the test was analyzed ((Quijano, 2021; Hidalgo, 2022).

5. Results

Figure 4 shows results for the station E1 and E2 in the rainy season (2021) and dry season (2022).

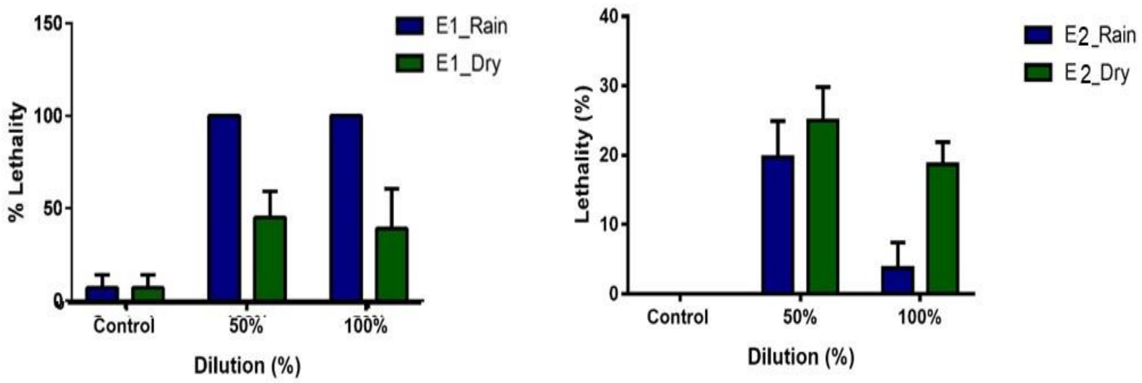

Figure 4 Lethality

Figure 4 shows the lethality percentage of nematodes 24 hours after exposure to contaminant extracts taken from sediment samples. Analyzing the graph, it is evident that point E1 2021 had a lethality of 100%, that is, none of the nematodes survived the exposure to the extract in any of the dilutions. Point E1 2022 had a lethality lower than 50% in both dilutions, however, it had a higher lethality in the pure extracts than in the 50% dilution. The absolute lethality of nematodes at point E1 2021 may be due to the fact that this sample was taken during the rainy season, a time when sediments are stirred up and all the runoff and streams from the city reach the Juan Angola channel loaded with garbage, emerging pollutants, heavy metals, among others. Figure 4 shows the percentage of nematode lethality 24 hours after exposure to the contaminant extracts taken from the sediment samples. Analyzing the results, it is evident that point E 1 in the rainy season is more contaminated than point E2 in the dry season and this is verified in both dilutions.

Establishing a comparison between both points, point E2 has a lower degree of contamination than E2, both in the dry season and in the rainy season, since the lethality of point E2 does not exceed 30%, while the lethality recorded at point E2 does not decrease by 40%. Therefore, it would be precise to assume that point E2 is the one that needs greater intervention to correct the Juan Angola channel. The sun emits radiation, and this has a degradation potential, this may be one of the reasons why in the dry season the conditions of the channel at point E2 are better compared to the rainy season, taking into account that Cartagena is one of the cities with more solar radiation in Colombia (4.5 - 5.0 Kwh/m^2). It should also be noted that point E1, where the Juan Angola channel begins, is directly connected to the *Ciénaga de la Virgen*, which is oxygenated by the stabilized tidal inlet.As previously mentioned, *C. elegans* is a biological model that is easy to handle for toxicological studies such as the one carried out in this document. It is easy to observe thanks to its transparent body; however, this can become a difficulty when carrying out tests with sediments since these have a dark tone. Therefore, it will generate problems while observing the behavior of the nematode when exposed to contaminants. The tests carried out in this research provide the necessary knowledge to share with the communities surrounding the Juan Angola channel and the authorities and thus contribute to a future restructuring and sanitation of the channel.

6. Conclusion

C. elegans was used to generate the toxicity profile of sediments taken from two sampling points along the Juan Angola channel in two field trips distributed according to climatic conditions. The aqueous extracts of the sediments, to which the nematodes were exposed, caused lethal effects on them, showing complete lethality at point E1 2021. However, at point E1 2022 the nematodes showed a better response to the stress caused by exposure, with a lethality of approximately 50%. The results showed that the body of water has high toxicity levels, displaying the need to generate remediation strategies that allows pollution decrease in the ecosystem and generate positive effects on the health of vulnerable communities settled close to this area and on mangroves, aquatic species, and birds that inhabit the system. Additionally, the results of this research could be helpful for the environmental authorities to focus their main strategies in order to contribute to the recovery of the Juan Angola Channel and establish protocols with the communities in the area to reduce anthropogenic impacts on the ecosystem.

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