UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

Ecotoxicological assessment of activated sludge

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

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Urcuquí, August 2021

Urcuquí, 18 de febrero de 2022

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ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA
CARRERA DE BIOMEDICINA
ACTA DE DEFENSA No. UITEY-BIO-2022-00014-AD

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Resumen

La ecotoxicología nació de la necesidad de evitar problemas de contaminación ambiental a gran escala por toxinas; a medida que ha ido avanzando, ha ido resolviendo problemas ya que en una sociedad tecno-industrial regida por el consumo. La ecotoxicología es una disciplina necesaria para reflexionar sobre los costes y beneficios de las decisiones tecnológicas e industriales. La industria minera y su notable auge hacen que se requieran bioensayos para verificar los impactos ambientales que pueden causar las perforaciones, la liberación de sedimentos al medio ambiente. Este proyecto minero presenta una cantidad de metales valiosos; por ello, estarán presentes en los lodos de perforación que lo conforman. La liberación de aguas de descarga provenientes de los procesos de depuración de las plantas de aguas residuales de los campamentos. Este trabajo tiene como objetivo analizar bioensayos de los sedimentos de perforación del pozo "D" y pozo "R", las aguas residuales del "campo X" y "campo Y", para verificar si afectan a los seres vivos del ecosistema en el caso que no exista una disposición y tratamiento final adecuado. Para verificar la toxicidad de lodos y sedimentos se realizaron pruebas de germinación a las 72 horas utilizando semillas de lechuga-rábano, desarrollo de plántulas para comprobar la inhibición, y presencia de anormalidades a los 10 y 20 días. Pseudomonas spp. fueron inoculados en medio sólido y líquido para verificar la existencia de inhibición a diferentes concentraciones de sedimentos de perforación. Las pruebas de inhibición en hongos se realizaron sembrando diluciones de suelo en agar de dextrosa de patata y sometiendo los hongos del suelo a concentraciones variables de sedimentos de perforación y lodos de aguas residuales.

Palabras clave: bioensayos, ecotoxicología, germinación y plantas de tratamiento de aguas residuales.

Abstract

Ecotoxicology was born from the need to avoid problems of large-scale environmental contamination by toxins; as it has progressed, it has been solving problems since in a techno-industrial society governed by consumption. Ecotoxicology is a necessary discipline to reflect on the costs and benefits of technological and industrial decisions. The mining industry and its notable boom make bioassays required to verify the environmental impacts that drilling can cause, the release of sediments into the environment. This mining project features a number of valuable metals; therefore, they will be present in the drilling muds that make it up. The release of discharge water from the purification processes of the wastewater treatment plants in the camps. The objective of this work is to analyze bioassays of the drilling sediments from well "D" and well "R", the wastewater from "Camp X" and "Camp Y", to verify if they affect the living beings of the ecosystem in the case that there is no proper disposal and final treatment. To verify the toxicity of sludge and sediments, germination tests were carried out at 72 hours using lettuce-radish seeds, seedling development to verify inhibition, and the presence of abnormalities at 10 and 20 days. Pseudomonas spp. were inoculated in solid and liquid medium to verify the existence of inhibition at different concentrations of drilling sediments. Fungal inhibition tests were performed by seeding soil dilutions on potato dextrose agar and subjecting the soil fungi to varying concentrations of drilling sediments and sewage sludge.

Key Words: bioassays, ecotoxicology, germination and wastewater treatment plants

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1 INTRODUCTION-JUSTIFICATION:

Ecotoxicology is a science that combines ecology with toxicology to predict and study the effects of pollutants within ecosystems. Likewise, bioassays are used primarily as they uses living organisms to recognize the concentration and effects of possible harmful contaminants[1]. Bioassays work with living organisms and offer rapid responses, are cost-effective, sensitive, and detailed compared to traditional methods. Likewise, an analysis must not be specific for a toxicant because it does not provide enough information to measure the biological impact on the ecosystem. However, it is essential to choose the test organisms because some are not ethically accepted, others are expensive, only serve for analyzing solid waste, among others[2,3].

Currently, the project has two camps "X and Y". The “X camp” has three wastewater treatment plants that have been increasing depending on the number of people in the camp, two plants are for black water and one for gray. In the case of “camp Y”, it has two plants with combined gray and black water.

The plants extract nitrates, ammonia, phosphates, organic compounds, fecal coliform bacteria, organic matter, which are pollutants to obtain discharge water that is suitable according to the country's regulations for evacuation. Likewise, during this process sludge-sediment is generated that can be used in agriculture. However, the sludge must be examined to obtain characteristics associated with a corrosive, reactive, explosive, toxic, flammable and biologically infectious analysis. [4].

Many countries make classifications considering elements such as the quantity and quality of the sludge to treat it later. The sludge treatment is due to the possible existence of germs and parasites that are harmful to humans [5]. Likewise, a management is carried out to take advantage of hazardous waste in non-hazardous resources for recycling, composting, energy recovery and dumping plans.[4]

The mining project has several drilling sites where the sediment passes through an SRU (Solid Removal Unit) and the liquid with sediment is mixed with xanthan gum to make it slimy. In Ecuador, any company that works with drilling fluids places these sediments that pass through SRU in pits where it is dried and closed [6]. Therefore, all this confined sediment can disturb nearby vegetation. Disposal methods for waste are expensive such

as pyrolysis due to its high energy requirement, and companies seek to reduce expenses preferring to bury the sediments [7].

The present work seeks to use ecotoxicology tools such as bioassays to analyze whether the resulting sludge from the treatment plants of the "camps X and Y" are toxic to the ecosystem. Nevertheless, currently this sludge is sent to an environmental manager for treatment and final disposal. Likewise, it seeks to solve the problem of confining all drilling sediments in pits, verifying if the sediments have an inhibitory character for the growth of plants, fungi and bacteria.

The mining company agreed with the YachayTech Research University of Experimental Technology to carry out this project. Where the company provided the ideal information and access to its camp for sampling and field experimentation. Experiments were also carried out in the university laboratory with sludge from gray and black water wastewater treatment plants and with drilling fluids from the D pit.

2 GENERAL AND SPECIFIC OBJECTIVES

2.1 General objective

- Analyze sewage plant sludge and drilling sediments using ecotoxicology bioassays to find out if they inhibit ecosystem organisms

2.2 Specific objectives

- Evaluate the germination, vigor of radish and lettuce seeds subjected to different doses of sewage sludge and drilling sediments.
- Examine characteristics of the discharge water and the one that enters the aeration process of the wastewater plants.
- Study of soil bacteria and fungi inhibition using different concentrations of drilling sediment.
- Determine the sedimentation rate of the black and gray wastewater plants of mining project camps.

3 THEORETICAL BACKGROUND

3.1 Ecotoxicology

The term ecotoxicology is implemented in 1975 by René Truhaut to replace the environmental toxicology that studies the dose. However, it does not relate the dose to its

effects making it a science for safety. Likewise, he separated the definitions of both since ecotoxicology refers to contamination in ecosystems and environmental toxicology specifically in pollution caused by humans[8,9]. Subsequently, Paracelsus related the toxins in the environment with the exposure time since an isolated dose would not have an impact for this type of study[9].

Ecotoxicology is a science that studies the evolution, origin and interaction of pollutants with ecosystems and the living beings that make it up. Likewise, it studies how physical agents and chemical compounds affect species under different exposure conditions and also takes into account pollutants that do not act as a toxin but are not desired for any ecosystem[9,10].

3.2 Germination

It is a test that allows finding the capacity of the seeds to form normal seedlings and their physiological quality. The physiological quality of seed allows determining the germination capacity, emergence, and development of structures to be considered a seedling. Therefore, the seeds must be in optimal light, temperature, water, and air conditions during the germination tests[11,12].

The first germination stage is the imbibition, where the tissues of the seed are absorbed and hydrated. Its time depends on the environment surrounding the seed (temperature, water deficit, or excess) and its permeability to water. Absorption occurs since the cell walls have matrix forces that carry out this process even though the seed is not viable[12,13]. Excess water hinders the permeability of oxygen, preventing germination in many seeds and allowing fungi that can infect the seeds. Likewise, if the embryonic tissue swells too quickly, it can suffer a lesion that prevents its formation[14].

In the second germination stage, there is an activation of the metabolism with less retention of water[13]. The internal reactions of the seed transform the macromolecules that reserve the seeds (carbohydrates, proteins, and lipids) into soluble molecules through the hydrolyzation of enzymes. After the enzymatic action of amylases, proteases, glucose lipases, amino acids, glycerol, and fatty acids, they will be converted into energy that will enter the embryo[12,14,15].

The last stage of germination increases the absorption of water and respiration. Finally, the growth of the radicle through the seminal sheaths is observed, signifying the end of

the process[11]. The extension is due to the pressure of the internal liquids contained by the walls of the embryonic axis. However, in the previous phases, they are reversible because if the conditions are not favorable, a hard seed can be produced. Still, if the necessary is not fulfilled at this stage, the kernel will die because it reaches irreversible physiology. The rupture of the seminal cover by the radicle implies the beginning of the development of the seedling. The reserve macromolecules will hydrolyze due to the high energy expenditure[12,14,15].

3.3 **Assays in Petri or Petrifilm dishes**

A culture medium is a mixture of components and nutrients to design an environment with optimal conditions for the development of organisms. However, each microorganism has different needs, so there is no universal culture. In practice, to obtain pure cultures, it is necessary to sterilize the medium and the work area since microorganisms are found in all types of ecosystems. The culture media are generally carried out in Petri dishes that allow different studies, such as the growth of viable microorganisms to form colonies known as plate count[16].

Among the most used methods for plate, counting is the pour plate and spread plate technique. In pour plate, a diluted sample containing bacteria is placed on the Petri dish, the sample is rotated, the medium is placed, generally cooled to 50 ° C, shaken to mix, and incubated when the medium is solidified. The spread plate is usually used to count aerobic bacteria where the medium is first placed on the plate. Then, when it is solidified, the known dilution is placed and spread throughout the medium[16,17].

A widely used microbiological method is Petrifilm plates that save time, reduce contamination, and are highly effective. The Petrifilm consists of two thin layers. The upper one is an adhesive plastic with a water-soluble gel, and the lower layer is a graph paper with nutrients depending on the needed method and water-soluble gel. The technique consists of lifting the plastic coating and placing 1ml of the dilution or sample on the middle of the lower film. Then it is covered with the first layer. Next, the sample is spread, incubated under conditions according to the microorganism, and the count is carried out using the grid. Among all the types of Petrifilm plates, there is one for counting E. coli / Coliforms with Violet Red Bile Glucose medium nutrients, a gelling agent, and an indicator. Most of the E. coli in the sample will have a blue residue due to beta-

glucuronidase production; there will also be bubbles produced by blue colonies or red colonies associated with coliforms[18,19].

The inhibition halos are a simple test where microorganisms of interest are inoculated on an agar plate. Around the inoculated medium, discs with substances that inhibit the growth of microorganisms or antibiotics are placed. The antibiotic diffuses radially, and discs are produced after 24 hours of incubation. The diameters are measured to find the minimum inhibitory concentration to know the antibiotic concentration that is between the phase of inhibition and the growth of the microorganism[20,21].

3.4 Drilling well

A well is an engineering system that seeks to have contact with minerals through a drilling process. Drilling is done by a rotational process applying thrust force with the use of an auger that cuts, sweeps, and lifts the rock. The thrust force and movement are given by the drill string transmitting from the rotating machinery using a motor. For the design of drilling well, it is essential to determine the formations to be perforated, stability, seismic sections, the size of the equipment necessary to drill, define coordinates and more over. However, it is crucial to know the formations to be traversed and the problems that may arise when selecting drilling fluids[6,22].

3.5 Drilling fluid

Drilling fluids are mixtures with solids, water, or oil that pass from the drill string to the bit and allow drilling to be facilitated according to the demands of the well to minimize formation damage[7]. Its composition varies according to the need to control pressures, not affect drilling, not damage circulation, avoid well erosion and improve penetration speed by keeping the well clean. Typical products for drilling fluid formulation are xanthic gum, an inhibitor polymer, polyanionic polymer, clay inhibitor, lutite inhibitor, latex starch, and surfactants[6,23].

Typical products for drilling fluid formulation are xanthic gum, an inhibitor polymer, polyanionic polymer, clay inhibitor, lutite inhibitor, latex starch, and surfactants[24].

Xanthan gum is a polysaccharide generally derived from the bacterium *Xanthomonas campestris* used as a thickener in liquids[23]. Its high molecular weight and suspending densifying solids provide a high viscosity that is not affected by high or low temperatures.

Its viscosity improves penetration capacity, reduces pressure while drilling, increases efficiency, avoids the use of clays due to their high suspension and moreover[25].

This low molecular weight acrylamide copolymer encapsulates cuttings and prevents clay scattering. Its principal function is to prevent clays and shale from hydrating. When they swell, they create instability in the well, reduce penetration, and stick to the pipes because the clays are fixed to the bit and all the materials at the bottom[26,27].

It is a product derived from cellulose with properties similar to carboxymethylcellulose that modifies the viscosity, elasticity, plastic limit, behavior, and flow of water-based fluids, allowing the use of fewer additives for quality drilling fluid[24]. Also, it avoids using too many additives, but it also requires little of the same for the fluid. In addition, it resists the presence of bacteria preventing the use of disinfectants to reduce the number of microorganisms[28].

This liquid polyamine prevents clays from absorbing water to damage the well by sticking to machinery. Likewise, it does not affect the viscosity, filtration, encapsulates cuttings, allows colloidal solids not to accumulate since it acts as a lubricant. However, to create a quality drilling liquid, the inhibitor must have the property of exchanging a cation with a large ionic radius for a lower one. This action prevents swelling that can crumble the walls of the drilling hole[29,30].

Shales are sedimentary rocks characterized by having fine grains that, when hydrated, expand, creating instability in the well, clogging pipes and machinery. The main mechanisms to avoid shale hydration are reducing water activity using polyols, developing the membrane with a phase that covers the formation surfaces, salinity, and sediment in the pore-surfaces of the shales with silicates. For inhibition, gilsonite is generally used, a stiff hydrocarbon that is not soluble in water, remaining as particles that can be used to seal microfractures of shales and that sand does not infiltrate due to its malleability[31,32].

This additive effectively plugs seal shales and filter cakes due to its copolymer property of joining different shapes using chemical bonds randomly distributed by monomers. Therefore, this control additive improves the performance of water-based drilling fluids by preventing swelling of shales from water absorption. Likewise, it supports high and low temperatures, and if there is salinity in the drilling fluid, it improves performance to avoid losing water by sealing soft permeability sands[6,33].

Surfactants are additives that have an amphiphilic characteristic, having a soluble part that migrates towards aqueous media because it is hydrophilic and a hydrophobic portion that moves away from water[34]. Therefore, in drilling liquids, they are concentrated by interfaces such as water-oil, water-air to perform their functions of preventing foaming, lubricating, moistening, improving drilling speed in the presence of shales. However, concentrations between 5% to 7% must be used in the drilling fluid not to affect its properties and not be very toxic to the ecosystem[35].

3.6 **Waste water treatment plants**

Wastewater is water altered with anthropogenic impurities of domestic and industrial origin. This water is discharged through surface or underground sewage systems and diluted by treatment plants to be exposed to purification and release[36]. However, the released water must meet specific standards so as not to cause damage to living organisms in the environment, such as pH, dissolved oxide, total chlorine and more over. Likewise, water sources such as lakes, seas, rivers cannot absorb or eliminate water pollutants with poor treatment and damage the balance to preserve natural conditions of these bodies of water[37].

Wastewater treatment plants are structures used to purify wastewater to obtain discharge water released into the environment and expected not to harm living beings. For the construction of a residual plant, the liquids or substances that are to be purified or reused, the concentration of the compounds in these substances, and the desired product as the discharge water must be reviewed[38].

The categories of these processes are chemical contact physics, where there is filtration, screening where large objects that can interfere with downstream equipment are removed, precipitation, and chemical destruction-removal of components[39]. Another category is biological because it relies on organisms to break down pollutants from wastewater. In these processes, there is the method of the stabilization lagoon where shallow excavations of rectangular or square shape are made surrounded by slopes that are typically used in agriculture for reuse. Second, the rotating biological contactor works using fixed films such as trickling filters or biological filters where microorganisms are placed on a support such as a rotating drum that rotates slowly and degrades the contaminants[38,40,41].

Activated sludge belongs to the category of biological processes because microorganisms degrade pollutants. An anaerobic system gives the purification with continuous aeration and recirculation of sludge to biodegrade the substances in the wastewater[4,42].

In the activated sludge process, a culture of various aerobic bacteria that have oxygen dependence and facultative heterotrophs that consume organic matter is carried out. The organisms adhere to a device similar to the trickle filter or biological contactors. Subsequently, all the material in the reactor with microorganisms or mixed liquor becomes by-products and more organisms known as "biomass." The biomass formed mixes with each other to form a large brown-colored biological mass. The content of the entire reactor with aeration passes a secondary clarifier where a part of the biomass is separated from the water to return to the reactor where the microorganisms will continue to degrade pollutants and the other, which is a surplus, will be removed as waste, also known as waste activated sludge "WAS." Then, the water or effluent will go to another treatment process[42–44].

3.6.1 Waste water organic and inorganic content

Wastewater has various inorganic contaminants, including sand, minerals, metals, and other non-biodegradable materials. Organic pollutants include carbon-based materials of animal or plant origin such as waste, food, oils, fats that can be a food source for living organisms. However, several organic products of artificial origin are not biodegradable[45]. Likewise, if not regulated effectively, several solids accumulate in the environment with each discharge water and increase the sedimentation of aquatic systems that harms animals and plants. Total solids are divided into settleable, suspended, colloidal, and dissolved[46]. Dissolved solids can only be noticed when the water in the sample evaporates, and when they pass through filters, they cannot be observed. The settleable solids will rest at the bottom of the sample after a particular time; they are observable and used in the sedimentation tests to control the operations of the treatment plants. Colloidal are microscopic in size, do not sediment, and provide the cloudy appearance of water. Finally, the suspended solids can be observed in the samples; they provide turbidity to the water and can be filtered[42].

3.6.2 Characteristics that must be regulated in wastewater

An essential parameter to measure the degree of wastewater pollution is the biochemical oxygen demand (BOD). It measures the amount of oxygen needed by microorganisms

that reproduce and feed on organic matter in wastewater, such as aerobic or anaerobic bacteria, fungi, and plankton[47]. As there are more pollutants to metabolize, the bacteria need more oxygen that will be extracted from the aquatic environment. Also, inorganic matter such as ammonia will nitrify to nitrate, depleting the amount of oxygen. However, there are several obstacles to determining the BOD since it depends on the microbial activity and therefore has low precision. Second, many toxic agents, such as high pH or chlorine, can inhibit the activity of bacteria, so it would be necessary to neutralize the pH and suppress the chlorine level to repeat the BOD calculation by inoculating new bacteria[48].

To measure the BOD of wastewater in the laboratory, a small sample must be diluted with a nutrient-rich buffer. Second, the initial concentration of the dissolved oxide of the dilute is calculated, and the sample is incubated at 20 ° C for five days. BOD is a process that requires a lot of time and depends on the temperature for decomposition; for them, this temperature and standard time is used, also known as BOD5. Subsequently, the final dissolved oxygen and BOD are calculated with the depletion of oxygen and the sample dilution amount. However, a nitrification inhibitor must be used to meet the Carbonaceous Biochemical Oxygen Demand (CBOD). Using CBOD corresponding to the organic part, the total BOD is subtracted to find the Nitrogenous biochemical oxygen demand (NBOD) corresponding to the ammonia nitrified to nitrate[47,49].

3.6.3 Nutrients

The sewage waste has nutrients such as phosphorus, nitrogen, potassium that are essential for living beings. They can also act as fertilizers, increasing the growth of aquatic plants in streams. Phosphorus is a necessary nutrient for plant development, so many farmers add phosphorus to stimulate their crops. However, nitrogen is naturally more abundant than phosphorus that appears from dissolved minerals[50]. However, nutrient-accelerated development will modify the stream into a swamp due to the enormous growth of weeds and sediment. In addition, the excessive growth of organisms due to the action of nutrients will deplete the oxygen in the bodies of water, increase the mud, and the smell that the excess of organic matter gives off can cause respiratory problems. Therefore, nutrient control is vital to avoid this eutrophication process when discharging the treated water to the environment[51,52].

3.6.4 Toxins

Many household wastes contain infectious agents that affect plants, humans, and animals. Likewise, they can affect treatment plant workers, so the necessary equipment to work in a treatment plant is essential. Discharge water that is not adequately controlled will spread toxic materials from pesticides, herbicides, acids, bases, and chlorine that will affect public health and the environment[42].

Among the halogens, chlorine is one of the most effective microbial agents due to its oxidative capacity, where it destroys cell walls and therefore eliminates cells. Likewise, it is often used in the form of hypochlorite gas that will react with water to create hypochlorous acid (HOCl) and hydrochloric acid (HCL), where the reactions with inorganic matter are faster than oxidation in organic matter[53]. When HOCL ionizes, it forms the hypochlorite ion, which, together with HOCL, are considered residual or free chlorine. Residual chlorine reacts with iron, magnesium, nitrogen, ammonia, bromides, organic matter, and reducing substances. First, when there is a small amount of residual chlorine, the reducing compounds will destroy the chlorine. Then, by increasing the amount, the chlorine will form compounds with the organic part and chloramines. Likewise, if more chlorine is added, the "breakpoint" is reached, where the formed compounds begin to be eliminated. Then, the free chlorine that remains becomes an active disinfectant agent[54,55]. Combined chlorine is the combination of free chlorine with ammonia and organic matter with nitrogen forming dichloramine, trichloramine, monochloramine and moreover. However, this chlorine has low disinfection, causes irritation, has a bad smell, indicating when the "breakpoint" is reached. When it exceeds the breaking point, the amount of combined chlorine is reduced, going an equilibrium[56,57]. The sum of the free chlorine and the residual chlorine gives the total chlorine that must not be more than 0.6 mg / l concerning the residual chlorine level in the discharge water. Therefore, it is recommended for wastewater chlorination to use 10mg to 30mg of chlorine with a contact time of 30 minutes. However, variables such as distribution, size, and concentration of the total suspended solids must be considered to find the necessary chlorine dose and optimal time. Next, it is required to know distribution factors, nature, and the number of organisms in the liquid. Finally, what chemical compounds will react with chlorine[54,58].

3.6.5 pH

The pH control is essential in the management and quantity of chlorine because at high pH values; the chlorine will be in the form of a hypochlorite ion with low disinfecting power. Therefore, it is vital to use a pH between 7.2 and 7.8 whereas you get closer to neutral pH, less residual chlorine will be needed[54,55].

The pH is a significant factor that affects the processes of microbial activity such as nitrogen removals such as nitrification, ammonification, and denitrification[59]. Ammonification is when nitrogenous compounds are degraded to simple compounds by microorganisms to ammonia or ammonium ion. The efficiency of this process will be affected if the sample does not have a pH of 6.5 to 8.5. Likewise, for aerobic microorganisms to use molecular oxygen to oxidize ammonium to nitrate or nitrification, a pH of 7 to 8 is required. However, if the pH of the sample is at 4.5, there is an inhibition of nitrification because it is necessary to consume alkalinity in this process. The denitrification process works optimally at a pH of 6.5 to 8, eliminates the nitrate that causes eutrophication, and impairs the potability of the water. However, the pH should not be less than 5 because the process is inhibited, damaging the discharge water since it is reduced to nitrite, which is carcinogenic[60,61].

3.6.6 Surfactants

Surfactants are substances that make it possible to reduce the surface tension of surfaces in contact between two phases, for example, liquid-liquid or liquid-solid. Generally, they have two structures where their head has hydrophilic groups and a hydrophobic-looking hydrocarbon chain. They are products commonly used for their detergent properties, solubility, emulsion, wetting previously processed, for which they are found in large quantities in wastewater[62].

It is crucial to control the level of surfactants because they have many significant effects on water bodies, such as increasing the pH of wastewater affecting the life cycles of aquatic organisms. Second, rivers that receive large amounts of these detergents raise their nutrients, causing excessive algae growth, causing reek due to the presence of phosphorus. Heavy metals will dissolve genetically, affecting the species. Chlorine and organochlorine compounds will increase their concentration, become carcinogenic, and affect the DNA structures of organisms. Likewise, it affects the sedimentation, coagulation, and flocculation processes, affecting treatment plants. Therefore, the

discharge water will demand an excellent demand for oxygen from the environment, causing the death of flora and fauna due to anoxia[63,64].

3.6.7 Fecal coliforms

Fecal coliforms are bacterial species formed by three genera *Escherichia, Klebsiella, and Enterobacter*. Its biochemical characteristics are essential to measuring the level of contamination present in water and food. They are generally transmitted through the intestines of warm-blooded people and animals. When released by feces, they remain in the water longer than many pathogenic bacteria[65]. However, they behave in the same way because they can even cause the death of individuals. Likewise, the coliforms present are difficult to isolate and identify because many coliforms do not come from feces, so the total coliform values are taken that include pathogens and those that are not dangerous[66].

The level of total coliforms is used as an indicator of water quality because most pathogens do not support being in seawater for a long time. Therefore, a low level of fecal coliforms would show few or no pathogens. In addition, in many Latin American countries, the maximum allowable is 1000 to 2000 fecal coliforms per 100 ml of wastewater from treatment plants[67].

4 MATERIALS AND METHODS

4.1 DRYING

Drying beds were made using cardboard and aluminum. The gray and black water sludge was extracted from the WWTPs of the "X camp" from the chamber that enters the aeration. In the case of "Y camp", the sludges are combined with gray and black water and was also extracted from the same aeration site. Subsequently, the sludge was expected to settle to extract it in the drying beds and leave it in a tent made of geomembrane near the composting area of the "X camp".

4.2 Soil pH measurement

The hydrogen ion concentration for the earth and drilling sediments was calculated using potassium chloride, an electrolyte that helps to avoid pH variations. Solutions were prepared with a ratio of 1: 1.1: 2.5 and 1:10 (soil: 1M KCL solution) and mixed for 10 minutes. Likewise, the same dilutions were prepared but using distilled water with soil to

observe the variations. Finally, the pH was calculated using two different pH meters that were previously calibrated (HI98129 and BOECO PT70).

4.3 WWTP measured parameters

The concentration of hydrogen ions in the case of WWTPs was carried out every three days, taking samples of the sludge in aeration and directly from the final discharge water for both camps. In addition, in the case of the “X camp”, the pH of the gray water plant and the black water plant were taken, and in the case of the “Y camp”, the samples were taken from both plants mixed with black and gray water. Likewise, the dissolved oxygen of the discharge water and the aerated sample was measured using the dissolved oxygen meter "HI 9146" that was previously calibrated. However, measurements were taken every six days in the case of residual chlorine and total chlorine parameters.

4.4 WWTP sludge bioassays

4.4.1 Bacterial identification

The TSB solution (tryptic soy broth) was prepared with 6 grams of powder and 200 ml of distilled water. It was heated and stirred constantly for 15 minutes, and 10 ml was placed in two test tubes. First, sludge water was extracted into 4-liter bottles that entered the WWTP aeration. Next, when they sedimented, 1ml of black water was taken for one test tube and 1ml of gray water for the other test tube. The type of bacterial growth in both tubes was monitored at 24 and 48 hours. Subsequently, the process was repeated three times with the “X camp” sludge and three with the “Y camp” sludge.

Five types of bacteria can be found in bacterial growth in test tubes according to their ratio or oxygen requirement. Obligate aerobe depends on at least 20% of the presence of atmospheric oxygen for their proper metabolism: Facultative anaerobe is generally pathogenic bacteria that can use oxygen to accept electrons for correct metabolism and can also be supplied with energy with fermentation reactions. Aerotolerant anaerobes are bacteria that use fermentation to obtain power for their proper metabolism; their main characteristic is that they support low concentrations of oxygen, for example, 2% to 8%. Obligate anaerobes are bacteria that oxygen is toxic. The strict ones tolerate only doses lower than 0.5% since they do not have cytochrome oxidase to metabolize molecular oxygen, require low potentials and obtain energy from fermentation; those that support more oxygen generally cause infections. Finally, microaerophiles require small amounts

of oxygen that are less than those present in the atmosphere of 2 to 10%, and in the case of anaerobic conditions, they grow little, as can be seen in the figure 1 [68–70].

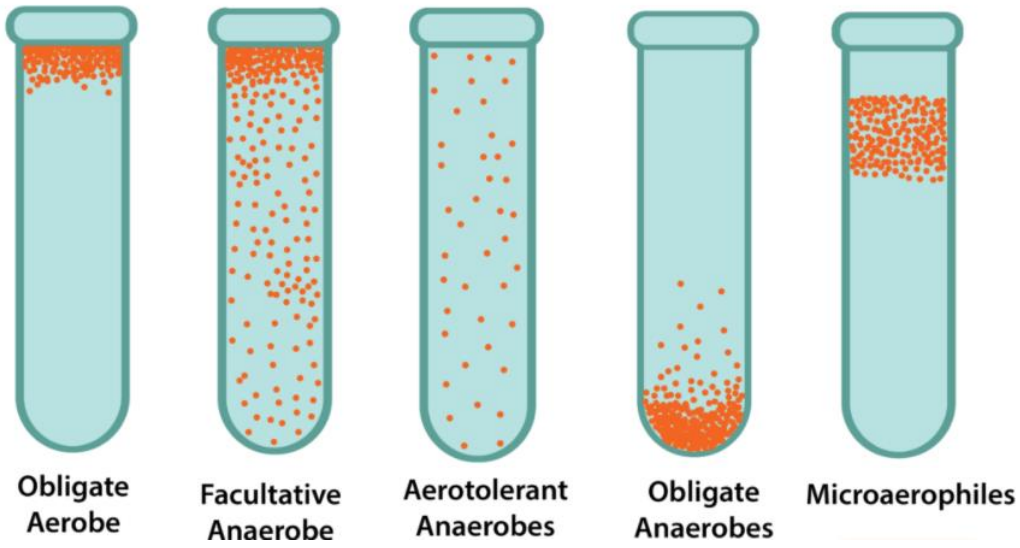


Figure 1: Types of bacterial growth in test tubes according to oxygen requirements[70].

4.4.2 Germination in Petri using WWTP

Firstly, dilutions were made for each WWTP 1: 150 (mud: soil) with 298 grams of soil and 2 grams of mud. Therefore, in the case of the plants of "X camp" there were 2 dry dilutions "one for gray water and the other for black water". The dry dilutions were mixed with 300 ml of distilled water and stirred for 20 minutes. 1 ml of solution was extracted and poured in 3 Petri dishes with absorbent kitchen paper. Having a total of three Petri dishes of black water, 3 of gray water and 2 controls with 1 ml of distilled water. Subsequently, 25 radish seeds were placed in each Petri dish and their growth was monitored for 3 days. However, seeds with green cotyledons were considered germinated. The process was repeated 3 times with radish and 3 times with lettuce seeds to validate the bioassay.

4.4.3 Germination and development of seedlings in plastic germinators using WWTP sludge

The development of seedlings in the presence of WWTP sludge was carried out in two plastic seedbeds with divided columns. The first column of both seedbeds was the control, the second for a 1: 150 dilutions in dry (black water mud: earth), the third a 1: 150 dilutions in dry (gray water mud: soil). Likewise, ten holes in each column were filled

using the respective dilutions. Next, holes were made in each hole of the columns to place one radish seed per hole in the first plastic germinator and one lettuce seed per hole for the second plastic germinator. Finally, the seedlings were watered and monitored for 30 days to observe the presence of abnormalities or growth inhibition of the seedlings due to WWTP sludge[71].

4.4.4 Coliform count in petrifilm in WWTP sludge

Black water sludge and gray water, were weighed in the presence of an alcohol burner to create an aseptic zone. Next, the laminar flow chamber was sterilized to place the sediment and sludge in labeled test tubes and with 9 ml of 0.9% saline solution. Each test tube was shaken for 2 minutes using the vortex. Five drops of black and gray water were placed on the 3M petrifilm plates to count coliforms. They were incubated at 37 ° C, and the plates were counted at 24 and 48 hours.

In the second Petrifilm test, 1: 100 dilutions were made (black or gray mud: saline solution), and three drops were placed in three Petrifilm for each WWTP mud Likewise, two Petrifilm controls were made with the physiological solution to observe contamination in the saline solution. They were incubated at 37 ° C and counted for 24 and 48 hours.

4.4.5 Fungi inhibition

PDA "Potato Dextrose Agar" was prepared and sterilized for 15 minutes at 100 ° C. Second, a solution was prepared with 20 grams of earth and 0.9% physiological solution until reaching 200 ml. Next, the soil was shaken, 1ml was placed in four Petri dishes, and each plate's content was spread by slowly moving it circularly. Subsequently, the PDA was placed at a tolerable temperature in each petri dish. Next, when the PDA of each plate was gelled, five holes were made using the reverse of the micropipette tips. Additionally, in the first plate and second Petri dish, one drop of dilution 1: 1 and 1:10 respectively of sewage sludge was placed in each hole. In the third and fourth Petri dishes, the same dilutions were placed using gray water mud. Finally, the Petri dishes were sealed with Parafilm, the samples were placed in a sealed box to prevent the entry of light, and the growth of fungi was observed on the first day and after one week.

4.5 Bioassays using drilling sediments

4.5.1 Germination in Petri using drilling sediment

For the germination tests of drilling sediment from the drilling near the road and the "D" trench, 7 different dilutions were made for the first test. The first 7 dilutions ranged from 1: 0 to 1:32 (drilling sediment: earth). 1 ml of each dilution was placed in a labeled Petri dish and with absorbent kitchen paper. However, for the following drill sediment tests, serial dilutions from 1:32 to 1: 1024 were made.

4.5.2 Evaluated variables for germination

The emergence speed indices and the total% emergence were calculated for each germination experiment. Likewise, a dose-response graph was performed using the total emergence of the germination experiments with drilling sediment.

Emergency speed index: For the emergence speed index (ESI), daily counts of the emerged seedlings were made for three days. However, for this experiment, seedlings with green cotyledons were considered[11].

$$ESI = \sum_{i=1}^{n} \frac{Xi}{Ni} \quad \textbf{(1)}$$

Where:

ESI = Emergence Speed Index; Xi = Number of emerged seedlings; Ni = Number of days after germinating; n = Number of counts.

Total percentage of emergence (TPE): When each repetition of the germination experiment was finished, the seedlings with green cotyledons were counted until the third day of evaluation[11,12].

$$TPE = \frac{E}{P} \quad \textbf{(2)}$$

Where:

TPE= Total percentage of emergence; E= Seeds that emerged on the last day of counting ; P= Total seeds that were used in the germination test or planted.

4.5.3 Construction of the dose-response curve.

First, the total emergence of the three Petri germination tests of Radish seeds exposed to drilling sediment was calculated. Only the second and third germination test data were used because the seeds died with high doses of drilling sediment (dilutions from 1: 0 to 1: 8). Second, the emergence total was found using the data, and the geometric mean of

each dilution was taken. Finally, the following Ligand binding equation was used for constructing the dose-response graph: sigmoidal dose-response[72].

$$f = minimum + \frac{(maximum - \text{minimum})}{1 + 10^{(\log(EC50) - x)}} \quad \textbf{(3)}$$

4.5.4 Germination and development of seedlings in plastic germinators using sediment

The second seedling formation test was carried out at Yachay Tech University using soil from the area. The dilutions with drilling sediment and dirt corresponded to the LD50, LD10, LD5 obtained from the germination graphs in Petri. 6 columns of 20 holes from the seedbed were filled with dilutions of drilling sediment: soil. The first, third, and fifth columns were filled with dilutions LD5 or 1: 1600, LD10 or 1: 700, LD50 or 1: 100 corresponding (pathway sediment: soil) where the first ten cavities were placed radish seeds and the following lettuces. The second, fourth, and sixth columns were filled with the same dilutions and seeds for LD5, LD10, and LD50 but with "D" sediment. Likewise, the presence of abnormalities or inhibition was checked and monitored for 20 days.

4.5.5 Bacterial inhibition

1000 ml of LB Agar was prepared and stirred while heating for 15 minutes. Subsequently, they were placed in the autoclave for 15 minutes at 121 ° C. Likewise, the dilution was prepared with 20 grams of soil from the Yachay Tech campus and saline solution up to 200ml. The solution was stirred with a magnetic stirrer for 15 minutes. Inside the laminar flow chamber, 1 ml of the soil solution supernatant was placed in 5 Petri dishes per pour plate. The Petri dishes were mixed carefully so that the content was spread throughout the plate. Likewise, the agar was placed in the labeled Petri dishes, and negative control was made without dilution. Subsequently, five previously sterilized absorbent paper discs were placed in the Petri dishes except for the positive and negative controls. In the first petri dish, one drop of 1:10 dilution (sediment of the route: saline solution) was placed in each circle, in the second a 1:100 dilution, in the third and fourth plates, the same dilutions were placed but with sediment. dry from the "D" fossa. Finally, the Petri dishes were sealed with Parafilm and left at room temperature to check for inhibition at 24 and 48 hours.

In the second inhibition test, dilutions were made 1: 1000 and 1: 10000 (sediments from perforation of the pathway and "D": saline solution). Likewise, the same solution was made with 20 grams of soil and physiological solution up to 200 ml. The supernatant from the already stirred solution was plated on a pour plate. Each box was gently shaken to spread the contents, and the agar was placed. Five discs were placed in each labeled Petri dish, and one drop of 1: 1000 dilution with "D" pellet was placed in one plate, 1: 10,000 in another plate, and the remaining dilutions of life pellet in other dishes.

In the third inhibition test, dilutions of 1: 1 and 1: 2 (drilling sediments: physiological solution) were made. However, 5 grams of soil were used for this test, and 200 ml were reached with the saline solution. The soil solution was constantly stirred, and 1 ml was taken to place it per poor plate in 5 Petri dishes. Next, the agar was placed on all plates with content and one with nothing as a negative control. Subsequently, the five sterilized discs were placed, and in each one, a drop of the sediment dilutions from the "D" pathway and fossa was pipetted.

4.5.6 Bacterial inhibition using TSB

First, 200 ml of TSB solution was prepared and sterilized in the autoclave for 15 minutes at 121 ° C. A dilution with 5 grams of soil and the physiological solution was prepared until reaching 200ml. Second, four dilutions, 1: 100, 1:0, 1: 740, and 1: 820, were made for each drill pellet. Next, 5ml of TSB solution was placed in 27 previously sterilized 10ml test tubes; where two were for positive control and one for negative control. Next, 0.1 ml of the soil solution supernatant was placed in twenty-six test tubes. Subsequently, 0.1 ml of each dilution of both drilling pellets were placed in three test tubes. Finally, stoppers were prepared for each test tube using gauze and cotton.

After 24 hours of liquid inhibition in TSB, 500 ml of Agar was prepared and sterilized in the autoclave at 121 ° for 15 minutes. A test tube was chosen from each dilution of drilling pellet and controls mixed with TSB and soil, and 0.1 ml per pour plate was placed in labeled Petri dishes. Therefore, we had four Petri dishes with dilutions 1: 100, 1:0, 1: 740, 1: 820 for road sediment, four for "D", a box for positive control, and another for negative. Next, the inoculation loop was placed in a glass of ethanol and then exposed to the flame to avoid contamination and used to spread the contents of the Petri dishes. Subsequently, Agar was placed in the Petri dishes and sealed with parafilm when it had gelled. Then, after 24 hours of rest at room temperature, each plate was counted. However, after 48

hours of rest of the tubes with medium and bacteria, 0.01 ml was taken from one tube of each dilution and placed in Petri dishes. Then, the content was spread using the inoculation loop, the Agar was set and sealed when it gelled. Subsequently, each Petri dish was counted after 24 hours of rest in Agar. Finally, to validate the test, the procedure was repeated two more times. However, to avoid a lot of bacterial growth, 100 microliters from each test tube were pipetted with the medium and bacteria per pour plate on Petri dishes.

4.5.7 Fungi inhibition

This test was carried out using the PDA (Potato Dextrose Agar), where the powder was first placed in distilled water, constantly stirred, and heated for 15 minutes at 100 °. When observing that the powder was completely dissolved, it was sterilized in an autoclave for 15 minutes at 121 ° C. A solution was made with 20 grams of earth, and it was filled with saline solution up to 200 ml. When the solution was well shaken, 1ml was placed in Petri dishes, and the boxes were moved to spread the content. Next, the PDA was placed on the plates, and when it was already gelled using sterile tips of a micropipette of 100 to 1000 microliters, five holes were made in the gel of each box. Likewise, 1: 1 and 1:10 dilutions were made for the drilling sediments with saline solution. Next, the tubes with the respective solutions were shaken using the vortex for 3 minutes each. Then, one drop of each dilution was placed on the Petri dishes according to the label. Finally, each Petri containing dilutions, positive and negative controls were sealed with Parafilm and they were placed in a sealed box avoiding light.

For the second fungal inhibition test, dilutions were made within the laminar flow chamber 1:0 and 1:2 (pathway perforation sediments or “D”: saline solution). However, the soil solution was made with 5 grams of soil and physiological solution until reaching 200ml while constantly stirring. Next, 1ml of the soil solution supernatant was placed in five Petri dishes, the dishes were shaken slightly to spread the content, and the PDA was poured into each one. Later, the agar was gelled, the holes were made with the micropipette tips, and one drop of the dilutions was placed in each hole. The four plates with dilutions and the two controls were sealed with Parafilm, set in a sealed box, and monitored every day for five days.

4.6 Composition and characteristics of the samples

The mining company sent samples of sewage sludge from “Camp X” and drilling sediment from Pit "D" to the Gruentec Chemical Laboratory for a total metal analysis report of the samples.

4.7 Statistics

The Boxplot graphs were made in the "IBM SPSS Statistics 21" program to observe the distribution, dispersion, and variability of the data obtained in the germination of Radish and Lettuce seeds exposed to WWTP sludge from the different camps. In addition, a two-way ANOVA statistical analysis was performed to simultaneously study the effects of drilling sediments to varying doses on radish and lettuce seeds. The dependent variables necessary for the ANOVA test were taken from the seedling development tests taking into account the biomass, root size, and the aerial size of the seedling or stem.

5 RESULTS

5.1 WWTP sludge results

5.1.1 “X camp” Sludges

5.1.1.1 Germination in Petri with radish

Assessment of germination in Petri using WWTP showed inhibition in the development of radish seeds when exposed for 72 hours to the dilutions of black and gray water. Something important to note is that radish roots exposed to gray water sludge were longer and thinner than controls and seeds exposed to black water sludge. Likewise, another important aspect is that the roots of the gray and black samples were bent and presented a more significant presence of root hairs compared to the controls (Figure 2).

It is essential to highlight that in the column of "blacks2" an example of inhibition is observed where one of the samples is in the process of breaking the seminal envelope. Additionally, several were observed in other samples exposed to black water that stopped in the water soaking process.

The seeds exposed to the dilution of black water sludge suffered a greater inhibition with respect to gray water samples, which is reflected in their mean. Most of the models had a very short-wide radicle, some began to break the seminal envelope, and others only remained embedded. According to the figure 3, the variability and data dispersion of the total emergence of seedlings in the presence of gray water sludge was more evident than the control and the samples with black water sludge. Likewise, it is observed that there was negative asymmetry in the samples with both sludge and a positive asymmetry for the control. Finally, there was a greater inhibition of germination when exposing the seeds to dilution with black water than gray water.

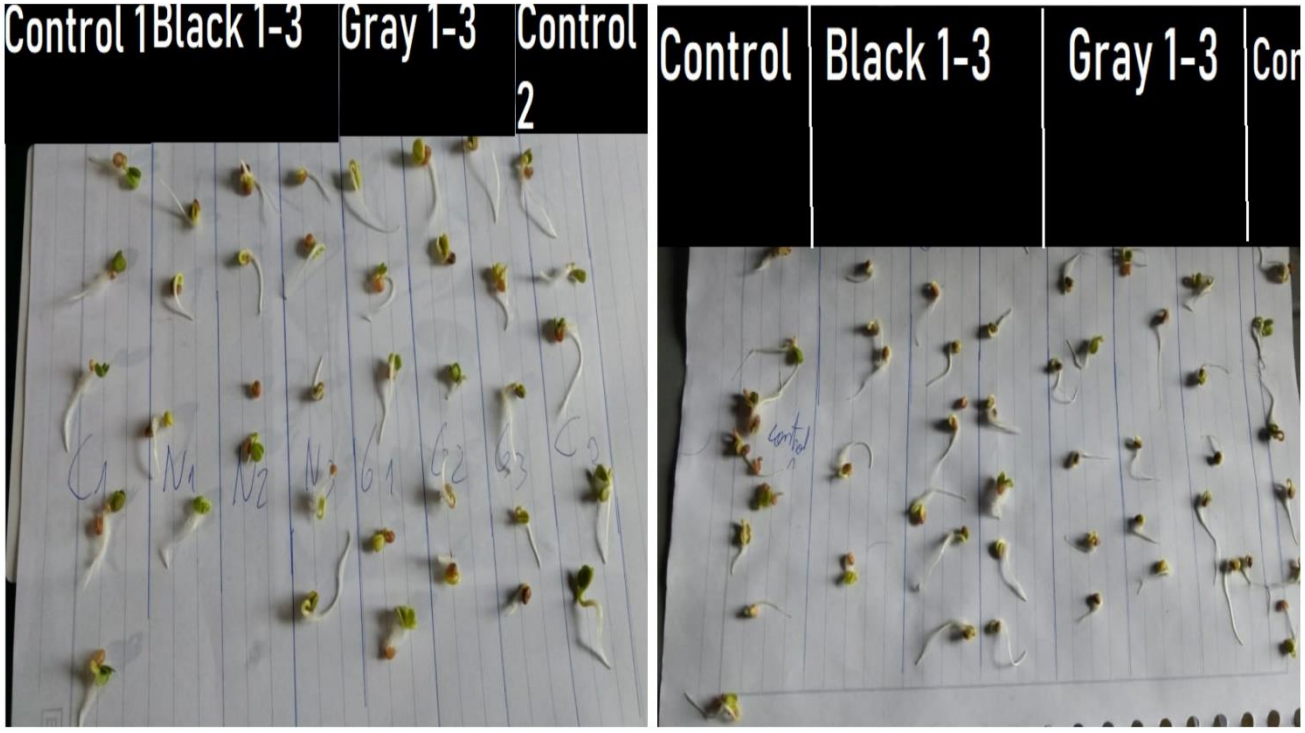


Figure 2: Germination of radish seeds exposed for 72 hours to black and gray water sludge from the "X camp". The seeds exposed to black water mud presented short roots or a remarkable inhibition of the germination stages in all the repetitions. In the case of the seeds exposed to gray water mud, they showed long and very thin roots. Finally, the control allowed to compare the process of inhibition and abnormalities of the seeds when germinating with WWTP sludge.

Table 1: Mean and standard deviation of the total emergence calculated from the three repetitions of the radish germination test in the presence of WWTP sludge from the "X camp".

	Control	Gray water Sludge	Black water sludge
Mean	0,78102287	0,41843433	0,12187323
Standard Deviation	0,05560276	0,09352362	0,06022181

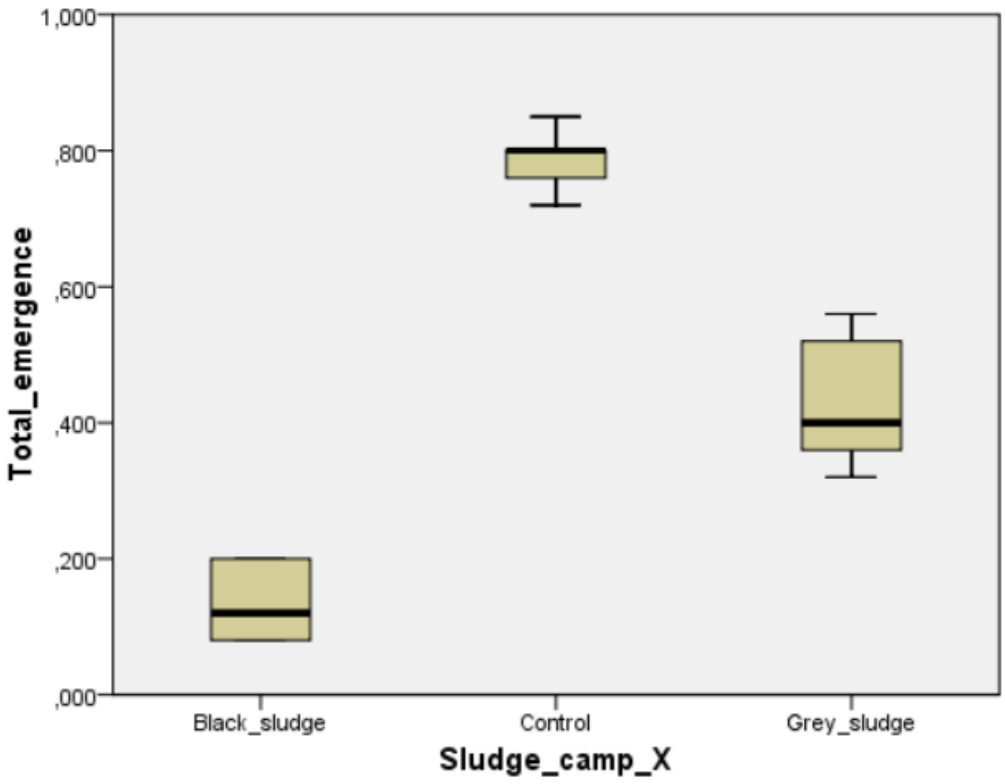


Figure 3: Boxplot of Radish germination tests using WWTP from "X camp".

5.1.1.2 Germination in Petri with lettuce

Germination experiments in lettuce Petri subjected to 1: 150 dilutions (WWTP sludge: water) showed the inhibition of seed development since some seeds remained in the soaking process after 72 hours of exposure. An important observation is that the type of abnormalities that occurred in radishes when exposed to mud from the "X camp" was repeated in the case of lettuces. Lettuce seeds exposed for 72 hours to gray water mud had longer and thinner roots. Lettuce seeds exposed to sewage sludge had broader and smaller roots than the control and the other sample. However, two similar characteristics were the presence of more root hairs and curved roots, unlike the control.

In figure 4, it is observed that there was inhibition in the lettuce seeds subjected to the dilution of black water since the data of the total emergence were very low. The dispersion and variability of the lettuce data subjected to black water were large and presented a

positive asymmetry. However, compared to the total emergence of radishes from the "X camp" shown in figure 4, the results of lettuce in the presence of gray water showed a significant increase in the total emergence showing that there were a more substantial number of seedlings after 72 hours and lower inhibition. Likewise, the means in table 2 present higher values, including the control.

Table 2: Mean and standard deviation of the total emergence calculated from the three repetitions of the lettuce germination test in the presence of WWTP sludge from the "X camp".

	Control	Gray water Sludge	Black water sludge
Mean	0,916681273	0,72279207	0,31826266
Standard Deviation	0,031471832	0,04995236	0,08591247

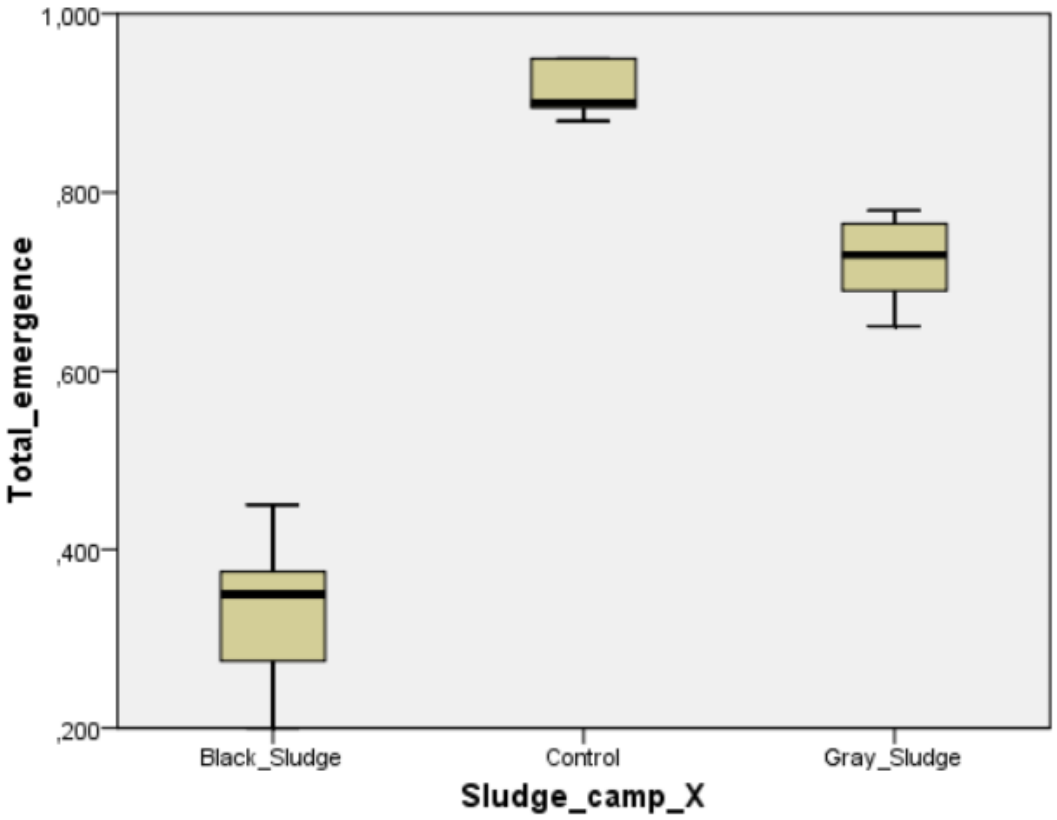


Figure 4: Boxplot of Lettuce germination tests using WWTP from "X camp".

5.1.1.3 Development of radish seedlings in plastic germinators

As shown in figure 5, the first column on the right is the control that shows ten green seedlings at 5 days; the other control is the final column counting from the right that had nine seedlings with leaves and one emerging seedling. The seeds exposed to sewage sediment located in the second column showed development inhibition since only five seedlings achieved growth comparable to the controls, three tiny seedlings, and small leaves. Then, one seed that was inhibited, which was beginning to break off the seminal envelope, and seed only soaked in water or dead. Finally, the seeds exposed to gray water mud at 5 days showed the growth of seven seedlings with leaves, one beginning to form the leaves and one without reaction since the seed was still in the process of hydration or dead.

At 10 days of development, exposed to a 1: 150 dilution of sewage sludge, an important observation is that the seed in the seedling break stage developed to a seedling, and the seed soaked in liquid progressed to the formation of leaves. At 10 days, all the seeds exposed to gray water sludge developed into seedlings showing that the seeds that had been inhibited were not dead. They only had a slowdown in their advancement. At 30 days of growth, it is observed that the column of seeds exposed to gray and black waters have several seedlings with better development than the controls and a greater aerial length. An important observation is that after 30 days of growth, no abnormalities were observed in the seedlings.

5.1.1.4 Development of lettuce seedlings in plastic germinators

It is observed from figure 5 that at five days, the lettuce seeds exposed to gray water sludge located in the third column counting from the right presented five tiny seedlings with green leaves and the rest were in the process of breaking the seminal envelope. In the case of those exposed to sewage sludge, 3 became seedlings, and the remaining seven were inhibited until the first germination or water absorption process. Thus, one fact to take into account is that the control also had five seedlings like the gray ones, but the rest were emerging.

At ten days of growth, eight seedlings of the seeds exposed to gray mud were observed, and the remaining two were still in the process of breaking the seminal envelope. In the case of the seeds exposed to sewage sludge, they showed a significant improvement since there were nine seedlings, and only one was still in the process of radicle growth. It is imperative to consider that the seeds exposed to gray water mud developed eight seedlings

to a considerable size at the control. The seeds that had slowed their development after 30 days showed a similar size to the control at five days.

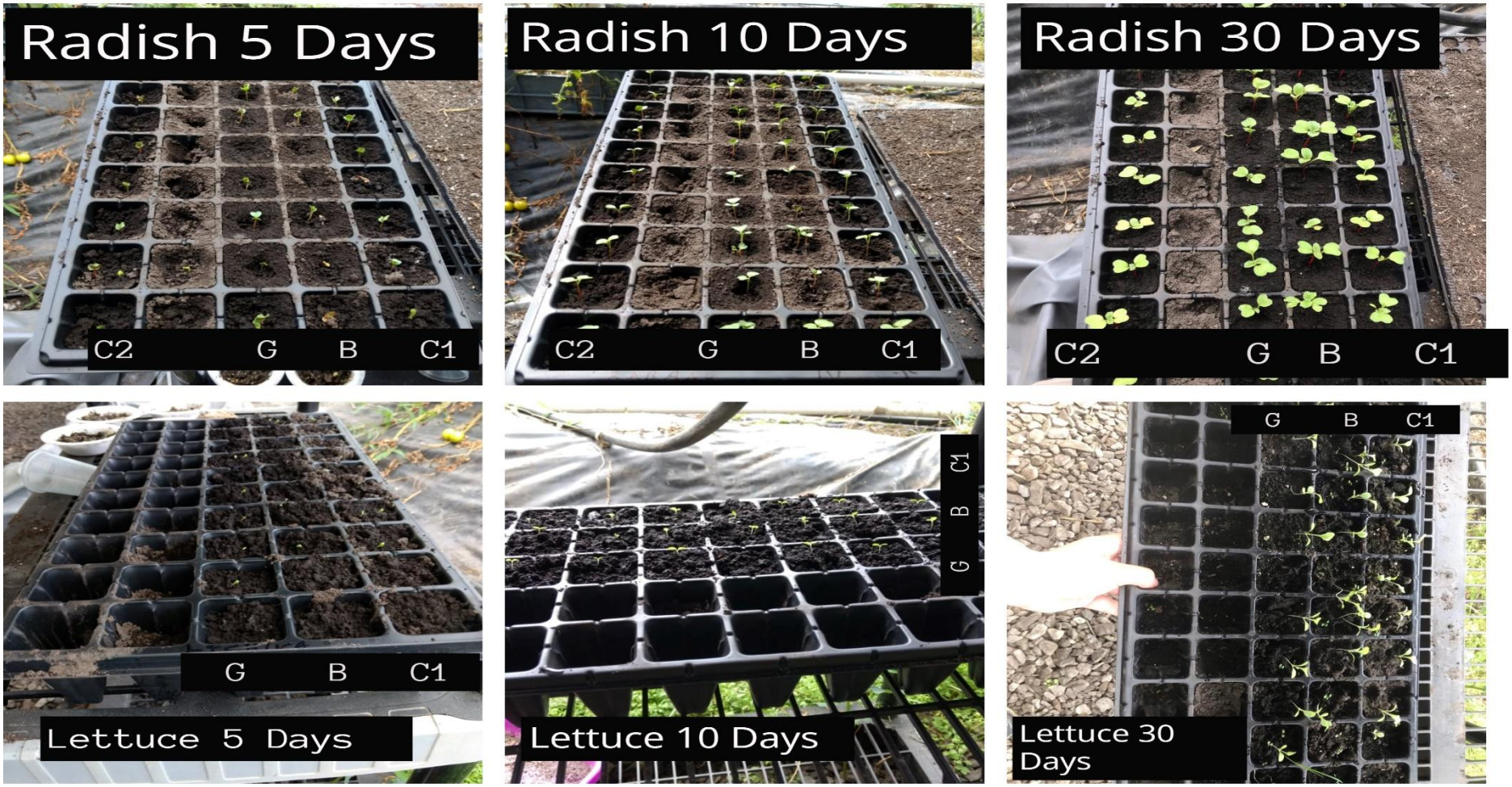


Figure 5: Growth of lettuce and radish seeds exposed to WWTP sludge from the "X camp" at 5, 10 and 30 days. In radishes the first column on the right is the control, then black, gray and finally the second control. In lettuce the first is the control, then black and finally gray.

5.1.1.5 Bacterial identification

The black and gray water sediments presented mainly bacteria at the facultative 24 hours. As detailed in the figure 6, a small percentage was close to the surface and others to the bottom, so there were also anaerobic and aerobic. At 48 hours, the TSB became cloudy in color, and facultative bacteria predominated. Again, only a tiny percentage were aerobic and anaerobic.

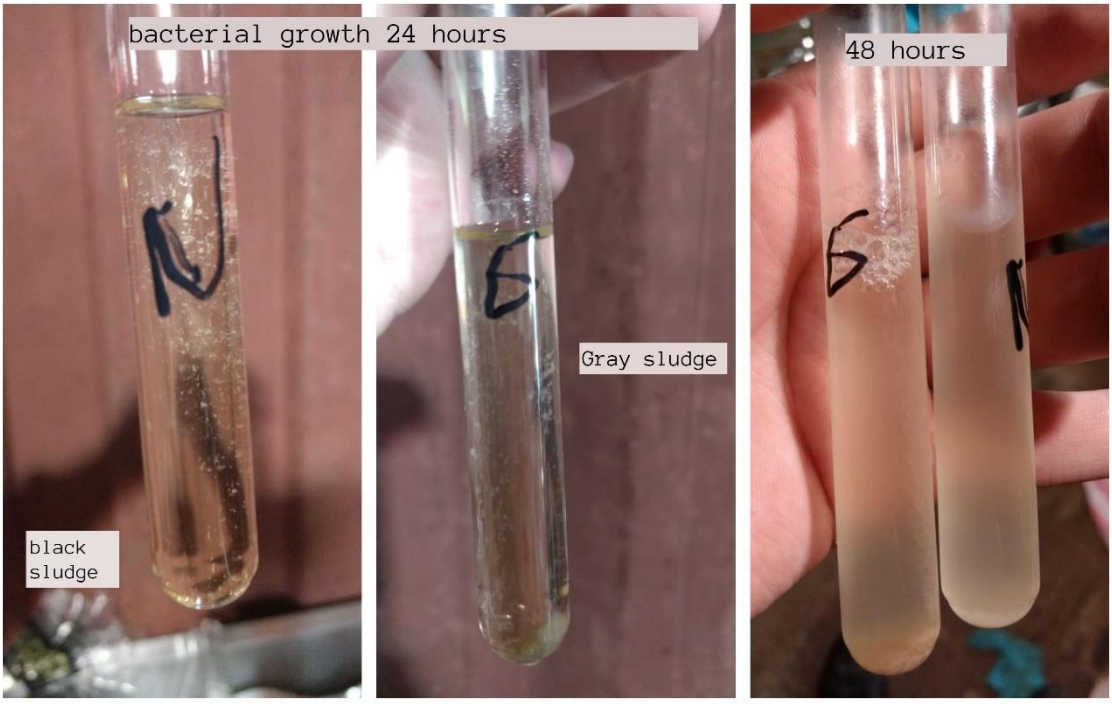


Figure 6: Bacterial growth in test tubes with gray water sludge and black water sludge at 24 and 48 hours. The analysis of these samples was qualitative to observe only the type of bacteria that grew in the tubes.

5.1.1.6 Petrifilm

An important fact showed in table and figure 7 is that the Petrifilm with dilutions of mud from gray water presented many bubbles and coliforms. Sewage sludge considerably reduced its number of bubbles with more significant dilution, as well as its number of total coliforms. One piece of information to consider is that the plates showed more than 300 estimated coliforms/ml despite the increase in dilution. This may be due to the fact that greywater sludge had higher humidity than the other sludge. As shown in the figure,

control was also carried out with the saline solution to establish possible contamination where it is delivered without bacteria present.

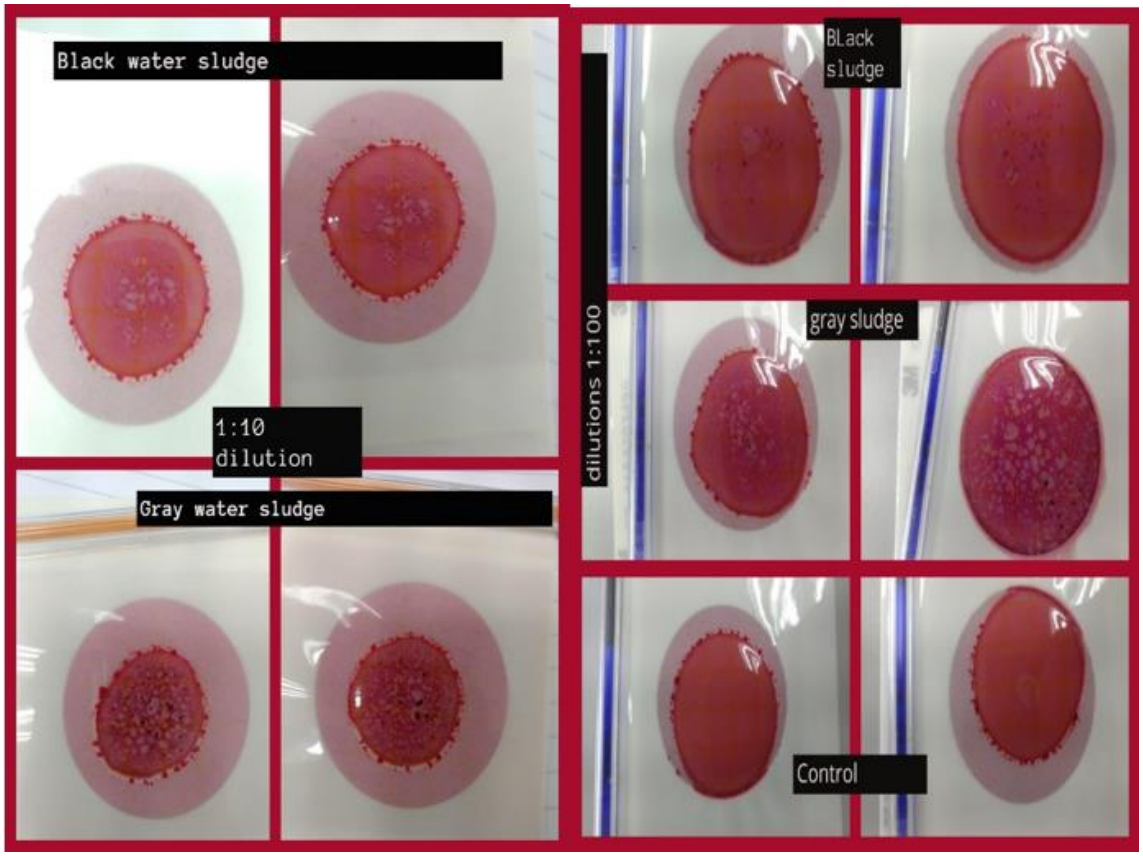


Figure 7: Petrifilm plates for counting coliforms from 1:10 and 1: 100 dilutions of gray and black water sludge at 24 hours.

Table 3: Coliform count and Colony forming unit obtained from Petrifilm plates at 24 and 48 hours of incubation at 37 ° C.

Sludge	Coliform count	Dilution factor	CFU	Hours
Black sludge	43	10^1	430	24
Gray Sludge	330	10^1	3300	24
Black sludge	56	10^1	560	48
Gray Sludge	358	10^1	3580	48
Black sludge	53	10^1	530	24
Gray Sludge	372	10^1	4800	24
Black sludge	60	10^1	600	48
Gray Sludge	359	10^1	580	48
Black sludge	36	10^2	3600	24
Gray Sludge	305	10^2	30500	24
Black sludge	21	10^2	2100	24
Gray Sludge	250	10^2	25000	24
Black sludge	45	10^2	4500	48
Gray Sludge	320	10^2	32000	48
Black sludge	30	10^2	3000	48
Gray Sludge	274	10^2	27400	48

5.1.2 “Y camp” sludges

5.1.2.1 Germination in Petri with radish

In the case of the “Y camp”, the gray and black water sludge is mixed in two treatment plants. Something to highlight is that the germination inhibition in radishes was lower than that presented in “X camp” since there was a higher percentage of seedlings with green leaves. Another notable observation is that the seeds using the mud from both plants had very long, thin, curved radicles with many root hairs than the controls. However, there were no seeds soaked in water or starting the seed-breaking process at 72 hours (figure 10).

Figure 8 shows a considerable range in the data from both “Y camp” treatment plants and a somewhat similar variability because the inter-quartile range was around 0.1. The asymmetry of plant B is positive, and plant A has a slight negative asymmetry.

5.1.2.2 Germination in Petri with lettuce

The seeds exposed to the 1: 150 dilutions of the mixed plants from the “Y camp” presented less inhibition than “X camp”. However, the abnormalities of the seedlings at 72 hours were similar since long, bent, and curved radicles were observed with many root hairs. Another important fact is that there was no seed in the embedding process after 72

hours; since all had radicles, the difference in the total emergence was due to the presence of green cotyledons (figure 10).

According to figure 9, the data from plants A and B are dispersed as they have a reasonably considerable range concerning the control. Nevertheless, the variability of both plants is similar since they have a similar interquartile range, and both show a positive asymmetry. A crucial data is that the WWTP of the “Y camp” maintains a high emergency total since there were only data greater than 0.65 and the means are greater than 0.70. Hence, the inhibition of lettuce seeds' development is less than the gray and black water sludge from the “X camp”.

Table 4: Mean and standard deviation of the total emergence calculated from the three repetitions of the radish germination test in the presence of WWTP sludge from the “Y camp”.

	Control	Plant A	Plant B
Mean	0,927202	0,72881641	0,74674798
Standard Deviation	0,0432435	0,04690416	0,04868265

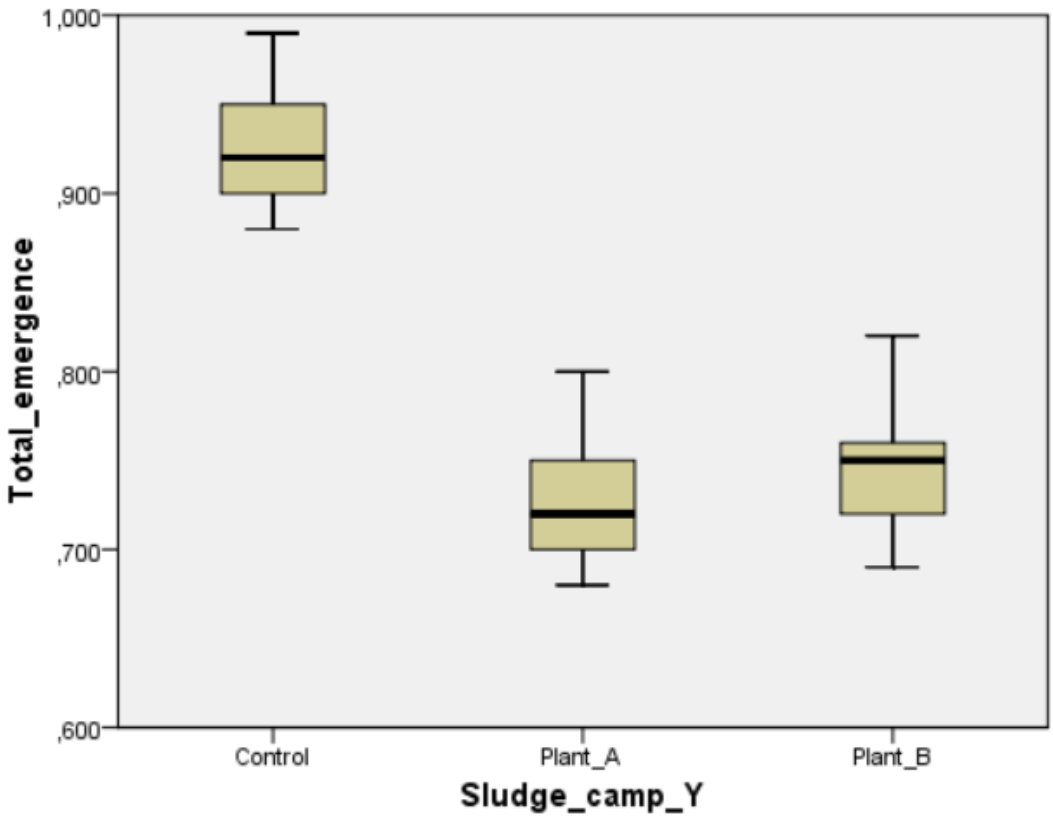


Figure 8: Boxplot of Radish germination tests using WWTP from “Y camp”.

Table 5: Mean and standard deviation of the total emergence calculated from the three repetitions of the lettuce germination test in the presence of WWTP sludge from the “Y camp”.

	Control	Planta A	Plant B
Mean	0,89973624	0,75914099	0,74188084
Standard Deviation	0,02380476	0,03872983	0,0411154

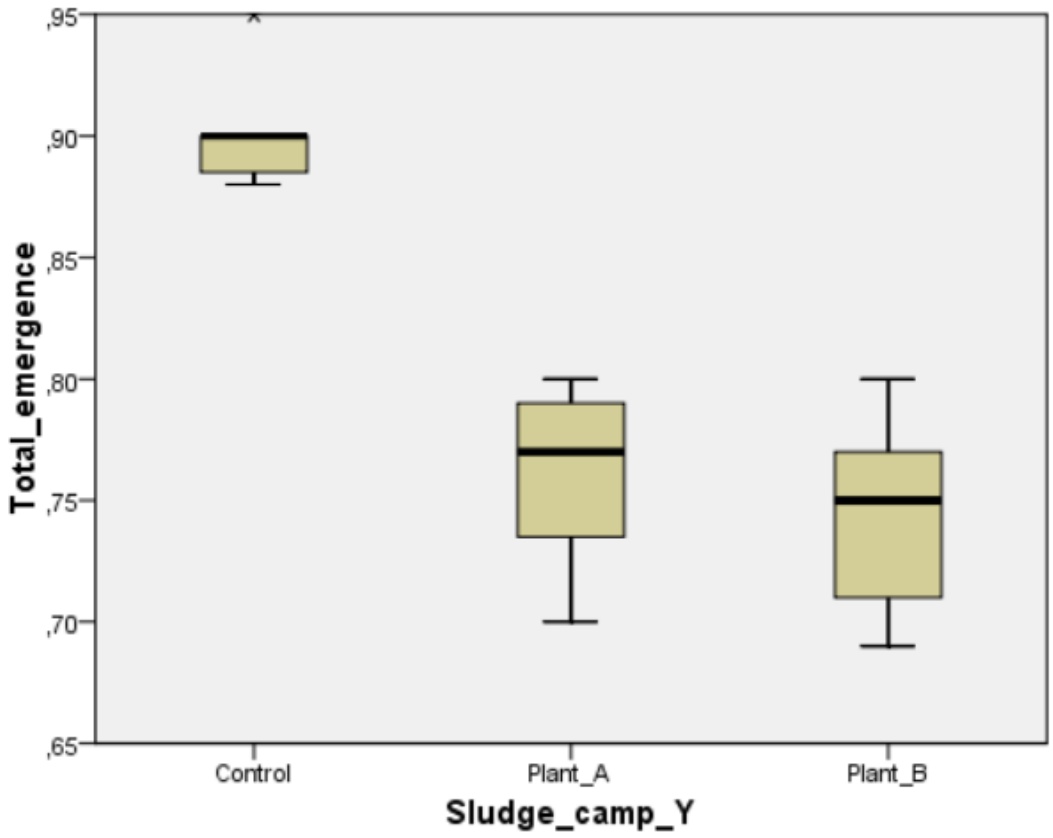


Figure 9: Boxplot of Lettuce germination tests using WWTP from “Y camp”.

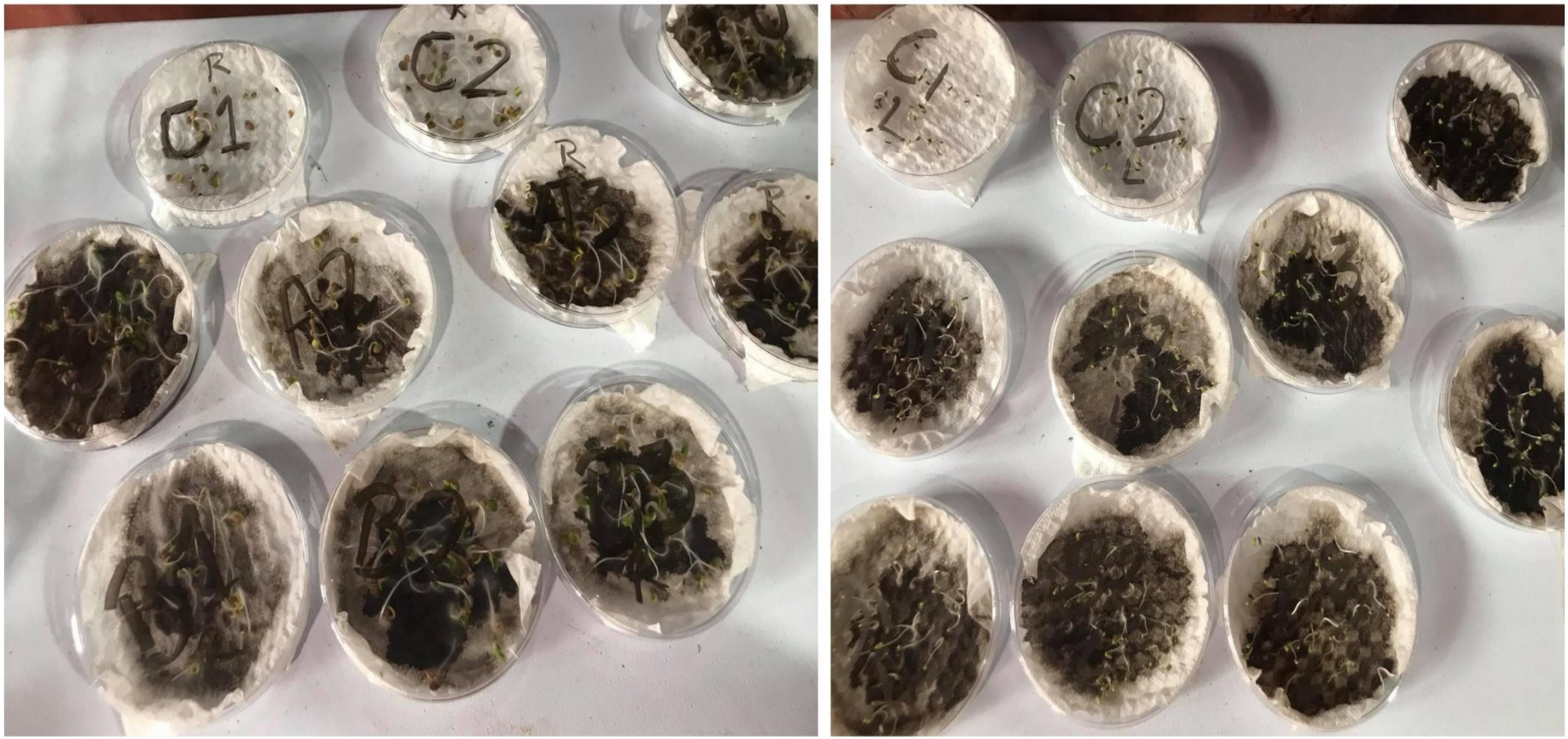


Figure 10: Germination of lettuce (right) and radishes (left) exposed to sludge from plants A and B of the “Y camp”.

5.2 Drilling sediment results

5.2.1 Germination in Petri with *Raphanus sativus*

As shown in figure 11, the seeds of *Raphanus sativus* exposed to dilutions of 1: 0 to 1:32 of drilling sediment from the well near via "Road" show a very evident inhibition of the germination process after 72 hours. One thing to consider is that most of the seeds remained in the process of breaking the seminal cover, and the rest remained as embedded seeds after 72 hours. Other essential observations are that the 1:32 dilution was the only one that presented seedlings with green cotyledons with a total emergence of 0.08 and the only Petri dish samples with the wet absorbent paper were the 1:16 and 1:32 dilutions. Therefore, having a minimal number of seedlings, the presence of abnormalities could not be observed, and the following tests were made from the 1:16 dilution that did not show green cotyledons but the formation of them and roots with many hairs root.

The change of dilutions from 1: 0-1: 32 to 1: 16-1: 1024 in seeds of *Raphanus sativus* showed a remarkable difference in the development of seeds after 72 hours. The lower concentration of drilling sediment made it possible to reduce the inhibition of the germination process and the observation of abnormalities such as the snail-shaped bending of the radicle, cotyledon formation with a root without root hairs without completely breaking the seminal envelope. As the sediment concentration in the well near the "Road" pathway decreases, the presence of longer and thinner radicles increases considerably. It is also observed that at a dilution of 1:32 and 1: 128 after 72 hours of exposure, some seeds did not advance from the first germination process since they only absorbed water.

The first germination test of *Raphanus sativus* in the presence of drilling sediment from the “D” well with serial dilutions 1: 0 to 1:32 showed a similar pattern since only two seeds of the 1:32 dilution developed until considered a seedling with green cotyledons. The inhibition caused by this sediment in the development of the seeds is evident since most of the test subjects remained in the stage of rupture of the seminal envelope; another small percentage had radicles. Still, no cotyledons, and the other seeds only embedded in liquid.

The following tests were done with serial dilutions from 1:16 to 1: 1024 to observe the abnormalities after 72 hours of exposure. The first test demonstrated enormous inhibition where the abnormalities could not be observed. Figure 11 shows a significant increase in

seedlings similar to the test using the pathway sediment. Another essential and comparable data is that the abnormalities were identical since the radicles rolled up. Others formed green cotyledons but did not have root hairs. One group had long radicles but no root hairs. Finally, an important observation is that when the seeds were removed from the Petri dishes, the absorbent paper was more humid as the sediment concentration was lowered further.

5.2.2 Germination in Petri with *lactusa sativa*

The germination tests of *lactusa sativa* with serial dilutions 1: 0 to 1:32 of the sediment from the well near the pathway showed enormous inhibition, as did the lettuce tests since only 3 seedlings were witnessed with a dilution of 1: 32 after 72 hours. Lettuces exposed to higher concentrations after 72 hours were characterized by embedded seeds, seeds with tiny roots and many initiating the breakdown of the seed coat.

The following tests were made from dilutions 1:16 to 1: 1024, where the increase of seeds that became seedlings at 72 hours was observed. An important observation is that only at 1:16, 1:32, 1:64 to 1:12 dilutions were embedded seeds that did not break the seed coat. After three days of germination, curved snail-like radicles were observed, with a greater presence of root hairs and radicules that were thinner and smaller than the control.

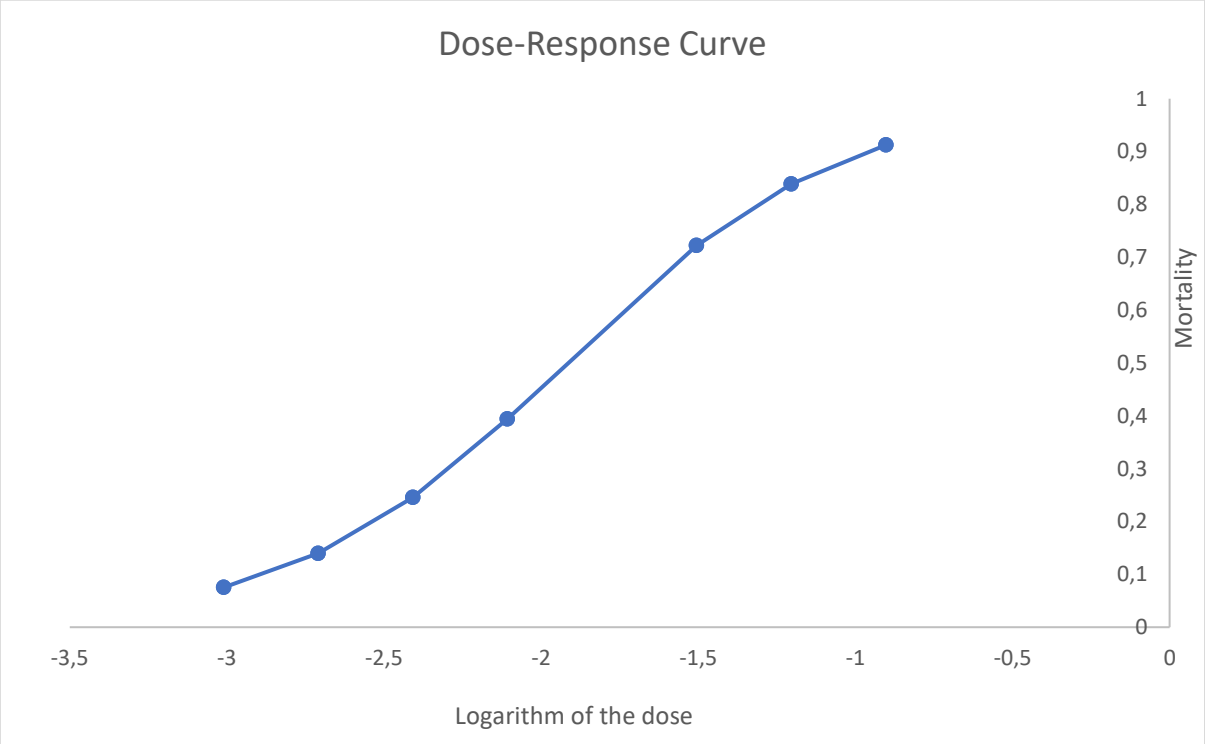
Like the results of *lactusa sativa* in the presence of sediment from the well near the road, the “D” sediment significantly inhibited the development of the seeds when using serial dilutions from 1: 0 to 1:32 to the point of only having two seedlings with green cotyledons at a 1:32 dilution and a 1:16 dilution seedling. Unlike the test with the other sediment, the radicles had a greater length. As a result, there was a smaller quantity of seeds only swollen with liquid and seeds beginning the rupture of the seminal envelope after 72 hours. The following tests that used a lower concentration 1:16 to 1: 1024, different characteristics of the control were observed such as longer, thinner radicle, the greater presence of root hairs, lettuces with long radicles, but without cotyledons, lettuces with short radicles, but with green cotyledons. Another important observation is that they had less curvature concerning the lettuce seeds exposed to the other drilling sediment.

Figure 11: Radish seedlings exposed to different concentrations of sediment for 3 days.

Figure 12: Lettuce seedlings exposed to different concentrations of sediments for 3 days.

5.2.3 Construction of the dose-response curve

Using the total emergence of three repetitions of the germination test at dilutions from 1:16 to 1: 1024 of the seeds of *Raphanus sativus*, the EC50 was calculated corresponding to a dilution of 1: 100, and equation number 3 was used for the Construction of the dose-response curve of figure 13. The dilutions of the first test were not used since 100% of the seeds did not complete the germination process or were seedlings with green cotyledons in most of the seeds. Likewise, solving equation number 3, it was found that at a 1:10 dilution of the drilling sediment from the well near the "Road" route, 90% of the seeds would die or remain in an embedded seed state.



$$f = 0.0045 + \frac{0.4925}{1 + 10^{(\log(-1.9203) - x)}} \quad \textbf{(4)}$$

Figure 13: Dose-response curve of radish seeds exposed to concentrations of drilling sediment from the well near "road"; where there is a 50% survival or EC50 at a 1:10 dilution.

5.2.4 Development of radish and lettuce seedlings in plastic germinators

Solving for equation number 3, the LD10, LD90, LD0.05 (1: 700,1: 10, and 1: 1600 correspondingly) were found, and the same EC50 value was used for both drilling sediments since they presented similar behavior in the tests germination.

After ten days of cultivation in the presence of sediment close to the pathway, most of the radish seeds had a small growth, and a small group had a considerable size but not close to the aerial length of the control. As expected, the seeds exposed to a higher sediment concentration had a slowdown and inhibition in their development. Three seeds did not develop at 10 days, which grew to seedlings with very dark leaves, and the leaves bent upwards. An important observation is that at 1: 1600 dilution, no seedling had a delay in its development since all reached a similar state with leaves and comparable aerial length.

A fundamental data of inhibition is that the seedlings of the three concentrations of drilling sediment had dry leaves, very thin-fragile stems, and lost their leaves after 20 days of exposure, as shown in figure 14.

After 10 days of exposure to the sediment from the well "Road", the lettuce seeds showed smaller leaves and closed upwards as the sediment concentration increased. Also, the difference in stem size decreased with increasing concentration. The concentration corresponding to the LD50 had only five seedlings with green leaves; the rest were still in the process of breaking the seminal envelope at 10 days. However, after 20 days of cultivation, all the seedlings of each concentration died and lost their leaves (figure 15).

The radish seeds exposed to the "D" sediment at 10 days showed more significant growth than those of the other sediment. All concentrations had wider, longer stems. The inhibition was reflected in the development of 4 seeds exposed to the 1: 100 dilution or LD50 that did not exceed the first germination stage at 10 days. In the other concentrations, all the seeds developed into a seedling at 10 days.

An important observation of the exposure of the sediment with the radish seedlings is that after 20 days, most of the seedlings lost their leaves or dried up, and the LD50 seedlings had longer, thinner, and more fragile stems concerning the other concentrations. Those exposed to "D" sediment were longer than those of the different sediment, but they were not comparable to the control where their aerial lengths were 5 to 6 cm (Figure 14).

The lettuce seeds exposed to the different dilutions of the "D" sediment showed less inhibition in development than the other sediment at 10 days since there were more seedlings and a larger stem and leaf size. An important observation is that as the concentration increases, the leaves lose their color, size and close upwards. Another introductory statement is that the phenomenon of the other sediment was repeated where

all the lettuce seedlings died after 20 days, lost leaves, the stems fell because they were too thin (figure 15).

In sediment, LD and their interaction was ($p=0\leq0.05$). Therefore, it is stated that with a significance level of 0.05 the types of sediment and the interaction with the amount of dose or LD will have seedlings with a different underground length according to table 6.

As shown in figure 16, as the concentration increases, the underground length or length of the root increases in the case of the road sediment; the control could not be estimated since it is a value without the dose of both sediments. An important observation is that in the “D” sediment, as the concentration increased, the root length decreased. However, at a concentration of LD50 or 1: 100 dilutions, the marginal mean root length was more significant than the LD10.

From table 7, it is observed with a significance level of 0.05 that the length of the stem or aerial length is different for each sediment and at different concentrations. However, there is no effect on the interaction of other sediments and concentration since ($p=0,725\geq0.05$). Likewise, it can be deduced that the model is correctly adjusted since the value of the "adjusted R squared" is close to 1.

It is observed from figure 17 that as the concentration is increased for both sediments, the size of the stem decreases. Another data to consider is that the seeds exposed to “D” sediment show higher values in the marginal means of the air length of the seedlings at 10 days.

According to table 8, it is stated with a significance of 0.05 that in the two types of materials and at different concentrations, the biomass of the aerial part of the radish seedlings is different. However, since the p-value is greater than 0.05, there would be no effect on the sediment-concentration interaction.

According to figure 18, the estimated marginal means of biomass increase as the concentration of drilling sediments decreases. Therefore, another necessary data is that the biomass of the aerial part of radishes exposed to “D” sediment was higher than the other sediment in the different concentrations.

Table 9 shows that at different sediments and concentrations, the root length or underground length differs with a significance of 0.05 since the p values are less than 0.05. Likewise, the interaction between both variables affects the size of the lettuce root, and having an adjusted R squared close to 1 shows that the model is well adjusted.

Lettuce seedlings exposed to both drilling sediments showed higher marginal root size means as sediment concentration increased. One thing to consider is that unlike the results in radish, this time, the roots exposed to the "D" sediment were longer than the other sediment and more significant than the control with LD10 and LD5 (figure 19).

According to table 10, the size of the aerial region of the lettuce plants differs when using the different sediments and the different concentrations with a significance of 0.05. Likewise, there is an effect on the interaction of both parameters on stem growth, and the model presents a good fit as it has an "adjusted R squared" close to 1.

The aerial height of the lettuce seedlings exposed to drilling sediments at 10 days increased as the concentration of the sediments was reduced, as detailed in figure 20. The seedlings exposed to "D" sediment showed higher aerial height with respect to the other sediment, but it had no comparison to those of the control.

The biomass values of the aerial part of the lettuce plants presented different values at different sediments and concentrations with a significance of 0.05. However, the p-value was greater than 0.05. Therefore, according to the calculations, the interaction of LD and sediments does not affect the dependent variable biomass expressed in table 11.

The marginal biomass means of lettuce seedlings increased as the concentration of drilling sediments decreased. The biomass values were higher for the seedlings exposed to "D" sediment concerning the other sediment, but their values were not close to those of the control (figure 21).

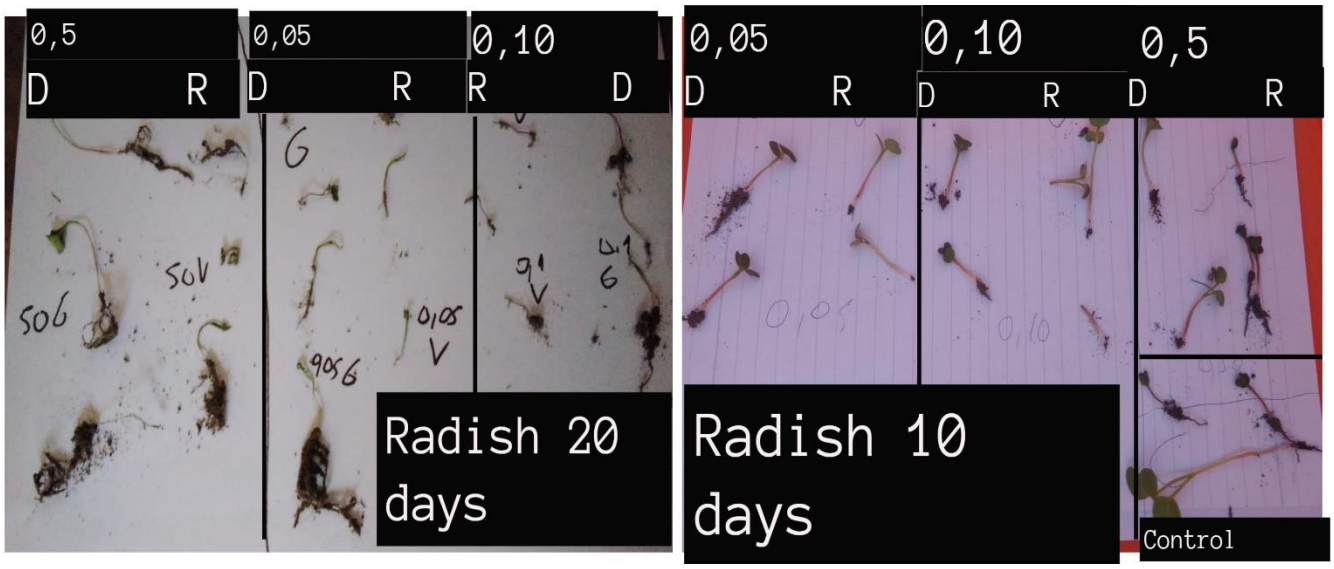


Figure 14: Development of radish seedlings exposed to sediment from "D" and Road at different concentrations for 10 and 20 days.

Figure 15: Development of lettuce seedlings exposed to sediment from "D" and Road at different concentrations for 10 and 20 days.

Table 6: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on root length in radish seeds at 10 days.

Dependent variable Length

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	24,047[a]	6	4,008	34,759	,000
Intersection	150,969	1	150,969	1309,341	,000
Sediment	4,320	1	4,320	37,468	,000
LD	2,233	2	1,117	9,685	,000
Sediment * LD	5,321	2	2,661	23,076	,000
Error	7,264	63	,115		
Total	182,280	70			
Corrected Total	31,311	69			

a. R squared = ,768 (corrected R squared = ,746)

Table 7: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on the aerial length in radish seeds at 10 days.

Dependent variable: Stem_length

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	103,060[a]	6	17,177	105,584	,000
Intersection	511,921	1	511,921	3146,750	,000
Sediment	,913	1	,913	5,610	,021
LD	10,201	2	5,101	31,353	,000
Sediment * LD	,105	2	,053	,324	,725
Error	10,249	63	,163		
Total	625,230	70			
Corrected total	113,309	69			

R-squared = .910 (corrected R-squared = .901)

Table 8: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on the biomass of the aerial part in radish seeds at 10 days.

Dependent variable:Biomass

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	,186[a]	6	,031	171,451	,000
Intersection	,312	1	,312	1728,243	,000
Sediments	,002	1	,002	12,273	,001
LD	,006	2	,003	16,084	,000
Sediments * LD	,000	2	5,796E-005	,321	,727
Error	,011	63	,000		
Total	,510	70			
Corrected total	,197	69			

R squared = .942 (corrected R squared = .937)

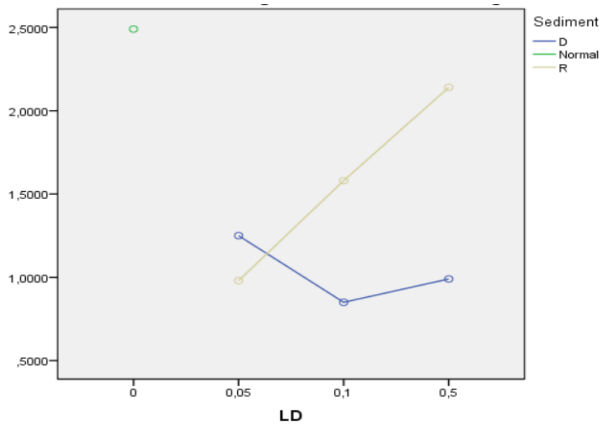


Figure 16: Estimated marginal means of radish root length against different sediment concentrations and the control.

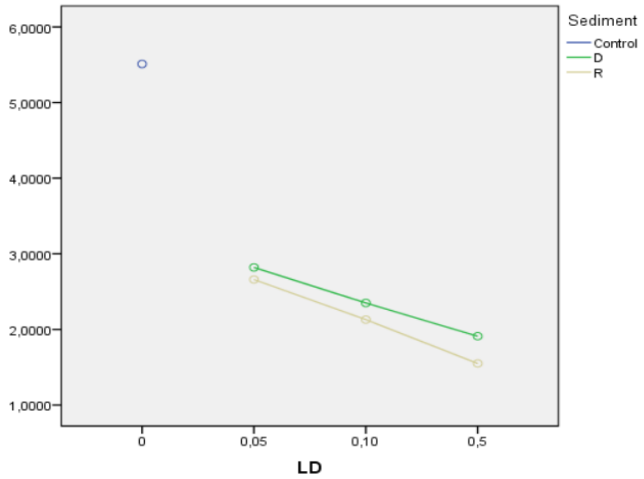


Figure 17: Estimated marginal means of radish seedling aerial length against different concentrations of sediment and control.

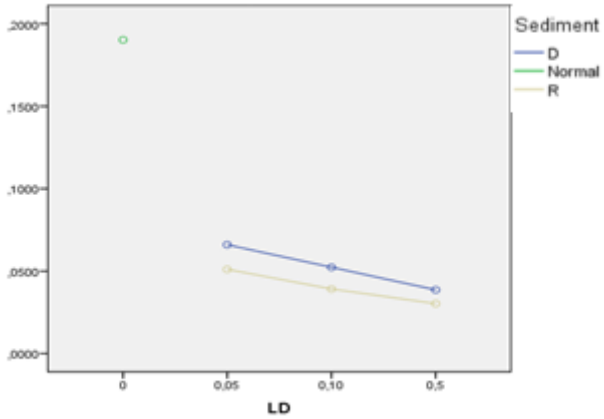


Figure 18: Estimated marginal means of the biomass of the aerial part of radish seedlings against the different concentrations of sediment and the control.

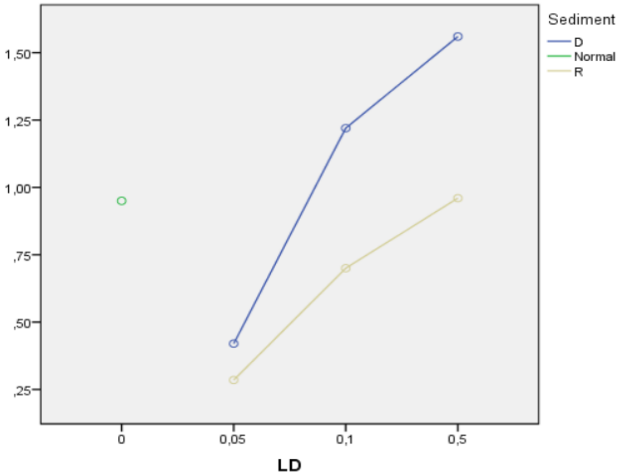


Figure 19: Estimated marginal means of the root length of lettuce seedlings against the different concentrations of sediment and the control.

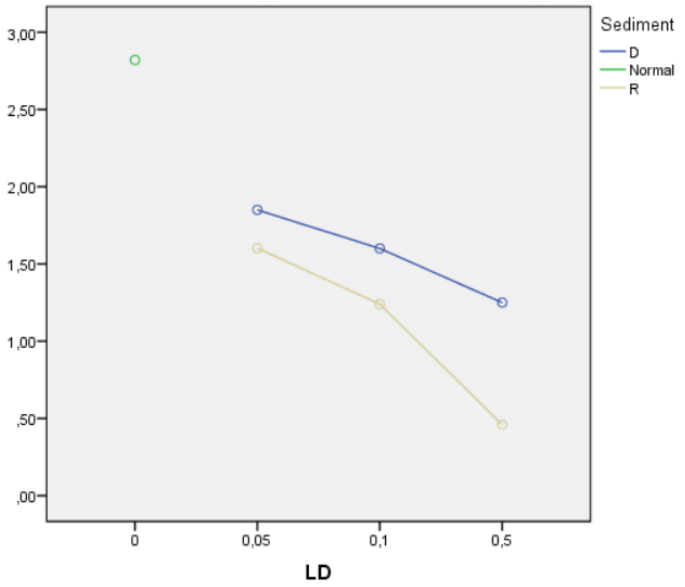


Figure 20: Estimated marginal means of the aerial length of the lettuce seedling against the different concentrations of sediment and the control.

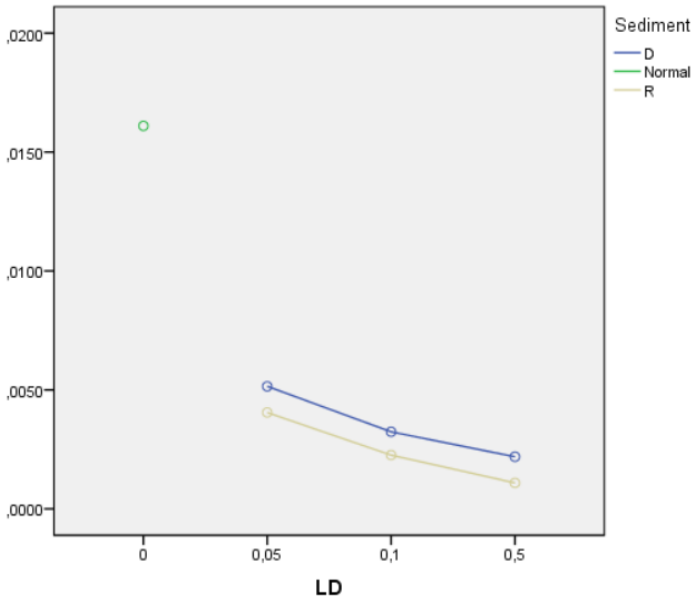


Figure 21: Estimated marginal means of the biomass of the aerial length of the lettuce seedling against the different concentrations of sediment and the control.

Table 9: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on root length in lettuce seeds at 10 days.

Dependent variable: Root_length

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	11,867[a]	6	1,978	72,435	,000
Intersection	53,070	1	53,070	1943,562	,000
Sediment	2,625	1	2,625	96,136	,000
LD	8,551	2	4,275	156,575	,000
Sediment * LD	,618	2	,309	11,318	,000
Error	1,720	63	,027		
Total	66,658	70			
Corrected total	13,587	69			

R squared = .873 (corrected R squared = .861)

Table 10: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on air length in lettuce seeds at 10 days.

Dependent variable: Stem_length

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	30,822[a]	6	5,137	144,713	,000
Intersection	167,308	1	167,308	4713,200	,000
Sediment	3,257	1	3,257	91,762	,000
LD	7,810	2	3,905	110,007	,000
Sediment * LD	,819	2	,409	11,531	,000
Error	2,236	63	,035		
Total	200,366	70			
Corrected total	33,058	69			

R-squared = .932 (corrected R-squared = .926)

Table 11: Two-way analysis of variance (ANOVA) on the influence of sediments and their LD-concentrations on the biomass of the aerial part in lettuce seeds at 10 days.

Dependent variable: Biomass

Origin	Sum of squares type II	gl	Quadratic mean	F	Sig.
Corrected model	,002[a]	6	,000	97,049	,000
Intersection	,002	1	,002	595,280	,000
Sediment	1,325E-005	1	1,325E-005	4,857	,031
LD	8,833E-005	2	4,416E-005	16,185	,000
Sediment * LD	2,128E-006	2	1,064E-006	,390	,679
Error	,000	63	2,729E-006		
Total	,003	70			
Corrected total	,002	69			

R squared = .902 (corrected R squared = .893)

5.2.5 Petrifilm

The sediment of the "Road" route had five coliforms shown as red colonies with their respective gas bubbles that they produced. In the case of “D”, an innumerable amount of bubbles and a large number of colonies similar to brown spots were shown, as can be seen in the figure 22. Thus, the test confirmed the presence of bacteria even though the sediments went through a drying phase in the sun for two days.

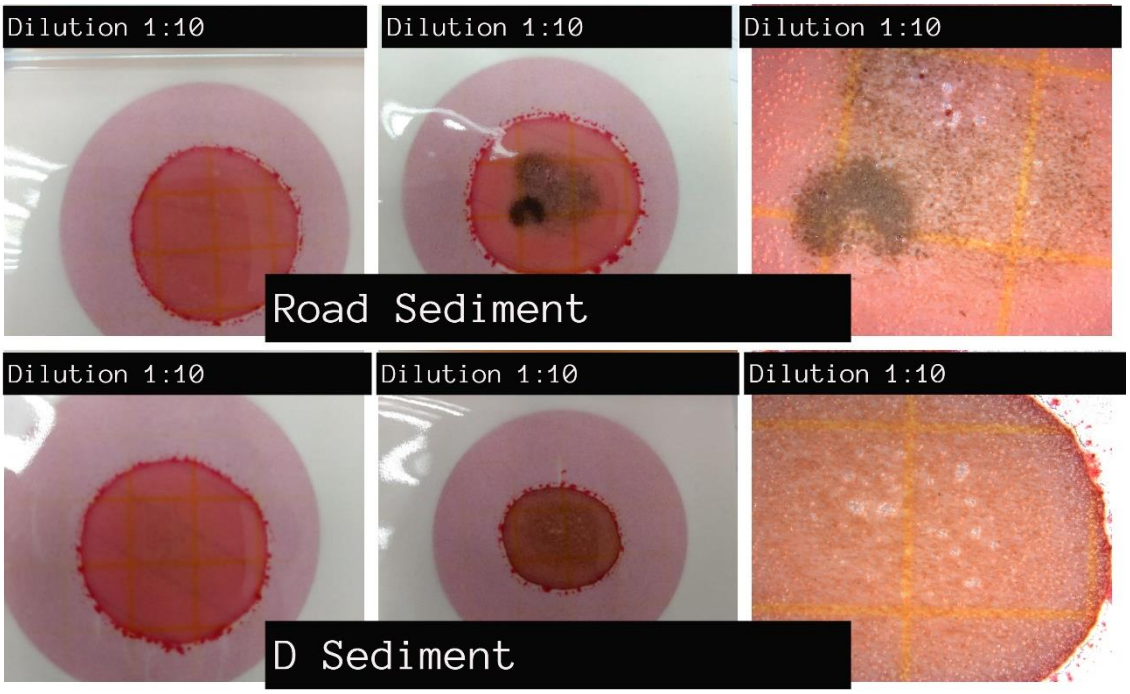


Figure 22: Petrifilm incubated at 37 ° C for 24 hours with dilution of drilling sediments from “D” and "Road". This test with drilling sediment dilutions was done only to identify the presence of bacteria qualitatively.

5.2.6 Bacterial inhibition

The growth of *Pseudomonas sp.* from soil dilution does not show inhibition when placing discs with 1: 1000,1: 1000 dilution of drilling sediments. Therefore, tests were carried out at a higher concentration of drilling sediments, where a kind of halo can be seen, and it was confirmed with dilutions 1: 1 and 1: 2 that it was not an inhibition halo. A noteworthy observation is that the bacteria usually grew on and around the halos, showing that drilling sediments may not affect the growth of ground *pseudomonas* as detailed in the figures 23-25.

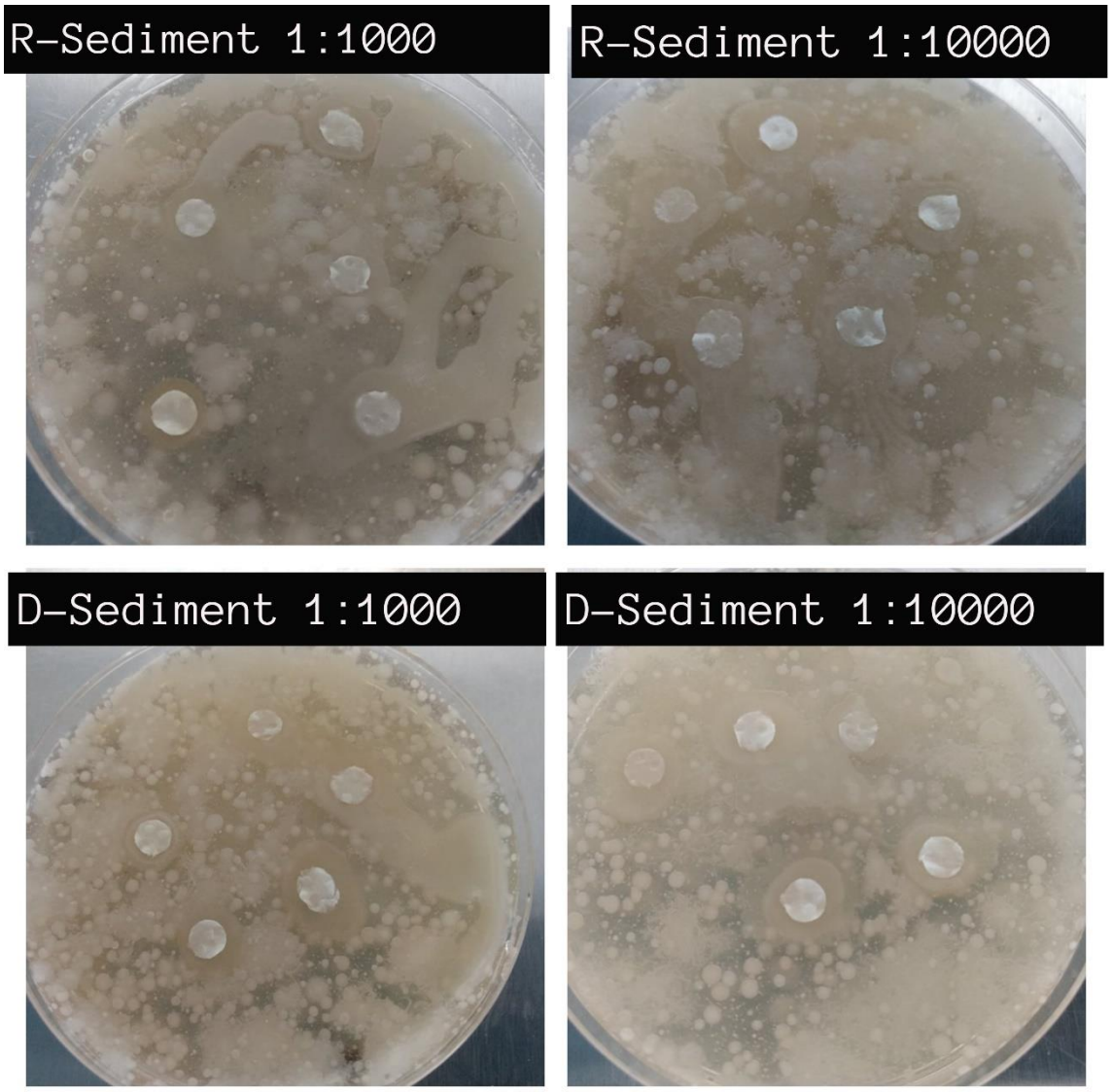


Figure 23: Assay of growth inhibition halos of *Pseudomona sp*. in the presence of drilling sediments at dilutions 1: 1000 and 1: 10000 (sediment: physiological solution). This test is qualitative since it was carried out to observe inhibition in bacterial growth when exposing the sample to drilling sediments. However, no inhibition halo was observed.

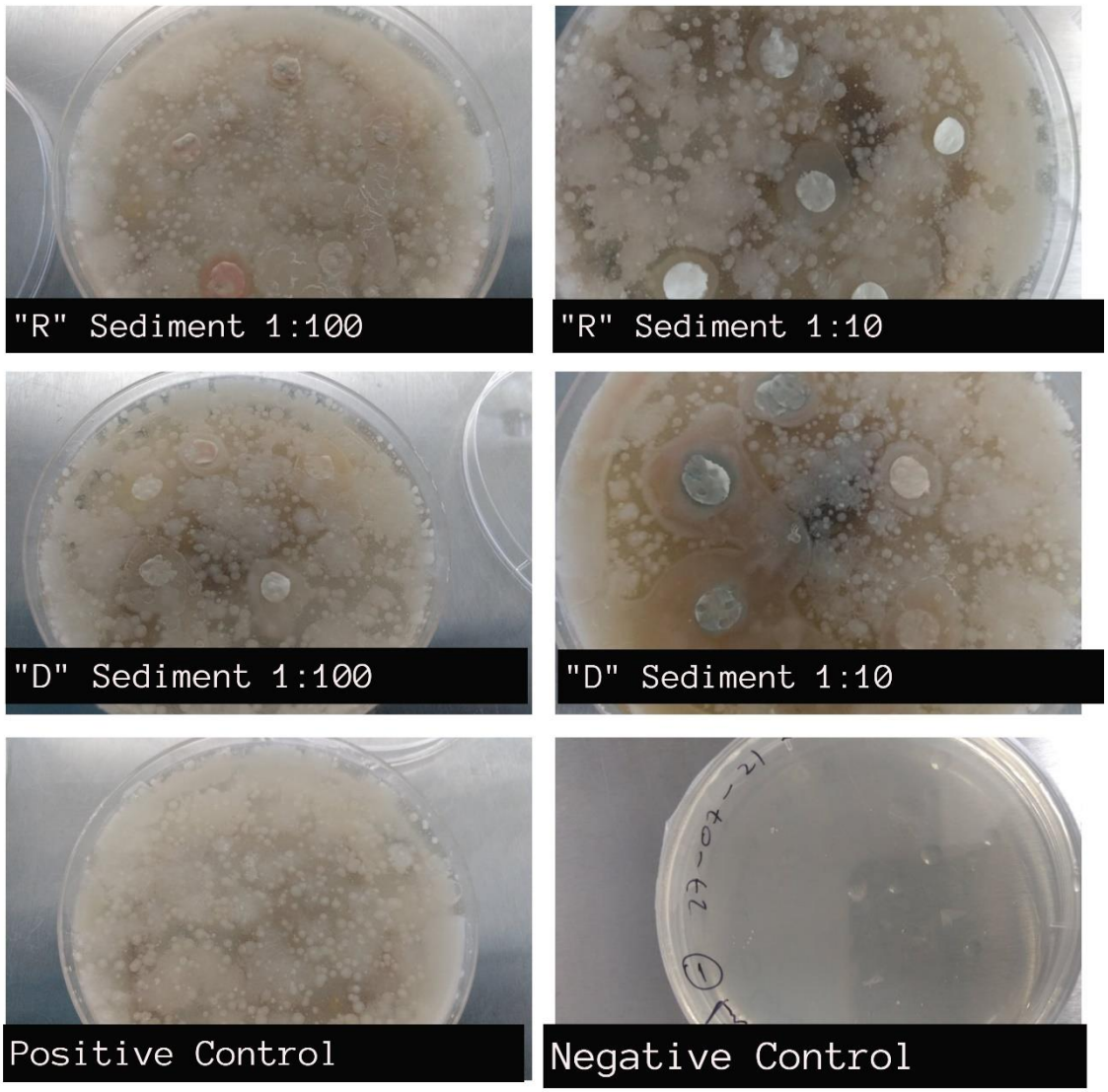


Figure 24: Assay of growth inhibition halos of *Pseudomona sp.* in the presence of drilling sediments at dilutions 1:10 and 1: 100 (sediment: physiological solution)

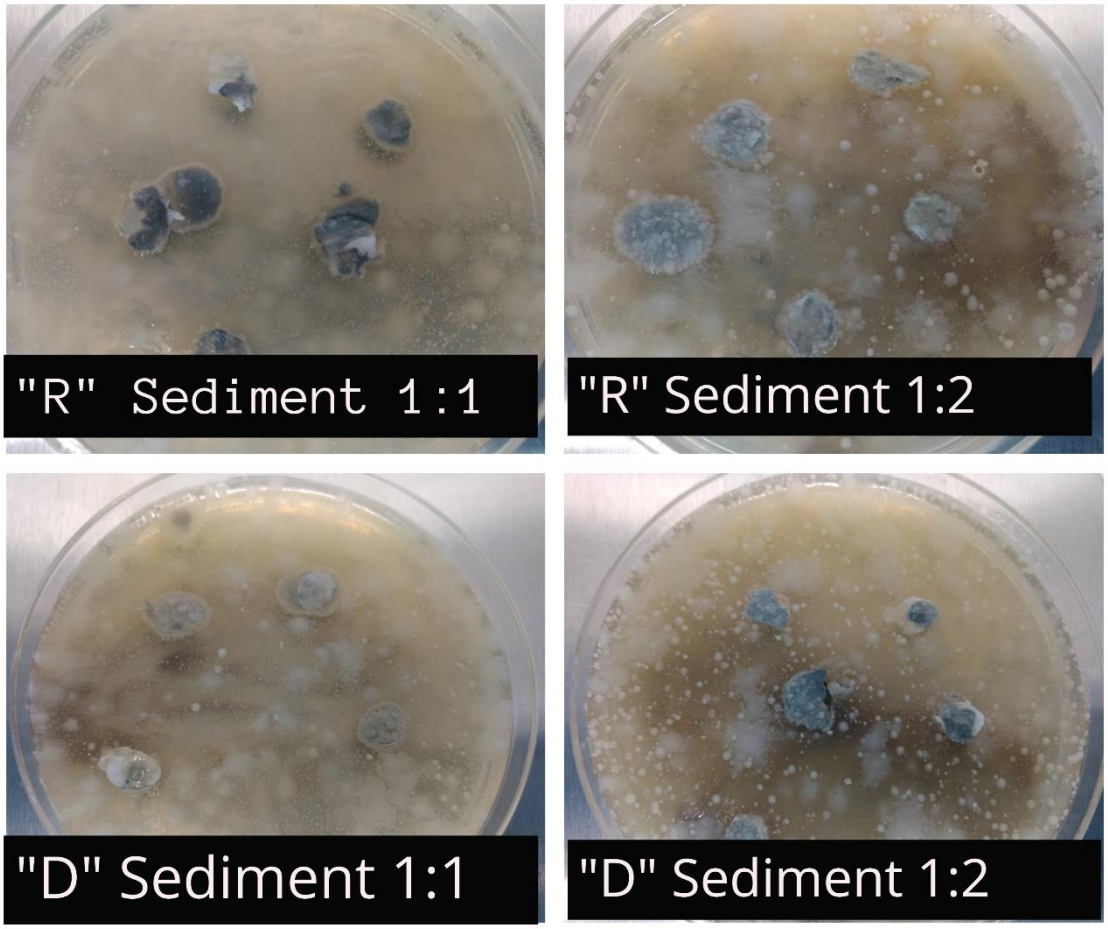


Figure 25: Assay of growth inhibition halos of *Pseudomona sp.* in the presence of drilling sediments at dilutions 1: 1 and 1: 2 (sediment: physiological solution).

5.2.7 Fungi inhibition

Petri dishes with PDA inoculated with soil dilution and exposed to drilling sediments had high fungal growth in 1 week, as did the positive control as shown in the figures 27. The high growth of fungi is possibly due to the high concentration of soil. The negative control showed no contamination in the used saline solution or the PDA medium.

An important observation is that the growth was just as enormous, although the soil concentration was reduced in the dilutions inoculated using the pour plate technique. No inhibition of fungal growth was observed despite using a large concentration of concentrating sediments in the medium holes (figure 27).

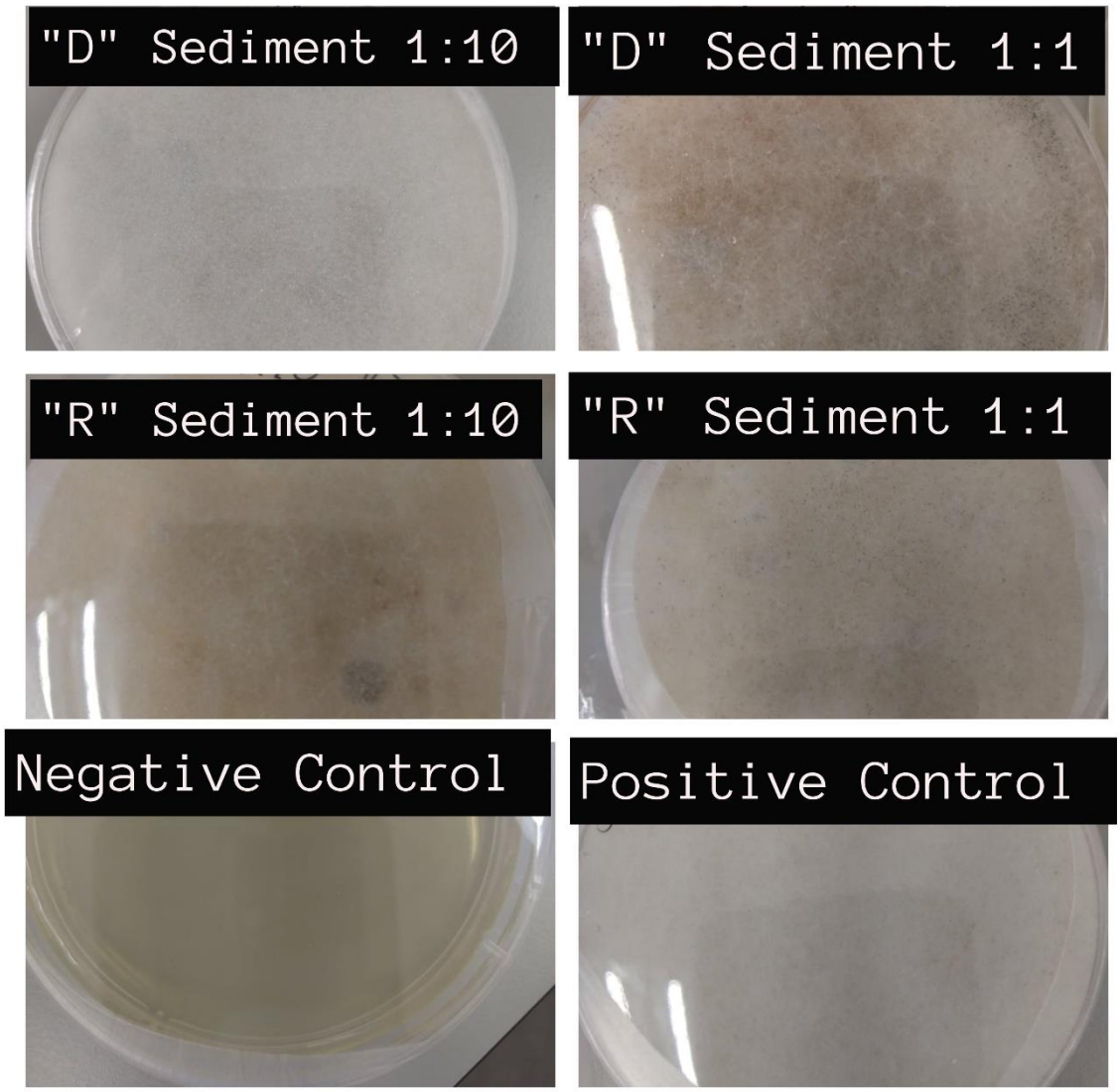


Figure 26: Petri inhibition test of the fungi present in the soil dilution against different dilutions 1:10-1:1 of drilling sediment. This test is qualitative since it was carried out to observe if there was inhibition in the growth of fungi when exposing the samples to drilling sediments. However, no inhibition was observed.

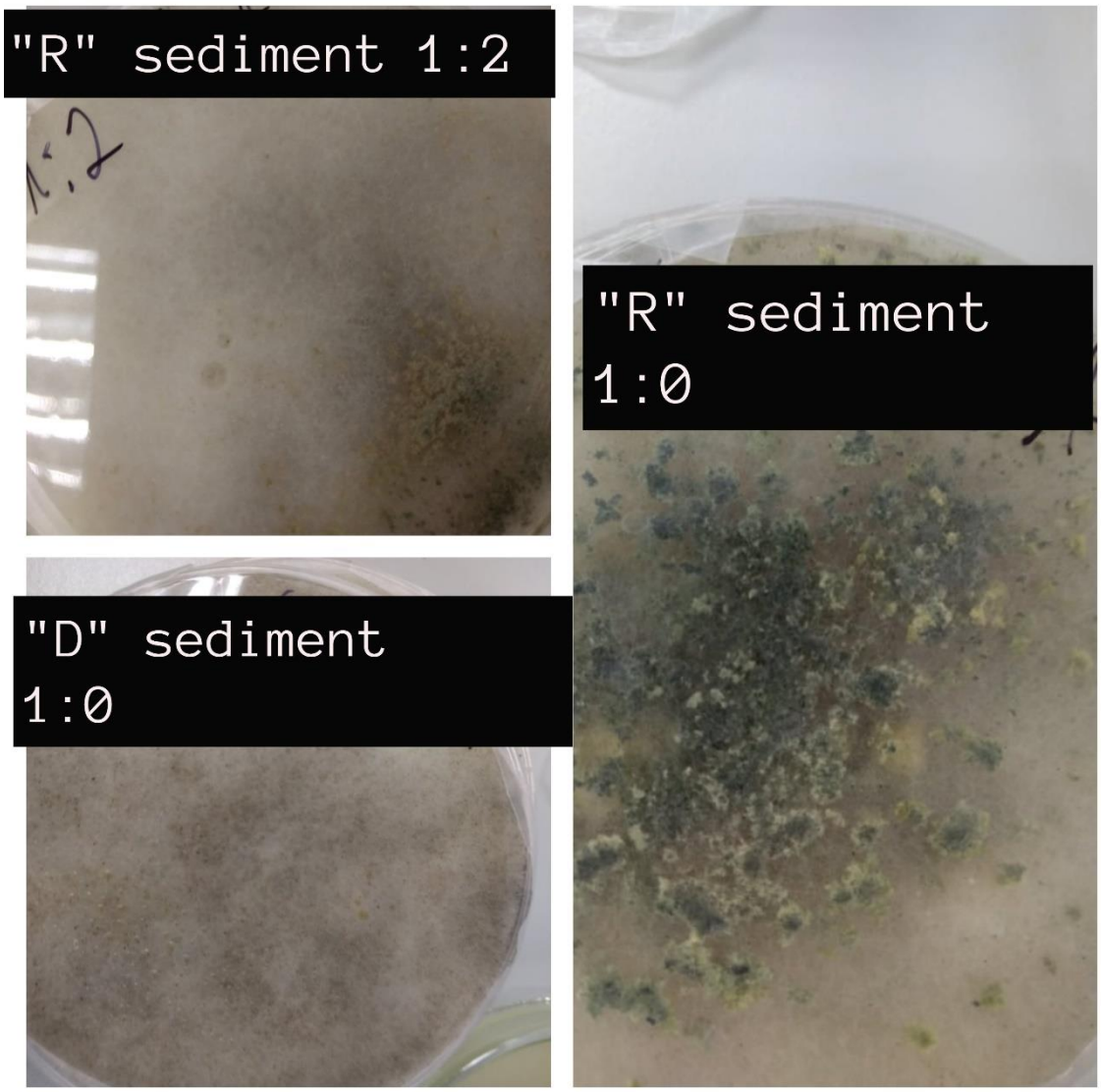


Figure 27: Petri inhibition assay of the growth of fungi present in a dilution with lower soil concentration in the presence of different 1: 0-1: 2 dilutions of drilling sediment.

5.2.8 Inhibition in TSB and Agar

The inhibitions carried out in liquid medium and inoculated in Agar showed that there is an inhibition of the growth of *"pseudomonas sp."* when exposed to drilling sediments since the positive control showed so much increase that it was impossible to count it. However, when it was exposed to different concentrations of sediments, the growth was lower and countable, as shown in the table 12. Another data to consider is that there was more remarkable bacterial growth in those exposed to “D”, indicating that the sediment of the "Road" pathway inhibits the growth of bacteria more. Likewise, the null presence of bacteria in the negative control showed no contamination in the test tubes covered with gauze and cotton.

Table 12: Count plate of *Pseudomonas sp.* exposed to different concentrations of drilling sediment at 24 and 48 hours in liquid medium TSB to Agar.

LD	Sediment	Bacteria count	CFU	Hours
1	Road	1	12200000	24
10	Road	15	20250000	24
50	Road	40	4000000	24
100	Road	104	104000	24
1	Road	7	85400000	48
10	Road	40	5400000	48
50	Road	70	7000000	48
100	Road	134	134000	48
1	D	6	73200000	24
10	D	28	37800000	24
50	D	40	4000000	24
100	D	80	80000	24
1	D	6	73200000	48
10	D	35	47250000	48
50	D	70	7000000	48
100	D	130	130000	48
Control +	No sediment	Uncountable	Uncountable	24-48
Control -	No sediment	0	0	24-48

5.3 Composition and characteristics of the samples

From table 13, it can be seen that there are large amounts of sulfur due to the use of surfactants from drilling sediments. Large amounts of aluminum can be detrimental to nearby bodies of water and plant growth. Other elements found in large quantities are barium, calcium, cobalt, copper, chromium, strontium, manganese, nickel, molybdenum, nickel, titanium, tungsten, and zinc.

Table 13: Analysis of metal types from a SRU drilling sediment sample with a humidity of 30.6% prepared by the Gruentec chemical laboratory.

Humidity % (1,2)	30.6	ASTM-4959-07 / MM-S-02	Plant B

Metals in dry weight:		
Aluminum mg/kg	57127	EPA 6020 A / MM-AG/S-39
Antimony mg/kg	<0.2	EPA 6020 A / MM-AG/S-39
Arsenic mg/kg	1.7	EPA 6020 A / MM-AG/S-39
Sulfur mg/kg	60041	EPA 6020 A / MM-AG/S-39
Barium mg/kg	80	EPA 6020 A / MM-AG/S-39
Beryllium mg/kg	0.3	EPA 6020 A / MM-AG/S-39
Boron mg/kg	<20	EPA 6020 A / MM-AG/S-39
Cadmium mg/kg	0.1	EPA 6020 A / MM-AG/S-39
Calcium mg/kg	40059	EPA 6020 A / MM-AG/S-39
Cesium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Cobalt mg/kg	53	EPA 6020 A / MM-AG/S-39
Copper mg/kg	954	EPA 6020 A / MM-AG/S-39
Chrome mg/kg	70	EPA 6020 A / MM-AG/S-39
Dysprosium mg/kg	0.9	EPA 6020 A / MM-AG/S-39
Erbium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Tin mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Strontium mg/kg	207	EPA 6020 A / MM-AG/S-39
Europium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
phosphorus %	0.1	EPA 6020 A / MM-AG/S-39
Gadolinium mg/kg	0.9	EPA 6020 A / MM-AG/S-39
Germanium mg/kg	0.8	EPA 6020 A / MM-AG/S-39
Hafnium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Iron	7.2	EPA 6020 A / MM-AG/S-39
Ytterbium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Lithium mg/kg	2.5	EPA 6020 A / MM-AG/S-39
Lutetium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Magnesium %	1.5	EPA 6020 A / MM-AG/S-39

Metals in dry weight:		
Magnesium %	1.5	EPA 6020 A / MM-AG/S-39
Manganese mg/kg	322	EPA 6020 A / MM-AG/S-39
Mercury mg/kg	0.3	EPA 6020 A / MM-AG/S-39
Molybdenum mg/kg	21	EPA 6020 A / MM-AG/S-39
Nickel mg/kg	64	EPA 6020 A / MM-AG/S-39
Silver mg/kg	28	EPA 6020 A / MM-AG/S-39
Lead mg/kg	2.7	EPA 6020 A / MM-AG/S-39
Potassium %	0.1	EPA 6020 A / MM-AG/S-39
Praseodymium mg/kg	0.6	EPA 6020 A / MM-AG/S-39
Rubidium mg/kg	3.4	EPA 6020 A / MM-AG/S-39
Samarium mg/kg	0.8	EPA 6020 A / MM-AG/S-39
Selenium mg/kg	5	EPA 6020 A / MM-AG/S-39
Sodium %	0.5	EPA 6020 A / MM-AG/S-39
Thallium mg/kg	<0.1	EPA 6020 A / MM-AG/S-39
Tantalum mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Tellurium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Titanium mg/kg	303	EPA 6020 A / MM-AG/S-39
Thulium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39
Uranium mg/kg	0.1	EPA 6020 A / MM-AG/S-39
Vanadium mg/kg	125	EPA 6020 A / MM-AG/S-39
Tungsten mg/kg	163	EPA 6020 A / MM-AG/S-39
Zinc mg/kg	212	EPA 6020 A / MM-AG/S-39
Zirconium mg/kg	<0.5	EPA 6020 A / MM-AG/S-39

Table 14: Analysis of types of metals of a water sample from the WWTP of sewage prepared by the chemical laboratory Gruentec.

Total metals:		
Aluminum mg / l	87	EPA 6020 B / MM-AG/S-39
Antimony mg / l	0.025	EPA 6020 B / MM-AG/S-39
Arsenic mg / l	0.017	EPA 6020 B / MM-AG/S-39
Sulfur mg/ l	96	EPA 6020 B / MM-AG/S-39
Barium mg/ l	1.3	EPA 6020 B / MM-AG/S-39
Beryllium mg / l	0.0009	EPA 6020 B / MM-AG/S-39
Boron mg/ l	0.08	EPA 6020 B / MM-AG/S-39
Cadmium mg / l	0.0025	EPA 6020 B / MM-AG/S-39
Calcium mg / l	21	EPA 6020 B / MM-AG/S-39
Cerium mg / l *	0.06	EPA 6020 B / MM-AG/S-39
Cesium mg/ l	0.0018	EPA 6020 B / MM-AG/S-39
Cobalt mg/ l	0.025	EPA 6020 B / MM-AG/S-39
Copper mg/ l	1.3	EPA 6020 B / MM-AG/S-39
Chromium mg / l	0.47	EPA 6020 B / MM-AG/S-39
Dysprosium mg/l	0.0035	EPA 6020 B / MM-AG/S-39
Erbium mg/ l	0.0019	EPA 6020 B / MM-AG/S-39
Tin mg /l	0.11	EPA 6020 B / MM-AG/S-39
Strontium mg / l	0.15	EPA 6020 B / MM-AG/S-39
Europium mg / l	0.0012	EPA 6020 B / MM-AG/S-39
Phosphorus mg/ l	127	EPA 6020 B / MM-AG/S-39
Gadolinium mg / l	0.0039	EPA 6020 B / MM-AG/S-39
Gallium mg / l	0.027	EPA 6020 B / MM-AG/S-39
Germanium mg / l	0.0052	EPA 6020 B / MM-AG/S-39
Hafnium mg / l	0.0005	EPA 6020 B / MM-AG/S-39
Iron mg / l	230	EPA 6020 B / MM-AG/S-39
Holmium mg / l	0.0008	EPA 6020 B / MM-AG/S-39
Lanthanum mg / l	0.032	EPA 6020 B / MM-AG/S-39
Lithium mg / l	0.044	EPA 6020 B / MM-AG/S-39

Total metals:		
Lutetium mg / l	0.0003	EPA 6020 B / MM-AG/S-39
Magnesium mg / l	23	EPA 6020 B / MM-AG/S-39
Manganese mg / l	0.95	EPA 6020 B / MM-AG/S-39
Mercury mg / l	0.0058	EPA 6020 B / MM-AG/S-39
Molybdenum mg / l	0.404	EPA 6020 B / MM-AG/S-39
Neodymium mg / l	0.021	EPA 6020 B / MM-AG/S-39
Niobium mg / l	0.0061	EPA 6020 B / MM-AG/S-39
Nickel mg / l	0.066	EPA 6020 B / MM-AG/S-39
Silver mg/ l	0.0086	EPA 6020 B / MM-AG/S-39
Lead mg /l	0.16	EPA 6020 B / MM-AG/S-39
Potassium mg / l	38	EPA 6020 B / MM-AG/S-39
Praseodymium mg /l	0.0052	EPA 6020 B / MM-AG/S-39
Rubidium mg / l	0.05	EPA 6020 B / MM-AG/S-39
Samarium mg / l	0.0045	EPA 6020 B / MM-AG/S-39
Selenium mg / l	0.026	EPA 6020 B / MM-AG/S-39
Silicon mg / l	65	EPA 6020 B / MM-AG/S-39
Sodium mg/ l	94	EPA 6020 B / MM-AG/S-39
Thallium mg / l	<0.0002	EPA 6020 B / MM-AG/S-39
Tantalum mg / l	0.07	EPA 6020 B / MM-AG/S-39
Tellurium mg / l	0.0004	EPA 6020 B / MM-AG/S-39
Titanium mg / l	0.75	EPA 6020 B / MM-AG/S-39
Thorium mg / l	0.0007	EPA 6020 B / MM-AG/S-39
Thulium mg / l	0.0002	EPA 6020 B / MM-AG/S-39
Uranium mg / l	0.0035	EPA 6020 B / MM-AG/S-39
Vanadium mg / l	0.23	EPA 6020 B / MM-AG/S-39
Tungsten mg / l	0.043	EPA 6020 B / MM-AG/S-39
Ytterbium mg / l	0.0021	EPA 6020 B / MM-AG/S-39
Zinc mg /l	1.6	EPA 6020 B / MM-AG/S-39

6 DISCUSSION

Since the emergence of living organisms, life has evolved to adapt to environmental conditions. Only the human being has used his intelligence to adapt the environment to his needs with the thought that resources were inexhaustible, degrading the environment with the activities of their recent industrialized societies. Ecotoxicology was born from the need for a science that studies the effects of toxics or natural or synthetic pollutants on the ecosystem and its components, such as humans, flora, animals, and microorganisms[73].

Mining has devised techniques to penetrate the ground with less effort and greater depth to avoid immense excavations. These machines drill by rotating and pushing the rocks but use chemical mixtures or drilling fluids that facilitate drilling, avoid damaging the well and machinery. However, many of these additives are harmful to aquatic or terrestrial organisms, so studying their harmful effects on the environment is necessary[23,74]. Likewise, the analysis of drilling cuts is vital because they usually are composed of heavy metals such as Cadmium, Zinc, Lead, Mercury, Arsenic, Copper and more over. These metals affect human health, affect the nervous systems of living beings, are carcinogenic, neurotoxic, can irritate the intestines and the death of aquatic species[75]. Large amounts of aluminum at highly acidic pH are detrimental because it solubilizes in ionic forms that inhibit root elongation growth. It is an abundant metal in the environment. The excess of copper in the plant substrates affects the development of the roots by burning their tips, causing the plants to have a negative geotropism because they grow laterally and for a prolonged time cause a deficiency of nutrients in the plant, causing deterioration of the same[76]. The element chromium can be presented as a mineral and in various valence states, but there is no evidence that metallic chromium has adverse effects on the environment. Its most unstable form is Cr (Vl), which can cause cancer in humans[73].

WWTPs must be monitored to avoid releasing inorganic substances such as minerals and metals that are not biodegradable. Likewise, organic substances are generally used as food for microorganisms. Still, many synthetic organic compounds are not biodegradable and increase the sedimentation rate of bodies of water, affecting the food chain, massively reducing species, and increasing algae growth[42].

Nitrogen and phosphorus are essential factors that must be controlled in wastewater discharges because they alter the average concentrations of nutrients in the environment

to which they are discharged, causing a reduction in dissolved oxygen levels in water bodies to a levels of anoxia that causes the death of species[51].

Dead seeds only go through the imbibition phase and the water absorption delay period shows only broadened seeds. Only the seeds that are germinating enter the protrusion phase of the root, breaking the seed coat. The root length depends on the characteristics of the seed and the levels of humidity found, such as permeability of the cover, oxygen absorption, size of the seed, composition of the medium where they grow, temperature and more over[13].

The release of fats and oils to bodies of water causes a high biochemical oxygen demand in the degradation of organic matter, and it is also difficult for bacteria to metabolize fats. In addition, fats and oils are soluble in water or immiscible, remaining on the surface in the form of foams or creams and affecting biological and physical-chemical treatments, so it is recommended to eliminate them in the first steps of wastewater treatment[77,78].

Grease traps are essential in wastewater pretreatment because they retain and slow wastewater streams within a chamber that cools the waters allowing grease to float and other heavy solid waste to sink. Then, the separated wastewater is treated in the wastewater plants[79]. The excess of organic matter, total coliforms, and *E. coli* represent a problem in the degradation of organic matter due to the antagonism and competition against beneficial microorganisms in the production of antioxidants. This competition prevents beneficial microorganisms from lowering the pH that makes the environment uninhabitable for pathogens[80].

The heavy metals arsenic, lead, Cadmium, and Mercury, are highly toxic and accumulative metals that can end up as a food source in the food chain and finally be consumed by humans causing blindness, amnesia, or even death. Lead has toxic effects on plants, plankton, and aquatic organisms such as fish, entering through gills, digestive tract, and the skin's surface, causing hematological alterations such as rapid mortality of red cells. Arsenic affects parameters linked to blood, biochemicals, and Cadmium is a more toxic metal in freshwater since it forms compounds with less bioavailability in saltwater. This metal deforms the skeletal system and damages the functioning of the kidney for fish and humans. Mercury is linked to a deterioration of the coating of the gills, loss of balance, reduction in growth-reproduction, and affects fish's metabolism and death[81–83].

In germination tests where the seedlings are exposed to different substances, they generate different variants in the development of seedlings, classifying them as normal, abnormal,

and ungerminated seeds. Abnormal seedlings are those that have damaged primary roots, without development, minimal vigor to break the seminal envelope, growth contrary to the ground, or negative geotropism. Other characteristics that show abnormalities are the presence of crooked hypocotyls, epicotyls, and mesocotyls, without development and deformed, damaged, dry cotyledons-leaves and dead tissue[11].

The vigor of the seeds determines the sum of properties and physiological activities carried out by the seed, as well as its genetic aspects. Vigor is linked to greater biomass production since it will have a better radical development as aerial, the decrease or less amount of humidity has a negative effect on the accumulation of biomass in the aerial part and physiological processes that affect the amount of biomass such as perspiration, stomatal conductance, and photosynthetic rate[84,85].

Bacteria with energy-dependent metal flow systems such as ATPases and proton pumps are associated with the resistance of metals such as Arsenium, Chromium, Cadmium, as is the example of *Pseudomonas putida*[86]. According to Bedoya et al.[87] *Pseudomonas spp.* isolated to wastewater with amounts of lead showed tolerance and resistance mechanisms up to 2500 mg / L concentrations. According to Rivet[88], the 18% chlorine dioxide disinfectant inhibits the growth of *Pseudomonas spp* at concentrations higher than 150 ppm, but the excessive use of these cleaning products generates resistant microorganisms. Many fungi obtain their food from dead organic matter, tolerate low humidity, and among them, protozoa can be used as bioindicators of residual plant functioning since they are rarely found in activated sludge flocs because they need environmental conditions with low pH, deficiency of nitrogen, and presence of toxic products. If a wastewater system has many sedimentable flocs, it is possibly due to protozoan fungi such as ciliates, flagellates, and rhizopods, as they consume bacteria from aquatic systems. This type of fungi improves the quality of the effluents, reduces the biochemical oxygen demand, the turbidity of the water, and preys on various pathogenic bacteria[89].

The recycling of materials has had remarkable growth to limit the discharge of waste into the environment and reduce the destruction of the environment to obtain virgin raw materials. In construction, the use of concrete consumes a large amount of raw material, having a tremendous environmental impact. A material that has lately been considered a

potential replacement for raw materials is drilling sediment that can be used as aggregate to produce concrete for non-structural applications only with controlled low-strength materials[90].

7 CONCLUSIONS AND RECOMMENDATIONS

- The growth inhibitions of the seedlings are possibly due to the presence of copper and aluminum that inhibit root growth and burn the roots causing lateral growth, as was the case with lettuce seedlings that died completely in 20 days.
- Seedlings exposed to drilling sediments showed low biomass values. Likewise, compared to the control, it is observed that exposing the seedlings to drilling sediment inhibits the physiological activities since the air-subterranean size was much smaller than the control; they had a skinny stem and worn leaves.
- The drilling sediments did not show inhibition in the growth of soil fungi when exposed to drilling sediments since an accelerated growth of the fungi was observed although the concentration of soil dilutions was reduced and the dose of sediments was increased.
- Many fungi consume organic matter, pathogens from sewage and are even used as a bioindicator of contamination in the WWTP process. Therefore, it is normal that gray and black water sewage sludge did not inhibit fungal growth in the PDA medium assay.
- Absorbent papers in Petri dishes with drilling sediment dilutions lost their moisture at high sediment concentrations. Therefore, the seeds had an early inhibition in the absorption of water since they lacked water for the development and protrusion of the roots.
- The wastewater plants of the “X camp” showed greater inhibition with respect to “Y camp”. Possibly this is due to the fact that the plants had not received a cleaning and maintenance of the fat bed for the gray water sediments.
- The *Pseudomonas spp.* inoculated in solid agar media did not show inhibition when exposed to drilling sediments at different concentrations. However, when carrying out the inhibition in liquid medium and after counting in a Petri dish with Agar at 24 and 48 hours, it was observed that the positive control had enormous and incalculable growth, but the inoculations with drilling sediments showed fewer colonies as sediment concentration was increased.

- “X camp” WWTP sludge was placed in drying beds and extracted with zero moisture for sewage and some moisture for gray water. However, it was evident that they still had live bacteria as colonies grew on the Petrifilm plates.
- After ten days, radish and lettuce seeds exposed to WWTP sludge from "camp X" showed delayed germination and seedling development. Some seeds did not break their seed coat and had tiny roots. However, at thirty days, the seedlings exposed to WWTP sludge showed greater development than the control.
- The gray water plants of the “X camp” presented no sedimentation and floating floccules due to contamination from the grease traps. The grease traps were not working due to a lack of maintenance and cleaning of the same. Likewise, there was a contamination of organic matter and excess total coliforms that entered the matter and liquor degradation chamber. The high presence of coliforms causes competition, preventing beneficial microorganisms from correctly degrading the organic matter in the wastewater. Also, eliminating fats in liquor is difficult since it is difficult to metabolize fats for many beneficial bacteria. For this reason, the discharge water from the treatment plants presented floating fat foams, high surfactants, small amounts of dissolved oxide due to the high biochemical oxygen demand, and a high number of total coliforms.
- The discharge water from the gray water plant releases discharge water with many organic residues, so the microorganisms will consume the available oxygen from the water bodies in case of being released.
- Beneficial microorganisms lower the pH by degrading organic compounds reducing the amount of pathogens in the water. However, as the gas trap did not work correctly, the liquor was contaminated. Therefore, there were many total coliforms in the Petrifilm Plates with the dilution of gray water sludge.
- It is imperative to try to recycle the drilling sediments of the mining project because they would contribute to the reduction of the consumption of virgin raw material by reducing pollution to obtain the raw material and the release of sediments since they have metals that inhibit the growth of plants and microorganisms affecting the environmental matrix.
- The water analysis of the “X camp” sewage plant showed small amounts of the metals most toxic to aquatic life, such as Lead, Arsenic, Mercury, and Cadmium.

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