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ECORFAN Journal Republic of Nicaragua

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Instructions for Scientific, Technological and Innovation Publication

Knowledge Area

The works must be unpublished and refer to topics of agriculture-forest, pathology-sustainable, forest, management, horticulture, engineering and integrated water useand other topics related to Biotechnology and Agricultural Sciences.

Presentation of Content

In volume eight, issue fifteen, as the first article we present, *Cultivation conditions of the Californian Red Worm in Ciudad Juárez, Chihuahua*, by NAVARRO-ENRÍQUEZ, Laura, RIVERA-MOJICA, Denisse, TOVAR-VÁSQUEZ, Amado and CHÁVEZ-MONTELONGO, Ana Lorena, with adscripction in the Universidad Tecnológica Paso del Norte, as a second article we present, *Lipopeptide produced by the bacteria Bacillus mojavensis with activity against the phytopathogenic fungus Colletotrichum gloeosporioides Penz & Sacc var. Minor*, by LÓPEZ-GUTIÉRREZ, Tomás Joel, SARABIA-ALCOCER, Betty, GUTIÉRREZ-ALCÁNTARA, Eduardo and AKÉ-CANCHÉ, Baldemar, with adscripction in the Universidad Autónoma de Campeche, as third article we present, *Characterization of Paenibacillus spp. CBRM17 as antagonist of phytopathogenic fungi and growth promoter of Capsicum chinense Jacq.*, by MEJÍA-BAUTISTA, Miguel Ángel, CRISTÓBAL-ALEJO, Jairo, PACHECO-AGUILAR, Juan Ramiro and REYES-RAMÍREZ, Arturo, with adscripction in the Instituto Tecnológico de Conkal and Universidad Autónoma de Querétaro, as fourth article we present, *Study on the generation and composition of household solid waste in Ciudad Valles, S.L.P.*, by ACOSTA-PINTOR, Dulce Carolina, VIDAL-BECERRA, Eleazar, MOJICA-MESINAS, Cuitláhuac and CÓRDOVA-OLGUÍN, Yuridia, with adscripction in the Tecnológico Nacional de México – Campus Ciudad Valles.

Content

Article **ECORFAN Journal-Republic of Nicaragua**
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Cultivation conditions of the Californian Red Worm in Ciudad Juárez, Chihuahua

Condiciones de cultivo de la Lombriz Roja Californiana en Ciudad Juárez, Chihuahua

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Abstract

The objectives of this research are to promote new knowledge related to sustainability in the interested university student community and in other students of different schools. Improve the green areas of the University and test the result of the Organic Compost in the plants of some teachers of the institution. Comply in part with the social commitment to transmit knowledge and care for the environment. In this investigation, it will be proved visually that the Red Worm only develops well under average temperatures of 30 °C (Edward & Bater, 1992). In the results obtained it was possible to observe that although in Ciudad Juarez, the summers are very hot, the winters are short and cold. During the course of the year the temperature varies from 1 °C to 36 °C in summer and up to 6 °C below zero on cold fronts. The earthworm reproduction did not stop if it did not double the initial amount placed in the worm compost.

Sustainability, Develops, Investigation, Reproduction, Compost

Resumen

Los objetivos de esta investigación son propiciar en la comunidad estudiantil universitaria interesada y en otros estudiantes de diversas escuelas nuevos conocimientos relacionados con la sustentabilidad. Mejorar las áreas verdes de la Universidad y probar el resultado del Abono Orgánico en las plantas de algunos maestros de la institución. Cumplir en parte con el compromiso social de transmitir conocimientos y el cuidado del medio ambiente. Sin embargo, en esta investigación se probará de manera visual que la Lombriz Roja solo se desarrolla bien bajo temperaturas promedio de 30°C (Edward & Bater,1992. En los resultados obtenidos se pudo observar que pese a que, en Ciudad Juárez, los veranos son muy calientes, los inviernos son cortos y fríos. Durante el transcurso del año la temperatura varia de 1°C a 36°C en verano y hasta 6°C bajo cero por frentes fríos. La reproducción de lombrices no ceso si no duplico su cantidad inicial colocada en los lombricomposteros.

Sustentabilidad, Desarrollo, Investigación, Reproducción, Composta

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Introduction

The indiscriminate use of pesticides in agriculture involves a series of contamination risks for farmers, soil, water, flora and fauna resources, and food intended for consumption by the population. There is substantial evidence of negative effects on human health and harmful impacts on the environment (MDRyT, 2017 and Mamani, 2020 and Martínez, 2022).

The beneficial effect of Californian redworms is not a recent discovery but has been known since ancient times. The Greeks also knew of their beneficial activity for agriculture and soil improvement; the Greek philosopher Aristotle called them "the intestine of the earth". Reines (1998) points out that soil without earthworms becomes cold, hard, unfermentable and therefore sterile. Later, the eminent biologist Charles Darwin spent more than 10 years studying the structure, feeding and life of earthworms and was the first to demonstrate the function of earthworms in nature in his book, "The transformation of vegetable detritus by the action of earthworms, in 1881" (Rodriguez,1998). (Rodriguez, 1998)

Thomas Barret (1948) was attributed the paternity of the breeding of earthworms in captivity. Finally, in the middle of the 20th century, worms started to be used for the production of organic fertilisers on a large scale. The present research takes place in Ciudad Juarez, Chihuahua because it has physical characteristics of an arid zone (extreme temperatures in winter and summer, as well as strong winds and scarce rainfall during the year). Due to all of the above and other social situations, it represents a great challenge for its development and the well-being of its inhabitants. The area of Anapra, where the Paso del Norte Technological University is located, is located in a desert zone, with sandy soils that, due to their characteristics, contain less than one percent organic matter. The vegetation is mainly composed of cactus and scrubland; with respect to rainfall, it is considered to be less than 250 mm per year, most of it falling in the summer months.

The objectives of this research are to provide the interested university student community and other students from different schools with new knowledge related to sustainability. To improve the green areas of the University and samples offered to teachers with the use of organic compost. Fulfilling part of the social commitment to transmit knowledge and care for the environment (Navarro L, 2018). Above all, to seek food sovereignty and security as a transcendental economic-social category (Altieri, 1999).

The need to conduct this research is due to the importance and the various opportunities offered by the university through the learning offered by the various careers offered, both in the area of technology and administration, all of them developed in the commitment to environmental care. (Corral, 2018), (Navarro L, 2018).

Vermicomposting is a process of bio-oxidation, degradation and stabilisation of the average organic matter by the combined action of earthworms and microorganisms, through which a stabilised, homogeneous and fine-grained final product called vermicompost, vermicompost or worm humus is obtained. (Villegas, 2017)

The annelid species most commonly used in the decomposition of organic waste is Eisenia fetida, particularly known as the Californian red worm. According to Chamorro and Romero (1996), the adult red worm measures between 6 and 8 cm in length and has a diameter of 3 to 5 mm. The exploitation of earthworms is carried out in two ways: for ecological or productive purposes (Somarriba, 2004).

Figure 1 Californian Red Worm
Source: Own elaboration

The extract or leached earthworm humus is the result of the digestion of all those materials such as crop residues, organic waste and all kinds of manure, which serve as food for the earthworm. The excreta resulting from the digestion process, when subjected to a washing and filtering process, gives a product that possesses properties (Rosales, 1998).

Description of the method

In order to carry out this project of starting a business in Ciudad Juarez, the following methodology was developed.

Key activities

First stage

The initial activities consisted in the elaboration of a 2x3.5 meters waterproofed wooden roof for the protection of two vermicomposters. For this purpose, a 200-litre drum was acquired, which was split in half and in each of the parts the "bed" was prepared with soil, manure and water for eight days to introduce the breeding stock donated by CONALEP II, an average of 20 to 30 kilos of substrate containing approximately 3 kg of earthworm.

Figure 2 Worm bedding
Source: Tovar, A., (2018)

The care of the earthworm culture consists of keeping the "beds" covered with black plastic in winter, and in summer uncovered but in the shade and protected with netting to avoid flies, birds or other organisms that could damage them; provide them with food (manure or vegetable waste with humidity between 50 and 80%, for this a layer of this material is spread between 3 to 4 cm along the worm culture, twice a week) and clean water until obtaining a humidity of 80% in the culture, with these cares it is expected that around three months the worms can be deposited in the definitive space. (Infante, 2016 and Somarriba, 2004).

For the commercialisation of the leachate, a campaign will be carried out at the University with students, teachers and administrative staff, to collect water containers with a capacity of one litre, as well as four-litre gallons, which can be milk or juice, the latter will be requested washed. After the containers have been collected, they will be checked to see if they need to be washed, and the original labels will be removed so that the project's own labels can be applied, and then they will be stored and filled with leachate according to demand.

Figure 3 Leachate produced from vermicomposting
Source: Tovar, A., (2018)

Analysis based on the method

The First Stage (developed in February and March)

Costs.

Waterproofed wooden roofing 2x3.5m = 2100.00

Labour = 800.00

Plastic drum with a capacity of 200 Lts. = 250.00

Gasoline (transport CONALEP II and purchase of = 200.00

200.00 material)

Total------------------------------ = 3350.00

With the above investment two worm composting bins were installed, which were obtained by cutting in half a 200 litre plastic drum with a hole at one end for the exit of the leachate, each one with a capacity of 0.1m3 each. with a capacity of $0.1m^3$

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They were also placed on blocks, considering the appropriate inclination.

Product	Production/year	Costs/production	Net profit/year
Leachate	552 litres/year Retail sale $35.00/litre TOTAL= $46, 080.00	Fixed costs/year $28,282.00 Variable costs/year $14,360.00 TOTAL= 42,642.00	$3,438.00
Humus	140 kg/year Retail $15.00/Kg. Total $2100.00	Fixed costs/year $ 1,718.00 Variable expenses/year $619.54 TOTAL $2,337.54	$-237.54
TOTALS	$48,180.00	$44,974.54	$3,205.46

Table 1 Cost and production table
Source: Own elaboration

The calculation in this section was based on the capacity of two installed and producing vermicomposters with a volume of 0.1 m^3 each.

As a first stage, layers of soil and manure and enough water were added little by little over a period of three weeks to keep the mixture moist.

After this process, the earthworms donated by CONALEP II were installed. To determine the number of worms, the born worms were separated from the introduced worms every 30 days until the 90th day, after 120 days all the worms, including the newly born ones, were quantified. With this production area, the results of the cost and production table were determined (see table 1).

Soil study in the community of Puerto Anapra in Juarez

The soil in the community of Puerto Anapra is composed mainly of sand, which means that water seeps through easily, which means that there is no process of decomposition of organic matter, which is necessary to maintain humidity in the soil, making it difficult for any plant species other than cacti and bushes to survive. A soil suitable for planting non-desert plants should have at least five percent organic matter. The use of vermicompost would provide part of this organic matter required by plants and trees for their development, in such a way that the use of this natural fertiliser generates a sustainable and ecological alternative for domestic gardening, public parks, urban gardens, greenhouses, fruit trees and crops in general.

Socio-economic study of Cd. Juárez

The Municipality of Juárez belongs to the Bravos Judicial District; it borders the municipalities of Ahumada to the south, Ascensión to the west and Guadalupe to the east, in the State of Chihuahua. To the north with the County of El Paso, Texas and the County of Doña Ana in New Mexico, the last two in the United States of America. It is one of the most populated municipalities in Mexico, as is its capital. According to projections, the population of the Municipality of Juarez in mid-2016 according to the Population and Housing Census has 1'407,959 inhabitants, of which 1'301,452 live in Ciudad Juarez (97.70% of the total). Of the 788,514 workers contributing to Social Security in Chihuahua, a total of 17,773 earn one minimum wage, another 310,251 earn twice the minimum wage, 186,488 earn three times the minimum wage, 83,361 earn four times the minimum wage and 46,191 earn up to five times the daily wage. The market study was carried out by applying a survey to a group of men and women of different ages, mainly from 18 to 40 years old and over 40 years old at UTPN campus ANAPRA (Navarro G, 2018) and also electronically through the survey monkey tool.

Results

A quantitative study was conducted by calculating the sample size using the probability method. The sample size was 200 people matching the scenario that would be used for a non-probability method. For which the following calculations will be made.

Sample size calculation:

Calculation for 1185 students

N = ((1.96) ^^{2} (.50) (1-.50) (1185)) / ((1185) (.05) ^^{2}+(1.96) ^^{2} (.50) (1-.50)) =290

Calculation for 199 students

n= ((1.96) ^^{2} (.50) (1-.50) (416)) / ((416) (.05)^^{2}+(1.96)^^{2} (.50)(1-.50))=199

Survey applied to quantify the consumption of naturist products

VERMICOMPOST SURVEY

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1 Personal data

Gender: Male or Female

Age:

2 Do you have a taste for gardening and/or urban agriculture?

YES
NO

3 Do you use any type of chemical fertilizer?

YES
NO

4 Have you had any problems at the time of application?

YES
NO

5 Have you heard about leaching and worm composting?

YES
NO

6 Would you like to use a 100% natural fertiliser that is environmentally friendly and easy to apply?

YES
NO

7 When you think of the product, do you think it is something you need or don't need?

Definitely need it
Probably need it
I don't care
I probably don't need it
Definitely don't need it

8 If the product were available today, how likely would you be to buy the product?

Extremely likely
Very likely
Somewhat likely
Not so likely
Not at all likely

9 How likely are you to replace your current product with the one offered?

Extremely likely
Very likely
Somewhat likely
Not very likely

Analysis of the survey results

Middle-class women, apart from being interested in flowers, also grow vegetables and/or fruit trees in their backyards, so that the activity in many cases also involves their husbands and children, so that their concern also includes caring for the harvesting of their crops.

It is important to note that the houses in this segment are in housing developments, so their backyards are restricted, but they optimise them for gardening. In this group, they are primarily interested in caring for the environment, since most of these households have highly educated people in the family, so that they are willing to invest part of their economic resources in favour of environmental conservation for this activity.

In this segment, the participation of men is not excluded; a large number of them, from young to old, are interested in the cultivation of vegetables and fruit trees in small gardens located in backyards.

Customers

The sale of organic products is generally offered to the middle- and upper-class segment.

By broadening the vision, the aim is to include low-income people as well. According to INEGI 2015 there is a ratio of 98.7 men to 98 women, 98 men for every 100 women, with a total population of 1,391,180 in Chihuahua, which represents 39.1% of the state population. 49.7% are men and 50.3% are women, who culturally are the ones most related to gardening. These segments do not exclude the participation of male farmers in the Juarez Valley.

Conclusions

Worm farming and marketing can become an excellent business with a small investment. However, few people know how to carry out such a project.

Temperature is one of the important factors, but it does not govern worm production.

We also ruled out that worm culture conditions with temperatures of 30°C are not only met but that they can reproduce in cold or warmer conditions and also with rain and wind gusts.

It was observed that the pH variation was in the neutral to slightly alkaline range during the first few days, but by the end of the study there was a rise in pH as well as humidity. The advantages of the vermicomposting production as an ecological alternative to have organic fertilizer and a way to diminish the environmental contamination by the solid residues that are generated day by day which generate problems in our society.

The results of applying the leachate to plants and trees can also be seen in the results, which show an increase in growth and strengthening of the trees.

Figure 4 Lemon plant with leachate applied (Before)
Source: TOVAR, (2018)

Figure 5 Lemon plant that leachate was applied in a period of 5 months (After)
Source: Own elaboration

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December 2022, Vol.8 No.15 1-7

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Article

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Lipopeptide produced by the bacteria *Bacillus mojavensis* with activity against the phytopathogenic fungus *Colletotrichum gloeosporioides* Penz & Sacc var. Minor Simmonds

Lipopeptido producido por la Bacteria *Bacillus mojavensis* con actividad contra el hongo fitopatógeno *Colletotrichum gloeosporioides* Penz & Sacc var. Minor Simmonds

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Abstract

The objective of this work was to determine the antifungal activity against *Colletotrichum gloeosporioides* Penz & Sacc var. minor Simmonds of the lipopeptide(s) isolated and purified from the culture of the marine bacterium *Bacillus mojavensis* (MC3B-22). The results will show that the extraction method with ammonium sulfate was the one that extracted the largest amount of biosurfactants with a yield of 3.1243 g/L compared to the acid precipitation method (0.3173 g/L). The crude extract in the presence of 103 conidia/mL of the fungus *Colletotrichum gloeosporioides* did not present inhibition, but the semi-purified extract obtained with ammonium sulfate reached a minimum inhibitory concentration of 12.5 µg/mL. In conclusion, the optimal method for extracting the biosurfactant was 40% ammonium sulfate, with methanol being a suitable solvent to semi-purify and obtain a minimum inhibitory concentration of 25 µg/mL against C. *gloeosporioides*.

Biosurfactant, Lipopeptide, *Bacillus mojavensis*

Resumen

El objetivo de este trabajo fue determinar la actividad antifúngica contra *Colletotrichum gloeosporioides* Penz & Sacc var. minor Simmonds del o los lipopéptido(s) aislado(s) y purificado(s) del cultivo de la bacteria marina *Bacillus mojavensis* (MC3B-22). En los resultados se determinó que el método de extracción con sulfato de amonio fue el que extrajo la mayor cantidad de biosurfactantes con un rendimiento de 3.1243 g/L en comparación con el método de precipitación ácida (0.3173 g/L). El extracto crudo en presencia de 103 conidios/mL del hongo *Colletotrichum gloeosporioides* no presentó inhibición, pero el extracto semipurificado obtenido con sulfato de amonio alcanzó una concentración mínima inhibitoria de 12.5 µg/mL. En conclusión, el método óptimo para la extracción del biosurfactante fue el sulfato de amonio al 40% siendo el metanol un solvente adecuado para semipurificar y obtener una concentración mínima inhibitoria de 25 µg/mL contra *C. gloeosporioides*.

Biosurfactante, Lipopetido, *Bacillus mojavensis*

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Introduction

Marine microorganisms have evolved particular metabolic and physiological capabilities to adapt to extreme habitats and in response produce metabolites that are generally not expressed in land-based microorganisms. For this reason, the marine habitat is a potential source for the search for new compounds such as antibiotics, antioxidants, bioemulsifiers, biosurfactants, enzymes, drugs, anionic surfactants, vitamins and other commercially important compounds (Sarubbo *et al*., 2022 and Satpute *et al*., 2010).

Synthetic surfactants have generated, in 2008, a market growth of 13 million tonnes worldwide equivalent to $23.9 billion, while in 2009 it increased by 2 % and is expected to increase by a further 3-5 % by 2025 (Mordor 2022 and Reznick *et al*., 2010). However, these surfactants are derived from petroleum, so there is great interest in the search for and application of bioactive marine compounds, such as biosurfactants, which are of great importance, due to their structural and functional diversity as well as their multiple properties, such as their antimicrobial and antiviral activity, plant disease biocontrol agents, high biodegradability and selectivity, low toxicity, environmental compatibility, detergency, dispersion, emulsification, specific under high temperature, pH and salinity conditions, foaming, wetting and solubilisation of hydrophobic compounds. (Nawazish 2022, Perfumo *et al*., 2010, Tapati and Dabashis, 2022,). Another advantage of biosurfactants is that they are obtained by microbial fermentation processes, where inexpensive substrates can be used and culture conditions can be controlled (Desai and Banat, 1997).

Biosurfactants, produced by a wide variety of microorganisms, are classified by their chemical composition into: a) high molecular weight compounds consisting of polysaccharides, lipopolysaccharides, lipoproteins, among others and b) low molecular weight compounds such as glycolipids, polypeptides, lipopeptides, among others (Ekambaram et al 2022; Smyth et al., 2010). Lipopeptides produced by the genus Bacillus have been studied for their broad spectrum of antimicrobial activity, and especially for their antifungal activity (Banat *et al*., 2010).

This is the case of lipopeptides belonging to the iturin family, which show remarkable haemolytic and antifungal activity in vitro. On the other hand, phengicins, although they do not have high haemolytic activity like surfactins and iturins, do have good antifungal activity (Ongena and Jacques, 2007).

Therefore, the aim of this work was to determine the antifungal activity against Colletotrichum gloeosporioides Penz & Sacc var. minor Simmonds of the lipopeptide(s) isolated and purified from the culture of the marine bacterium Bacillus mojavensis (MC3B-22).

Description of the method

Micro-organism

The antagonistic strain B. mojavensis (MC3B-22) used in this work is preserved in deep freeze at -80°C in the Collection of Microbial Environmental Cultures (CCMA) of the Department of Environmental Microbiology and Biotechnology (DEMAB).

Culture conditions

A 24-hour pre-culture of strain B. mojavensis (MC3B-22) in Luria Bertani Miller broth (Fluka) supplemented with sea salts (Sigma) (LBMSM) was performed at 25°C, 140 rpm and constant light. After the time had elapsed, the pre-culture was adjusted to an optical density (OD) of 3 at 520 nm. Ten millilitres of the pre-culture, previously adjusted, was inoculated into blafeated Erlenmeyer flasks containing Luria Bertani Miller broth. The conditions established for the culture were the same as those used for the pre-inoculation. The fermentation time for obtaining the maximum crude extract was 84 hours. Subsequently, the cell-free supernatant (SLC) was obtained by centrifugation (Eppendorf Ultracentrifuge 5810-R) of the culture medium at 4000 rpm at 4°C and filtered with 0.45 μm Millipore membranes (Durán, 2010).

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Biosurfactant extraction

Acid extraction: SLC was adjusted to an acidic pH with 6 M HCl and allowed to stand overnight at 4°C for complete precipitation of the biosurfactant. Subsequently, it was centrifuged at 4000 rpm for 45 minutes at 4°C and the precipitate was collected. The precipitate was resuspended in basified water and then frozen and lyophilised (Labconco). The yield was obtained with respect to the volume of the medium and expressed in gr/L.

Extraction with ammonium sulphate: SLC cooled to (4-5°C) and under slow stirring was added to ammonium sulphate, until a final concentration of 40% was obtained. The extraction was kept under refrigeration and stirring overnight. Hours later it was centrifuged at 4000 rpm for 3 minutes and the packet was suspended in 5 mL of water, frozen and freeze-dried. The yield was estimated with respect to the volume of the medium and expressed in g/L.

Semi-purification of crude extracts

Pure methanol was added to part of the crude extracts obtained from the different extraction methods and the methanolic fraction obtained was transferred to another vial, previously weighed, to determine the yield of the semi-purified extracts.

Biosurfactant activity: collapsed droplet (CG) technique

Three polystyrene microplate coverslips (12.7 x 8.5 cm) of 96 microwells were washed three times with hot water, 96% ethanol and distilled water, and allowed to dry. Subsequently, 2 µL of mineral oil was added to each plate and allowed to stand for 24 hours to allow the oil to spread homogeneously over the surface of the wells. After the stabilisation time, 5 µL of the crude and semi-purified extracts were added at a concentration of 1mg/100 µL, positive control sodium dodecyl sulphate (SDS) at 1.84 mg/mL and negative control DMSO-SS (50/50 v/v). The assay was performed three times in quintuplicate. The shape and size of the droplet was observed on the microplates after one minute of applying the extracts and measured with a vernier under a stereo microscope. An increase of the droplet larger than 1 mm with respect to the negative control was considered to have biosurfactant activity (Youssef *et al*., 2004).

Purification of the semi-purified extract by preparative thin-layer chromatography

The semi-purified extract was dissolved in methanol to a concentration of 5% and 250 µL was applied to the whole plate. The plate was eluted with the nBuOH: MeOH: H_2O system (3:2:1), allowed to dry and then scraped off the silica gel in the area of the Rf where haemolytic activity was observed. The entire scraping was placed in a beaker containing 50 mL of chloroform: methanol ($CHCl_3$: MeOH), 2:1, and left to stir in this solvent system for extraction of the metabolites from the silica. The mixture was filtered to separate the silica and the solvent was concentrated under reduced pressure with a rotary evaporator. The excess solvent was allowed to dry and its yield was determined and expressed as mg/mL (*Satpute et al*., 2010).

Determination of the minimum inhibitory concentration by the microdilution method

For this assay, a suspension of conidia was obtained at 3.5 X 105 conidia/mL of C. gloeosporioides (ATCC 42374) in RPMI 1640 medium with L-glutamine, without bicarbonate and supplemented with 165 mM 3-(n-morpholino)-propanesulfonic acid (MOPS) and adjusted to pH 7.0. The assay was performed in a sterile 96-well microplate.

The semi-purified fractions were dissolved in DMSO-SS to a final concentration of 4 mg/mL. 10 µL (40 µg) were transferred to the first well previously containing 190 µL of RPMI 1640 medium, carefully mixed until a homogeneous suspension was obtained and 100 µL were transferred to the next well containing 100 µL of RPMI 1640 medium, and so on up to well 12. All wells were then inoculated with 100 µL of the conidial suspension. The assay was performed in triplicate and the microplate was incubated at 25°C for 48 hours.

The 24 hours was determined as the cut-off time of the assay, since at that time the inverted microscope observation showed total germination of the conidia (germination is considered when the size of the hyphae coming out of the spore is one and a half times larger than the conidium) and when developing with TTC the reaction is strongly observed. The addition of TTC to each well of the microplate allowed visualisation of the MIC of the extracts, which coincided with the observation with the inverted microscope.

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Results

In this research work, three biosurfactant extraction methodologies were used: acid extraction, ethyl acetate extraction and ammonium sulphate extraction. These techniques allowed to establish that the ammonium sulphate extraction was the one that presented the highest amount of biosurfactants with a yield of 3.1243 g/L compared to the acid precipitation (0.3173 g/L) and ethyl acetate (0.110 g/L) methods.

Confirmation of the surfactant nature of each extract was performed by the collapsed droplet assay with an increase of the droplet in the semi-purified extracts as visualised in Figure 1 (Dehghan-Noudeh et al., 2005; Youssef et al., 2004).

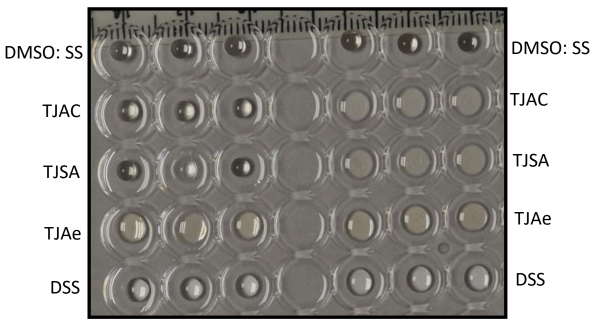

Figure 1 Biosurfactant activity of crude and semi-purified extracts. Negative control: DMSO: SS (Sodium Dimethyl Sulphate with Salt Solution (1:1)), Positive control: DDS (Sodium Dodecyl Sulphate), TJAC: extract obtained by acid extraction, TJSA: extract obtained by extraction with ammonium sulphate, TJAe: extract obtained with ethyl acetate

The determination of the active metabolites was determined by thin layer chromatography and haemolytic bioautography. This methodology allowed the Rf corresponding to the areas with haemolytic activity of the extracts to be located after 12 hours of incubation. Simultaneously to this assay, another plate was run to reveal the presence of peptides with ninhydrin, as well as cupric sulphate in phosphoric acid to reveal the presence of lipids (Figure 2).

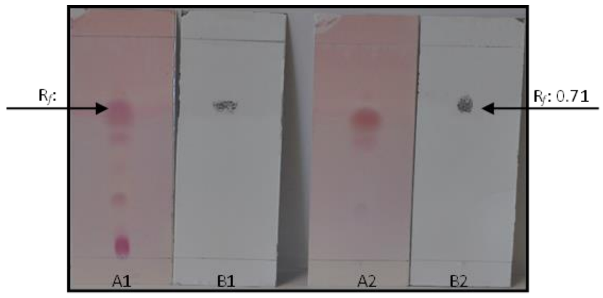

Figure 2. Thin layer chromatography of the semi-purified (A1 and B1) and purified (A2 and B2) extract developed with ninhydrin (A1 and A2) and with 10% cupric sulphate in 8% phosphoric acid (B1 and B2)

The determination of the minimum inhibitory concentration (MIC) and observation under inverted microscope and interpretation using the NCCLS numerical scale showed the following results: None of the tested concentrations of the TJSA extract inhibited the growth of the fungus, therefore, the MIC corresponds to a value higher than 100 µg/mL. These high values may be associated with the presence of impurities present in the crude extract. This was evident when the crude extract was evaluated, as no germination of the conidia of C. gloesoporioides ATCC 42327 was observed at a concentration of 12.5 µg/mL. These results demonstrate the sensitivity of this pathogen against this antifungal agent.

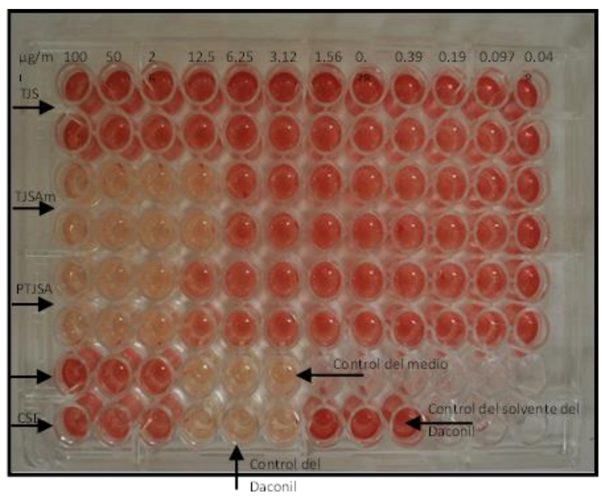

Figure 3 MIC (Minimum inhibitory concentration) of TJSA, TJSAm and PTJSAm extract. CCH: fungal growth control, CSE: extract solvent control. Daconil: positive control

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Conclusions

As conclusions we can mention that the optimal method for the extraction of the biosurfactant produced by *B. mojavensis* was determined as precipitation with 40% ammonium sulphate, the biosurfactant is of lipopeptidic nature and methanol was a suitable solvent for the semi-purification since the lipopeptide remained in this fraction increasing the haemolytic and antifungal activity. After successive purifications the total yield obtained by preparative thin layer chromatography was 92.5 mg/L and the minimum inhibitory concentration of the purified extract was 25 µg/mL against C. gloeosporioides.

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Characterization of *Paenibacillus* spp. CBRM17 as antagonist of phytopathogenic fungi and growth promoter of *Capsicum chinense* Jacq.

Caracterización de *Paenibacillus* spp. CBRM17 como antagonista de hongos fitopatógenos y promotor de crecimiento de *Capsicum chinense* Jacq.

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Abstract

Studies of the plant-microorganism relationship have made it posible to explore the potential of rhizospheric bacteria to improve the health and quality of plants. In the present study, *Paenibacillus* sp. CBRM17 was characterized *in vitro* for its ability to inhibit the growth of phytopathogenic fungi that cause diseases in horticultural crops in the tropics, finding a reduction in the mycelial growth of *Alternaria*, *Fusarium* and *Helmintosphorium* strains in the range of 50 to 70 %, additionally its biochemical properties related to the promotion of plant growth were characterized, registering the production of 0.36 µg/mL of indole acetic acid (IAA), the solubilization of tricalcium phosphate with solubilization index (SI) of 2.41 mm and solubilization efficiency (SE) of 140.6 %, producing in the supernatant 63.5 mg/L of soluble phosphorus, in addition to being positive for ACC deaminase activity. Inoculations trials of habanero chili (*Capsicum chinense* Jacq.) seeds with *Paenibacillus* sp. CBRM17 showed its potential to be used as an inoculant in the growth promotion of this crop, since it increased all growth variables; increasing the total fresh and dry biomass by 93.3 and 96.4 %, respectively.

Microbial antagonism, Indole acetic acid, Phosphate solubilization

Resumen

Los estudios de la relación planta-microorganismo han permitido explorar el potencial de las bacterias rizósfericas para mejorar la sanidad y calidad de las plantas. En el presente estudio se caracterizó *in vitro* la cepa *Paenibacillus* sp. CBRM17 por su capacidad para inhibir el crecimiento de hongos fitopatógenos causantes de enfermedades en los cultivos hortícolas del trópico, encontrando reducción del crecimiento micelial de cepas de *Alternaria*, *Fusarium* y *Helminthosporium* en el orden 50 al 70 %, adicionalmente se caracterizaron sus propiedades bioquímicas relacionadas con la promoción del crecimiento vegetal, registrando la producción de ácido indol acético (AIA) con 0.36 µg/mL, la solubilización de fosfato tricálcico con índices solubilización (IS) de 2.41 mm y eficiencia de solubilización (ES) del 140.6 %, produciendo en el sobrenadante 63.5 mg/L de fósforo soluble, además de resultar positiva para la actividad ACC desaminasa. Ensayos de inoculación de semillas de chile habanero (*Capsicum chinense* Jacq.) con *Paenibacillus* sp. CBRM17 mostraron su potencial para ser usado como inoculante en la promoción de crecimiento de este cultivo, ya que incrementó todas las variables de crecimiento; aumentando la biomasa fresca y seca total en un 93.3 y 96.4 %, respectivamente.

Antagonismo microbiano, Ácido indol acético, Solubilización de fosfatos

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Introduction

Plant pathogenic fungi are a group of microorganisms that cause diseases in tropical crops, mainly *Fusarium* spp., *Alternaria* spp., *Cercospora* spp., *Curvularia* spp. and *Helminthosporium* spp. highly damaging genera that cause different crop pathologies (Vásquez-López *et al*., 2009; Badía *et al*., 2011). In order to control them, organosynthetic fungicides are applied more frequently, which continuously contaminate the environment, affect human health and generate new strains of fungicide-resistant phytopathogens. Therefore, there is an urgent need to search for more agro-ecological alternatives, including the use of antagonistic microorganisms such as *Paenibaillus* spp., which is a rhizospheric bacterium capable of exerting a biocontrol effect on phytopathogenic fungi; due to the production of molecules that exert antifungal activity, this, together with the fact that the genus *Paenibaillus* is capable of producing endospores that ensure its survival in the environment, makes it a viable alternative (Annapurna *et al*., 2013). Some species such as *P. polymyxa* also show growth promoting effect on crops such as pepper (*Capsicum annuum*), soybean (*Glycine max* L.) and tomato (Solanum lycopersicum) (Lamsal *et al*., 2012; Annapurna *et al*., 2013, Mei *et al*., 2014). This is attributed to different microbial mechanisms such as: production of indole acetic acid (IAA), a hormone that induces root and vegetative growth, solubilisation of mineral elements such as phosphorus, atmospheric nitrogen fixation, siderophore production and ACC deaminase activity have also been documented (Çakmakçi *et al*., 2007; Luna *et al*., 2013). In this study, the antagonistic potential of Paenibacillus sp. CBRM17 towards different plant pathogenic fungi affecting tropical crops was determined, as well as its plant growth promoting capacity on habanero pepper (*C. chinense* Jacq.) plants.

Methodology to be developed

Biological material

Paenibacillus spp. CBRM17 belongs to the collection of the Microbiology Laboratory of the Technological Institute of Conkal, previously identified by Mejía *et al*., (2016), and nutrient agar medium (NA) was used for its cultivation. Meanwhile, the fungi *Alternaria* spp. isolated from tomato (*Solanum lycopersicum* L.), *Fusarium* spp. from sugar cane (*Saccharum officinarum*), *Corynespora cassicola*, *Curvularia* spp. *F. equiseti* and *F. solani* from habanero chili (*C. chinense*) and *Helminthosporium* spp. from kerpis palm (*Veitchia merrili*) were provided by the Phytopathology Laboratory of the same institute and were grown on potato dextrose agar medium (PDA) for cultivation and assays.

Antagonism tests

In vitro biocontrol tests of *Paenibacillus* spp. against tropical plant pathogenic fungi were carried out in triplicate on PDA medium. First, a 0.5 cm diameter disc of active mycelium of the plant pathogen was seeded in the centre of the Petri dish, then 6 µL of a bacterial suspension ($1x10^7$ cells/mL) was inoculated at four points at 2 cm equidistant around the fungal disc. Finally, the plates were incubated at 28 °C, as negative control plates were used without the bacterial inoculation only growing the fungus, the assay was terminated when the mycelium covered the entire surface of the medium on the control plates, determining the percentage of inhibition of radial growth (ICR) of the fungus and the inhibition halos generated by the bacterial inoculum (Ezziyyani *et al*., 2004).

Plant growth promoting activities

The activity of ACC deaminase in *Paenibacillus* spp. was first performed by seeding it on Dworkin and Foster (DF) mineral minimal medium containing $(NH_4)2SO_4$ as nitrogen (N) source. After 48 h at 30 °C, a colony was reseeded on the same medium containing 1-aminocyclopropane 1-carboxylate (ACC) (Sigma-Aldrich®) as N source replacing $(NH_4)2SO_4$. The plate was incubated for 72 h at 30 °C to observe the growth (Penrose and Glick 2003).

For the determination of AIA production by *Paenibacillus* spp., 5 mL of a bacterial suspension containing 1x108 cells/mL were inoculated in triplicate into flasks containing 50 mL of nutrient broth supplemented with L-tryptophan (1 g/L), the flasks were shaken at 180 rpm for 72 h at 30 °C. The supernatant was then recovered by centrifugation at 3,000 xg for 15 min, and 1 mL of Salkowski reagent was added to 1 mL of the supernatant, and the mixture was kept in the dark at room temperature for 30 min for complex development (Almoneafy *et al*., 2012).

Upon completion, the samples were read at 535 nm on a Genesys 10UV spectrometer to determine the concentration of AIA produced (Badía *et al*., 2011).Siderophore production was determined by inoculating the bacterial strain in triplicate into tubes containing 7 mL of M9 liquid minimal salts medium, after 16 h of growth, a subculture was performed by taking an aliquot of 70 µL which was transferred to new tubes containing the same medium, which were placed in agitation at 120 rpm and 30 °C for 24 h. The supernatant was then recovered by centrifugation at 8,000 Xg for 10 min, and 1 mL of this was taken and mixed with 1 mL of chromium azurol-S (CAS), the change in colour of the CAS reagent from blue to orange was considered positive (Alexander and Zuberer 1991).

Phosphate solubilising capacity was tested by quadruplicate inoculation of 0.8 µL of a 1x108 cell/mL suspension of *Paenibacillus* spp. in Petri dishes with Pikovskaya solid medium (Pradhan and Sukla, 2005), the plates were incubated at 30 °C, recording phosphate solubility daily after three days up to 15 days, subsequently calculating the solubility index (SI) and solubility efficiency (SE) of each strain according to Qureshi *et al*. (2012). For the quantitative phosphate solubilization method, bacteria were grown in triplicate on NBRIP (National Botanical Research Institute's Phosphate growth medium) (Mehta and Nautiyal 2001) and at the end of the culture, pH was recorded and soluble phosphorus was determined in the supernatant by molybdenum blue method (Mussa *et al*., 2009).

Growth promotion of habanero pepper (Capsicum chinense Jacq.)

For the plant growth promotion trials, habanero chili (C. chinense Jacq.) was used as a model plant. Seeds of chili cv. naranjo criollo were inoculated with a bacterial suspension of Paenibacillus sp. (1x108 cells/mL) and then sown in trays with commercial substrate Cosmopeat®. Once germinated, they were reinoculated at 15 and 28 days after germination (DDG) with 1 mL of Paenibacillus sp. per plant at a concentration of 1x108 cells/mL (Luna *et al*., 2013). After 28 DDG, transplanting was carried out in 32 oz (0.95 L) unicel cups containing a 1:1 mixture of Luvisol-type soil with bobinaza, taking the plants to the greenhouse.

Subsequently, eight days after transplanting, they were re-inoculated with the same dose (Canto-Martín *et al*., 2004), fertilisation was carried out based on the regional recommendations of the crop (Soria *et al*., 2002) and at the end of 60 DDG the experiment was completed. Each plant was evaluated for height, stem diameter, number of leaves, leaf area, root volume, root length, fresh and dry biomass of the aerial part and root, as growth variables. A completely randomised design with ten replicates was applied, leaving a control lot without inoculation. The data obtained were subjected to analysis of variance (ANDEVA) and mean comparison test (Tukey, $p \leq 0.05$), using SAS software version 9.3 for Windows (SAS Institute Inc. 2010).

Results

Antagonism of Paenibacillus spp. towards tropical plant pathogenic fungi

The *in vitro* antagonism assays show that Paenibacillus sp. CBRM17 inhibits five of the seven fungi tested, presenting ICR ranges from 50.6 to 70.0 % (Table 1), with Fusarium equiseti being the most inhibited. The antagonistic and antifungal activity of Paenibacillus genus towards fungi of agricultural interest has been reported previously, Zhai et al. (2021) show inhibitions from 61.8 to 79.2 % of P. polymyxa HX-140 towards Fusarium, Colletotrichum, Sclerotinia, Phytophthora and Rhizoctonia strains, while Zhang et al. (2018), achieve 69.8 % inhibition of Botrytis cinerea using P. polymyxa ShX301. The inhibitory activity of Paenibacillus also extends to bacterial pathogens such as Clavibacter michiganensis (Chávez et al., 2020), although the inhibition percentages reported are lower (21.7 %).

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The production of diffusible antagonist molecules in the culture medium delimits the mycelial growth of the pathogen. In the present study, inhibition halos were recorded for three of the seven fungi evaluated (Figure 1), with values of 2.77, 1.87 and 6.67 mm for Alternaria sp., *Fusarium* spp. and *F. equiseti*, respectively (Table 1). The nature of these molecules can be diverse, Ran *et al*., (2020), report from *P. polymyxa* 7F1, the production of a 36 kDa enzyme with glycosyl hydrolase domain that inhibits the growth of *Fusarium species*; *F. equiseti*, *F. verticillioide*, *F. semitectum*, *F. graminearum*, *F. proliferatum*, *F. oxysporum*, *F. nivale*, *F. oxysporum* and *Colletotrium nivale* and *Colletotrichum gloeosporioides*, while Soo et al. (2016), found in *Paenibacillus polymyxa*, an increase in protease and amylase activity, which could be related to the biocontrol activity of anthracnose in apple caused by *Colletotrichum gloeosporioides* and *C. acutatum*. Peptide production is another biocontrol strategy identified, Beatty and Jensen (2002) report the production of fusaricidin in Paenibacillus polymyxa PKB1, a cyclic depsipeptide of 882 kDA that inhibits *in vitro* the growth of Leptosphaeria maculans, the fungus causing blackleg in canola.

Mushrooms	Halos (mm)	ICR (%)
Altenaria spp.	2.77 b	60.41 b
Corynespora spp.	0.00 d	0.00 e
Curvularia spp.	0.00 d	0.00 e
Fusarium spp.	1.87 c	50.67 d
F. equiseti	6.67 a	70.04 a
F. solani	0.00 d	54.19 c
Helminthosporium spp.	0.00 d	52.12 dc
DMS	0.56	2.92
ICR= Radial growth inhibition, LSD = Least Significant Difference.		

Table 1 Antagonism of *Paenibacillus* spp. CBRM17 against different tropical phytopathogenic fungi

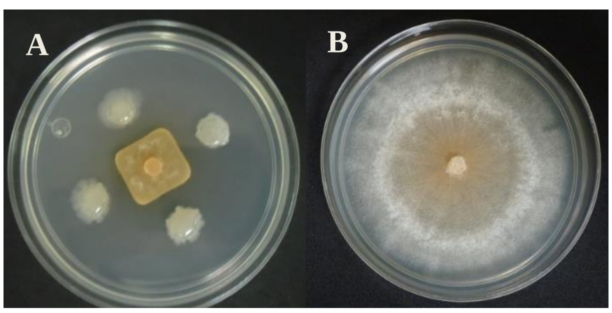

Figure 1 Mycelial growth inhibition in *F. equiseti* and the presence of inhibition halos by *Paenibacillus* spp. CBRM17 A) and control B)

Mechanisms related to the promotion of plant growth

In the test for ACC deaminase enzyme activity, *Paenibacillus* spp. strain CBRM17 was positive when grown on DF medium and used 1-aminocyclopropane-1-carboxylic acid (ACC) as the sole source of nitrogen. Various PGPR such as *Enterobacter cloacae*, Pseudomonas putida and *P. fluorescens* also have the ability to synthesise this enzyme, having effects on the growth promotion of Oryza sativa and Glycine max L. seedlings (Penrose and Glick 2003). The importance of the activity of this enzyme is because it reduces the production of ethylene in plants, due to the degradation of its precursor (ACC), thus avoiding the deleterious effects of ethylene, such as the suppression of root growth, among others; the synthesis of this enzyme is encoded in the acdS gene (Mayak *et al*., 2004; Hernández-Forte *et al*., 2015). Regarding the ability of *Paenibacillus* spp. CBRM17 to solubilise phosphates, after seven days, in the plates with Pikovskaya medium, the formation of solubilisation halos was observed with IS values of 2.41 mm and ES of 140.6 %, while in the tests in liquid medium a concentration of 63.5 mg/L of soluble phosphorus was found and a decrease in the pH of the filtrate to 4.75. Almoneafy *et al*., (2012) and, Xu and Kim (2014), in evaluations of phosphate-solubilising *Paenibacillus* spp. *Bacillus* spp. and *Pseudomonas* spp. also observed the formation of clear zones around bacterial colonies on plates after seven days of incubation. The IS reported in our research is lower than those of Bacillus spp. with values of 3.3 mm and efficiencies higher than 233 % (Qureshi et al., 2012). However, this evidence needs to be corroborated with quantitative analysis, analysis of supernatants of *Paenibacillus kribbensis* UFLA 03-46 and *Paenibacillu* spp. UFLA 03-12 yield values of 25 mg/L soluble phosphorus and a decrease in pH to 5.3 and 5.8, respectively (Marra *et al*., 2012). These values are lower than those obtained in the present study, while Luna et al. (2013) report the concentration of 56.0 mg/L soluble phosphorus in B. megaterium MA06, values similar to those obtained in our trials. The decrease of pH in the media is due to the production of organic acids, responsible for solubilising the mineral phosphate, such as: lactic, isovaleric, isobutyric and acetic acid (Mehta and Nautiyal, 2001).

In the quantification of AIA and indoles production, *Paenibacillus* spp. at 72 h had a production of 0.36 µg/mL AIA; while in the CAS assay, siderophore production was not detected (Table 2). Studies on Paenibacillus sp., P. polymyxa CF05 and P. polymyxa RC05, found AIA concentrations in the range of 5.0 to 25 µg/mL (Çakmakçi *et al*., 2007, Xu and Kim 2014, Mei *et al*., 2014) higher than those obtained in our study.

Caracter de *Paenibacillus* sp. CBRM17	
IS (mm)	2.41 ± 0.04°
ES (%)	140.6 ± 4.20
P (mg.L^{-1})	63.50 ± 4.29
pH	4.75 ± 0.09
AIA (µg mL^{-1})	0.36 ± 0.15
ACC deaminase	+
Siderophores	-

IS: solubilisation index; ES: solubilisation efficiency; P: phosphorus (solubilised); IAA: indole acetic acid and +/-: positive or negative to these tests. ○Means and standard error.

Table 2 Properties of *Paenibacillus* spp. CBRM17 determined for growth promotion in habanero peppers (*Capsicum chinense* Jacq.)

Growth promotion of habanero peppers (*Capsicum chinense* Jacq.)

The results of the growth promotion trial show that the inoculation of *Paenibacillus* spp. CBRM1 in habanero peppers had beneficial effects at 60 DDG, increasing stem diameter, leaf area, root length and volume, as well as biomass through total fresh weight, root and aerial part, and dry weight, respectively, with respect to the control (Table 3 and 4). For fresh and dry biomass, these increases were 93.3 % and 96.4 %, respectively. These increases are dependent on the plant species and crop conditions, Annapurna *et al*., (2013) report that inoculation of soybean with *P. polymyxa* HKA-15 in G. max L. increased the dry weight of root and aerial part by 29.2 and 49. 2 % respectively, higher values found in our study, while inoculation of tomato (*S. lycopersicum*) with indole acetic acid producing *P. polymyxa* CF05 significantly increased root fresh and dry weight and total fresh and dry weight by 21.9, 60.0, 29.4 and 38.3 % respectively (Çakmakçi *et al*., 2007, Mei *et al*., 2014), values lower than those obtained in this study.

Other microorganisms such as Azospirillum sp and Bacillus are also able to promote the growth of *C. chinense* Jacq. *and C. annuum* L. plants, increasing plant height, leaf area, aerial and root dry weight as reported by Canto-Martin *et al*., (2004) and Lim and Kim (2009). The growth-promoting characteristics are mainly attributed to AIA acid production and phosphate solubilisation according to Sanchez-Lopez *et al*., (2012).

Treatment	AL (cm)	DT (mm)	AF (cm^2)	LR (cm)	VR (m^3)
CBRM17	13.9 a°	3.2 a	223.7 a	23.9 a	2.7 a
Witness	10.1 b	2.7 b	128.8 b	20.9 b	1.3 b
DMS	2.4	0.38	82.6	3.0	0.81

AL = height; SD = stem diameter; FA = leaf area; RL = root length; RL = root volume. LSD = Least Significant Difference; ○Means with different letters in each column are statistically different. (Tukey, 0.05).

Table 3 Effect of Paenibacillus sp. CBRM17 on the growth variables of habanero pepper plants. (*Capsicum chinense* Jacq.)

Treatment				weight (g)		
	PFR	PFPA	PFTo	PSR	PSPA	PSTo
CBRM17	2.9 a°	5.7 a	8.7 a	0.27 a	0.86 a	1.1 a
Control	1.3 b	3.8 b	4.5 b	0.11 b	0.44 b	0.56 b
DMS	0.95	1.9	2.7	0.08	0.26	0.29

PFTo = total fresh weight; RWW = root dry weight; AWW = aerial part dry weight; TWD = total dry weight. LSD = Least Significant Difference; ○Means with different letters in each column are statistically different (Tukey, 0.05).

Table 4 Biomass found in habanero pepper (*Capsicum chinense* Jacq.) plants inoculated with *Paenibacillus* spp. CBRM17

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Conclusions

Paenibacillus spp. CBRM17 has antagonistic activity to reduce *in vitro* mycelial growth of phytopathogenic fungi, probably due to the production of diffusible metabolites that generate inhibition halos during dual confrontation. In addition, it exhibits biochemical properties related to plant growth promotion, which was proven to generally increase the biomass of *Capsicum chinense* plants, an activity that can be exploited to improve crop productivity.

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Study on the generation and composition of household solid waste in Ciudad Valles, S.L.P.

Estudio sobre la generación y composición de los residuos sólidos domiciliarios en Ciudad Valles, S.L.P.

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Abstract

The problem of the generation and composition of household Urban Solid Waste (RSU) in Ciudad Valles, San Luis Potosí was addressed. The information obtained could be used in making decisions about management and treatment for final disposal. To define the dwellings under study, the city was divided into socioeconomic strata with the support of the kmz tool, to visualize the Basic Geostatistical Areas (AGEB) according to the percentage of poverty. The generation of waste in the study area was determined through the NMX-AA61-1985 standard. For seven days, the selected dwellings were sampled, the quartering method was applied to the waste generated by means of the NMX-AA-15-1985 standard, and the samples were obtained for the physical-chemical analyses. The quantification of the by-products was carried out according to the NMX-AA-22-1985 standard. The organic fraction residues were the predominant in all the strata, being the medium marginalization stratum (EMM) the one that presented the highest percentage (49%). The physical-chemical characterization of the residues showed a moisture content of 52.48% and organic matter of 61.28%, results derived from the high composition of food residues that provide a higher percentage of water.

Quarting method, Volumetric weight, By-products, Organic fraction, Physicochemical analysis

Resumen

Se abordó el problema de la generación y composición de Residuos Sólidos Urbanos (RSU) domiciliarios en Ciudad Valles, San Luis Potosí. La información obtenida podría utilizarse en la toma de decisiones sobre la gestión y tratamientos para su disposición final. Para definir las viviendas objeto de estudio, la ciudad se dividió en estratos socioeconómicos con apoyo de la herramienta kmz, para visualización de las Áreas Geoestadísticas Básicas (AGEB) según el porcentaje de pobreza. Se determinó la generación de los residuos en el área de estudio a través de la norma NMX-AA-61-1985. Durante siete días se muestrearon las viviendas seleccionadas, a los residuos generados se les aplicó el método de cuarteo mediante la norma NMX-AA-15-1985 y se obtuvieron las muestras para los análisis físico químicos. La cuantificación de los subproductos se llevó a cabo de acuerdo a la norma NMX-AA-22-1985. Los residuos de fracción orgánica fueron los predominantes en todos los estratos, siendo el estrato de mediana marginación (EMM) el que presentó el mayor porcentaje (49%). La caracterización físico-química de los residuos mostró un contenido de humedad del 52.48% y materia orgánica de 61.28%, resultados derivados de la alta composición de residuos alimenticios que aportan un mayor porcentaje de agua.

Método de cuarteo, Peso volumétrico, Subproductos, Fracción orgánica, Análisis fisicoquímicos

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Introduction

In Mexico, the General Law for the Prevention and Integral Management of Waste (LGPGIR) classifies waste according to its characteristics and origin into municipal solid waste (MSW), special management waste (SMW) and hazardous waste (HW). MSW are those generated in households when disposing of materials used in domestic activities, those coming from activities carried out inside establishments or on public roads, with household characteristics, and those resulting from public roads and places, as long as they are not considered as other types of waste (LGPGIR, 2003).

The increase in the generation of MSW is related to human activities and is potentiated by factors such as population growth and changes in consumption habits, migration to cities and poor waste management by waste management agencies and society itself (Buenrostro & Bocco, 2003).

Likewise, the accumulation of MSW causes environmental risk factors associated with the generation of "biogases" classified as greenhouse gases (GHG), such as methane (CH4) and carbon dioxide (CO2) mainly (Kiss and Encarnación, 2006). Most of these accumulated wastes also generate liquids (leachates) during their degradation process, and although they are leachates of organic origin, they represent a source of contamination of soil and adjacent water bodies, which can cause problems of toxicity, eutrophication and acidification (Allen, 2001; Torres, et al., 2011). In addition to these environmental problems, there is also the proliferation of harmful fauna and the consequent transmission of diseases to the population (Jaramillo, 2002; Hernández-Rejón, 2014).

Studies on the subject have been carried out in Mexico; however, some of them have not been disseminated. Studies carried out in the 1980s and 1990s by agencies such as SEDESOL stand out; and more recently, the Basic Diagnoses for Integrated Waste Management, carried out by SEMARNAT and INECC in 2012 and the most recent one, carried out in 2020.

Likewise, there are some research studies carried out in some regions, such as in the Cuitzeo Lake Basin, in the State of Michoacán (Buenrostro and Israde, 2003); others, carried out in small cities, such as in Berriozábal, Chiapas (Araiza et al., 2017) and others carried out in the final disposal sites themselves, such as the one carried out in the Tepic Landfill, Nayarit (Saldaña et al., 2013).

In the State of San Luis Potosí, some studies related to the subject have been carried out. In the capital, a master's degree thesis was carried out by the Colegio de San Luis, A.C. (García, 2010). Likewise, in the Magical Town of Real de Catorce, a bachelor's degree thesis was carried out by the National Polytechnic Institute (Martínez, 2018); however, both works were not published as scientific articles.

The studies of MSW generation and quantification are based on methodologies to estimate the quantity and by-products generated in the localities. The resulting information can support municipal authorities in planning and management activities. Likewise, the physical-chemical characterisation allows to know the composition of the waste through the contents of: humidity, organic matter, ash, carbon, hydrogen, oxygen, nitrogen, caloric content, mainly; as relevant characteristics to evaluate the possibilities of utilisation (Tchobanoglous, et al., 1994).

Currently, in Ciudad Valles San Luis Potosí, there is no study of generation, quantification and physical-chemical characterisation; the only document that provides information on MSW is the Municipal Development Plan of Ciudad Valles 2021-2024, which only presents the national figures provided by SEMARNAT in the basic diagnosis for integrated waste management.

In this context, the present study was carried out, which shows the results of the generation, quantification of by-products and physicochemical characterisation of MSW of household origin, generated in the City, with the aim that these results are disseminated and can be considered by decision-makers in the municipality for better waste management.

Methodology to be developed

The study was carried out in Ciudad Valles, S.L.P., which is located at the geographical coordinates 21° 59' north latitude; 99° 01' west longitude and an altitude of 70 m above sea level. Its population is 179,371 inhabitants according to information from INEGI, 2020. The main economic activities are livestock, agriculture and services, with tourism services predominating in recent years.

The objective of this research was to carry out a study on the generation of household MSW in Ciudad Valles, S.L.P., the quantification of by-products and their physical-chemical composition, so that the results generated can support the design and orientation of public policies in decision-making on the management, use and/or treatment for their final disposal. To this end, three specific objectives were established: to determine the generation of household MSW in the study area by socio-economic strata, to quantify the by-products of MSW and to carry out the physico-chemical characterisation of MSW.

The following research questions were also posed: Is the per capita generation of household waste in Ciudad Valles lower than those registered in the state of SLP and at the national level? What are the percentages of organic fraction waste and usable inorganic waste by stratum? What is the physical-chemical composition of the waste? In order to answer these questions, the following methodology was applied:

Statistical sampling

In order to obtain the study sample, proportional stratified sampling was used. The population was defined as the 53,323 urban dwellings of Ciudad Valles, S.L.P. reported in the Population and Housing Census, 2020, and the socioeconomic strata of that population were considered according to the CONEVAL (2019) classification: low, medium and high marginalisation strata. To determine the sample size, a confidence level of 95%, an estimation error level of 10% and a population variance value of 0.04 kg^2 /inhab/d of waste, considering a standard deviation of 200 g/inhab/d, were used.

The population variance value was estimated according to previous studies on per capita waste generation, therefore, the per capita value of 0.663 kg/inhab/d of waste from the State of San Luis Potosí reported in the Basic Diagnosis for Integrated Waste Management (SEMARNAT, 2020) was considered. The equation used to estimate the sample number for the study was as follows:

$$n = \frac{Z^2_{1-\frac{\alpha}{2}} N\sigma^2}{(N-1)E^2 + Z^2_{1-\frac{\alpha}{2}} \sigma^2} \quad (1)$$

Where:

N= population size

n= sample size

Z_(1-α/2)^2=confidence coefficient

E= permissible error

σ^2= variance

Therefore:

$$n = \frac{(1.96)^2(53323)(0.04)}{(53323-1)(0.0663)^2+(1.96)^2(0.04)}$$

$$n = \frac{8193.82}{234.35}$$

$$n = 35$$

Consequently, the study sample consisted of 35 dwellings, and was distributed proportionally, with 8 dwellings for the Low Marginalisation Stratum (LMS), 11 for the Medium Marginalisation Stratum (MMS) and 16 for the High Marginalisation Stratum (HMS), as shown in the table below 1:

Strata	Number of dwellings	%	Number of dwellings to be sampled
EBM	12,129	22.75	8
EMM	16,879	31.65	11
EAM	24,315	45.60	16
Totales	53,323	100.00	35

Table 1 Statistical sampling by socio-economic stratum
Source: Own elaboration

For the sampling, the Google Earth programme and the kmz tool were used to visualise the Basic Geostatistical Areas (AGEB) with the ranges according to the percentage of poverty, available on CONEVAL's official website. In this way, it was possible to identify the neighbourhoods of the municipality by socio-economic stratum (Figure 1). These tools made it possible to choose the classification in the following levels

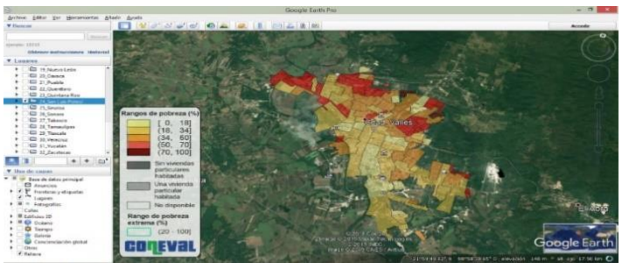


Figure 1 View of the Municipality of Ciudad Valles in kmz file at AGEB level in the Google Earth interface
Source: INEGI, CONEVAL, (2015)

The selection of households to be sampled was made using a weighting matrix with three selection criteria: ease of access, knowledge of the locality and current risk situation.

The selection of houses to be sampled was made using a weighting matrix with three selection criteria: ease of access, knowledge of the locality and current risk situation. For the EAM, the selected households were located in the colonias: El Consuelo, Emiliano Zapata, Santa Lucia and Vista Hermosa. For the EMM the dwellings were located in the colonias: Bugambilias and Nelly Zulaiman. For the EBM, the homes selected were located in the colonias Mirador and Rotarios. A work plan was drawn up for the presentation of the field survey form to the inhabitants of the dwellings sampled, and a daily sampling procedure was established for the delivery of polyethylene bags identified by dwelling, as well as the timetables established for the collection of samples from 14 to 21 October 2021, contemplating a first day for the cleaning operation in each dwelling and on the following seven days the collection of household waste for its quantification and characterisation.

Collection and quantification of solid household waste

In accordance with the work plan, household waste was collected and taken to the TecNM Campus Ciudad Valles to determine the physical composition and physical-chemical characterisation. For the waste obtained for each of the strata, the procedure of the NMX-AA-015-1985 Municipal solid waste, quartering method (SECOFI, 1985) was applied; once the bag with waste arrived at the place of analysis, its weight was recorded in the logbook; this weight represented the total amount of daily waste (Wt) generated in the dwellings sampled by strata. Based on the data collected on the number of persons per dwelling (ni), the total number of persons involved (Nt) in the sampling per stratum was determined and the total weight of the bags (Wt) was divided by the total number of persons (Nt) to obtain the average daily per capita generation of the sampled dwellings (kg/inhab/d). According to formula 2:

$$Per\ capita\ waste\ generation = \frac{Total\ weight\ of\ waste\ (Wt)}{Total\ number\ of\ persons\ (Nt)} \quad (2)$$

The bag was then torn open and the waste was dumped in a heap. With the help of hand tools, the bulky waste was chopped up to a size of 10 cm or less. The mound of solid waste was then shovelled into a homogenous heap. The mound was then divided into four approximately equal parts, and two of the opposing parts were separated, reserving them for volumetric weight calculation according to NMX-AA-019-1985 (SECOFI, 1985).

The other two parts of the residues were weighed and their weight was recorded in the logbook for the selection and quantification of by-products according to NMX-AA-022-1985 (SECOFI, 1985). For the determination of the volumetric weight by strata, a clean cylindrical container was selected, free of damage (dents or breakages), as well as a TORREY Model L-EQ scale. The empty container was weighed, taking this weight as the tare weight of the container (W_1). The volume was determined, using the height (h) of the vessel and the geometry of the vessel based on the formula 3:

$$V=\pi r^2 h \quad (3)$$

The waste selected for this process was deposited homogenised in the container, hitting it against the ground three times to add waste up to the top, taking care not to press on it so as not to alter the volumetric weight that was determined. The container with the waste was weighed, gross weight (W_2) and the volumetric weight of the waste was calculated by strata using the formula 4:

$$Pv = P/V \quad (4)$$

Where:

Pv= Volumetric weight of solid waste (kg/m^3)
P = Net weight of the solid waste (gross weight

minus tare weight, kg)

V = Volume of the container occupied by the waste (m^3)

Once calculated, it was recorded in the corresponding logbook and a sample of this waste was set aside for physical and chemical characterisation tests.

For the selection and quantification of by-products by strata, these were classified by type in labelled bags. With the aid of a TORREY Model L-EQ scale. The waste was weighed and recorded in the logbook by weight of the sorted component. The percentage of each by-product was calculated taking into account the weight of the last heap resulting from the quartering (Wq) and the weight of each by-product (Pi) according to the formula 5:

$$\% \, Subproducto = \frac{Pi}{Wq} * 100 \quad (5)$$

Where:

Pi= weight of each by-product considered in kg (minus the weight of the bag used)

Wq= total weight of the sample

Physico-chemical characterisation of household solid waste

Proximal analysis was carried out in the Chemistry Laboratory of the TecNM Campus Ciudad Valles on the waste samples by strata; determining moisture, ash, organic matter, and percentages of C, H, O, N and S through gravimetric methods.

Results

Table 2 shows the quantities of household solid waste used for quantification by the quarantine method according to the NMX-AA-015-1985 standard, for each of the strata:

	Low Marginalisation Stratum Weight (kg)	Medium Marginalisation Stratum Weight (kg)	High Marginalisation Stratum Weight (kg)
Quantity harvested	30.63	26.81	77.89
Quantity intended for volumetric weighing	16.24	13.62	37.73
Quantity intended for laboratory analysis	2.57	3.02	1.00
Quantity intended for sorting and quantification of by-products	8.62	7.95	9.54

Table 2 Quantities used for the quantification of household MSW by stratum
Source: Own elaboration

Table 3 shows the per capita generation of household MSW in the urban area of Ciudad Valles, as well as the volumetric weight of this waste for each stratum.

	Low Marginalisation Stratum	Median Stratum Marginalisation	High Marginalisation Stratum	P average
Household per capita generation in urban area (kg/inhab/d)	0.828	0.725	0.980	0.844
Volumetric weight (kg/m^3)	159.0	207.8	141.1	169.3

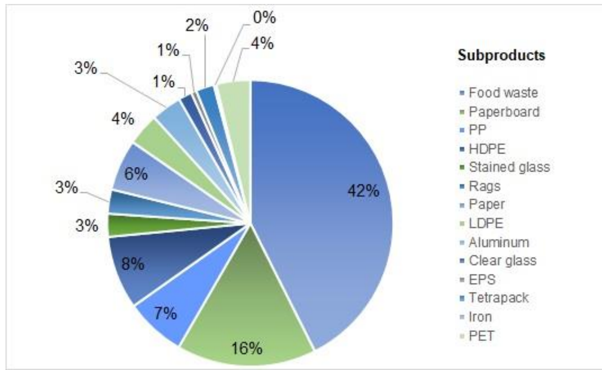
Table 3 Per capita generation and volumetric weight of household MSW by stratum
Source: Own elaboration

The per capita household waste generation in the urban area in Ciudad Valles, S.L.P. averages 0.844 kg/inhab/d (Table 3), which is above the national indicator reported in the Basic Diagnosis of Integrated Waste Management (DBGIR, 2020); which is 0.653 kg/inhab/d. It is considered that this difference may be due to the fact that the Ciudad Valles data considers only the urban area whose population is greater than 170,000 inhabitants and the national data comes from an analysis that includes different socio-economic strata in the different urban and rural regional areas. With respect to generation by socio-economic stratum, it was the high marginalisation stratum that presented the highest per capita generation and the medium marginalisation stratum that presented the lowest. These results confirm the socio-economic stratification of households in the City, but not the relationship of household income with the generation and composition of solid waste. There is no consensus on the influence of socioeconomic variables on solid waste generation.

On the one hand, some authors report that there is no statistical relationship between income and the rate of waste generation, although there are differences in the composition of solid waste (Getehun et al., 2012), (Monavari et al., 2012). Likewise, Xue et al. (2011) report that gross domestic product (GDP) has no significant correlation with production. On the other hand, there is a broad consensus that high-income population strata generate more solid waste (Gómez et al., 2009 and Ogwueleka, 2013), and also that the content of the organic fraction is higher in developing countries (Akinci et al. 2012) and in rural areas.

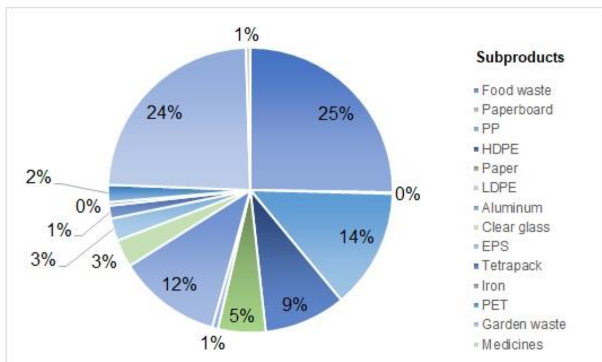
Now, with respect to volumetric weight, the average of the three strata was 169.305 kg/m³, a value above the national average weight which is 140.447 kg/m³ (SEMARNAT,2020). It is worth noting in Table 3 that the stratum of medium marginalisation was the furthest from the national average (207.8 km/m³); a situation that can be attributed to the fact that in this stratum there was a greater quantity of garden waste, such as leaves, which accumulated greater humidity and registered a higher weight.

Knowledge of the composition of waste is mainly used to determine which by-products or fractions of waste can be used or recovered, and which systems would be the most appropriate for this purpose. The quantification of the composition of household waste by strata, according to NMX-AA-022-1985, is presented in graphs 1, 2 and 3 below:



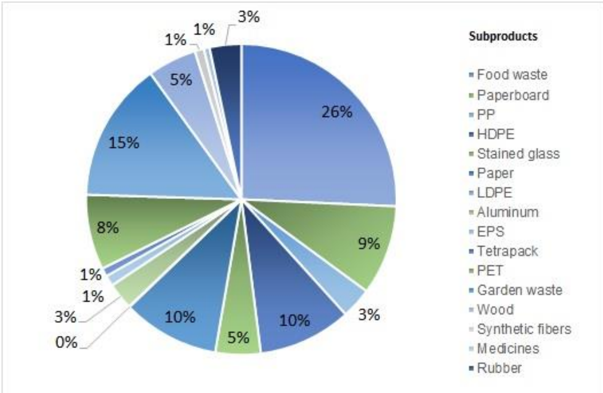
Graphic 1 Waste composition (%) in EAM
Source: Own elaboration

In the case of EAM, food waste by-products made up 42% of the total quantified waste, cardboard 16%, high-density plastics 8%, polypropylene plastics 7%, paper 6%, PET plastics 4% and the rest of the other waste.



Graphic 2 Waste composition (%) in MME
Source: Own elaboration

For the EMM, food waste by-products constituted 25%, around 24% was garden waste, polypropylene plastics 14%, aluminium 12%, high density plastics 9%, paper 5%, glass 3%, expanded polystyrene plastics 3%, PET plastics 2%, and the rest of other waste.



Graphic 3 Waste composition (%) in EBM
Source: Own elaboration

In the case of EBM, the composition of by-products was 26% food waste, 15% garden waste, 10% paper, 10% high-density plastics, 9% cardboard, 8% PET plastics, 5% wood, 5% coloured glass and the rest other waste.

With regard to the composition of household MSW and its percentages, it is noticeable that the largest generation is the organic fraction derived from food waste, whose percentages are the highest in the three socio-economic strata analysed, and garden waste in the medium and low marginalisation strata.

Finally, the results of the physical-chemical characterisation of the sampled waste are presented in Table 4:

Physical-chemical analysis	Percentage (%)
Moisture	52.48
Total solids	47.51
Working Moisture	6.11
Ash	11.16
Organic matter	61.28
Carbon	35.54
Hydrogen	4.08
Oxygen	20.99
Nitrogen	0.66
Sulphur	0.2
Calorific content (KJ/kg)	12000.1

Table 4 Results of the physical-chemical characterisation of waste
Source: Own elaboration

It can be observed that the waste contains a high percentage of moisture, on average 52.48%, due to the high composition of food waste, which contributes a higher percentage of water. The percentage of organic matter was 61.28%, as the presence of organic material is predominant, mainly due to the presence of food and garden waste. The ash content was 11.16%, which is related to the organic matter.

The nitrogen result was 0.66% and the theoretically calculated calorific value was 12,000 KJ/kg, which is mainly due to the amounts of carbon and hydrogen present.

Acknowledgement

We would like to thank the inhabitants of the colonies who actively participated in this study for their support.

Conclusions

The analysis of the composition of household MSW shows an interesting opportunity for recovery through composting, since the organic fractions are the ones with the highest percentage; in EAM about 42%, in EMM about 49% and in EBM about 41%, corresponding to food and garden waste.

There is also a diversity in the generation of plastics (PP, PS-E, HDPE, PET), paper, cardboard, glass and aluminium. The highest generation of plastics was found in the EMM with 28%, in the EAM with 19% and 18% in the case of EBM. Cardboard waste was identified in the high marginalisation and low marginalisation strata with 16% and 9% respectively. Paper generation was identified in all strata, with 6% for EAM, 5% for EMM and 10% for EBM. In the case of metals, aluminium accounted for 12% in the EMM and 3% in the EAM and EBM strata. Glass was 3% of the waste generated in the high and medium marginalisation strata, and 5% in the low marginalisation stratum. From the physical-chemical characterisation carried out in the Chemistry Laboratory of the Tecnológico Nacional de México Campus Ciudad Valles, it is observed that the percentages of moisture, total solids and organic matter of the samples are very uniform, due to the high percentage of food waste by-products detected in each of the strata.

However, when compared with results from other research, differences are apparent. For example, at the national level, the per capita generation of household MSW, the organic fraction and the usable fraction is 0.653 kg inhab/d, 46.42 % and 31.56% respectively (SEMARNAT, 2020). In Berriozabal, Chiapas, the per capita generation of household MSW, the organic fraction and the usable fraction is 0.619 kg inhab/d, 54.88 % and 78% respectively (Araiza et al., 2017).

Saldaña, et al. (2013) report a recoverable fraction percentage of 30.81% and organic matter content of 37.56%. While, for the present study, the per capita generation of household MSW obtained was 0.844 kg inhab/d, the organic and recoverable fractions were 44% and 50.7% respectively and the organic matter was calculated at 61.28%. The results obtained in Ciudad Valles, S.L.P. show significant percentages of usable waste (organic waste, various plastics, paper, cardboard, glass and aluminium). Currently, this waste arrives at the sanitary landfill without prior separation, and is collected in situ by the collectors for subsequent sale. This situation generates operating costs that the transfer of waste implies and a reduction in the useful life of the landfill. Therefore, collaboration between the municipality and academia is necessary to continue carrying out studies that provide data for the definition of relevant waste management strategies for the economy, the environment and, therefore, a better standard of living for the population, and thus contribute to the proper management of solid waste.

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General explanation of the subject and explain why it is important.

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Clearly focus each of its features

Clearly explain the problem to be solved and the central hypothesis.

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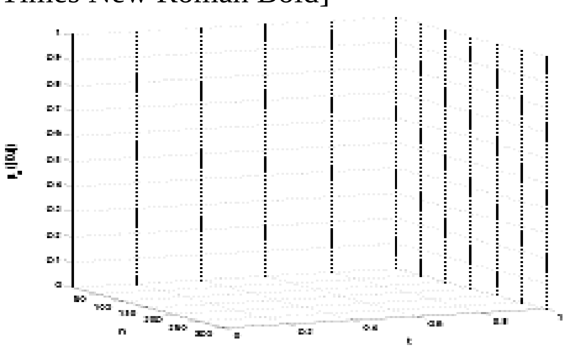

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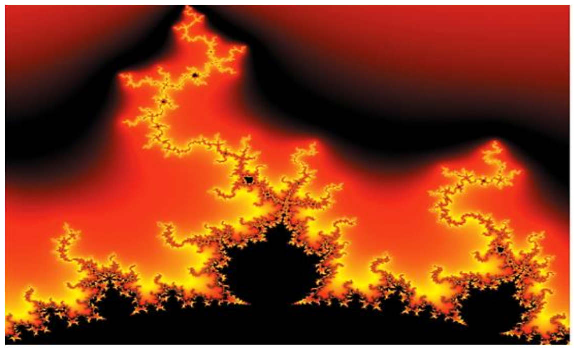

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