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Article

Microbiological analysis of biosolids treated with different techniques for agricultural use

Análisis microbiológico de biosólidos tratados con diferentes técnicas para su aprovechamiento agrícola

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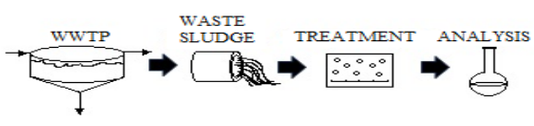
Abstract

Microbiological analysis of four biosolid samples (A, B, C, D) was carried out in accordance with current regulations. In addition, microbiological tests were performed on water, with percentage of untreated biosolid (E) and on water with sludge treated with microalgae (F) after a period of 42 days. The results obtained show that samples B, C and D were within the L.M.P., sample A was found to be above with respect to Salmonella spp., with 1,100,000 NMP/g ST of Fecal Coliforms, 1,100,000 NMP/g ST of Salmonella spp., and 1 helminth egg in 2 g ST. Salmonella spp. was present in samples E and F. Subsequently, an analysis was carried out on a sample of sorghum and another of corn treated with biosolid for germination and growth, obtaining results of 250,000 CFU/g and 2,200,000 CFU/g respectively.

Objetive:	**Contribution:**	**Results:**
Use of sludge from a WWTP.	Environmental improvement.	Elimination of microorganisms by drying.

Metodology:



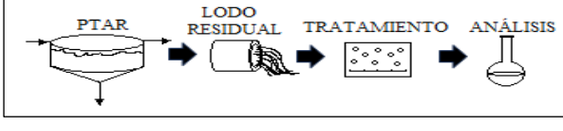
Coliforms, Fecal, *Salmonella*

Resumen

Se efectuó el análisis microbiológico de cuatro muestras de biosólidos (A, B, C, D) conforme a la normatividad vigente. Además, se realizaron pruebas microbiológicas al agua, con porcentaje de biosólido no tratado (E) y al agua con lodo tratado con microalgas (F) después de un periodo de 42 días. Los resultados obtenidos muestran que las muestras B, C y D estaban dentro de los L.M.P, la muestra A se encontró por encima con respecto a Salmonella spp., con 1 100 000 NMP/g ST de Coliformes Fecales, 1 100 000 NMP/g ST de *Salmonella spp*., y 1 huevos de helminto en 2 g ST. Hubo presencia de *Salmonella spp*., en la muestra E y F. Posteriormente se realizó el análisis de una muestra de sorgo y otra de maíz tratadas con biosólido para su germinación y crecimiento, obteniendo los resultados de 250,000 UFC/g y 2,200,000 UFC/g respectivamente.

Objetivo:	**Contribución:**	**Resultados:**
Aprovechamiento de lodos de una PTAR.	Mejoramiento del medio ambiente.	Eliminación de microorganismos mediante secado.

Metodología:



Coliformes, Fecales, *Salmonella*

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Introduction

Significant volumes of sludge are produced in WWTPs, which can be defined as urban waste from wastewater treatment whose organic matter can be used in agriculture and soil improvement (Porta-Casanellas *et al*., 2003), as they contain a nutritional value that improves soil fertility and crop production; they are rich in organic matter (60 to 70%) and essential nutrients for plant growth, such as nitrogen (N), phosphorus (P) and potassium (K) (Kara *et al*., 2003).

Their proper use would determine the solution of environmental problems such as the reduction of sludge disposal to landfills, as well as the reduction of excessive use of chemical fertilisers (Cogger *et al*., 2000). Due to the pollutants present in the biosolids, it is necessary to apply a treatment that focuses on two fundamental aspects such as volume reduction that can be obtained by simple thickening (the dryness of the product can in some cases reach 10 or very exceptionally 20%), dewatering by natural drainage, mechanical draining, thermal drying or also dewatering by incineration.

On the other hand, fermentation reduction or stabilisation consists of reducing its biological activity (tendency to putrefaction) and its content of disease-causing microorganisms, which can be obtained through processes such as: anaerobic or aerobic digestion, chemical stabilisation, pasteurisation, cooking, treatment with lime, etc. (Rojas-Remis & Mendoza-Espinosa, 2012).

Justification

The world is currently experiencing alarming environmental problems; one of these is the final disposal of large quantities of sewage sludge from the Intermunicipal System for Wastewater Treatment and Disposal Services for the Rincon Municipalities (SITRATA) (Soto-Alcocer *et al*., 2018).

Most are taken to landfills as they contain contamination characteristics such as heavy metals, toxic waste and pathogenic microorganisms, parasites and bacteria that can affect the environment and human health, however, they can be used in agriculture, as most contain elements such as nitrogen, phosphorus and trace elements that are beneficial for plant growth and development.

For this reason, it is reasonable to look for treatments for the sludge generated in SITRATA, evaluating its quality through microbiological characterisation in order to use it as fertiliser for short-stemmed crops, allowing its rational use and also having an economic benefit, as it reduces the use of commercial chemical fertilisers, offering the opportunity to rehabilitate soil at a low cost and complying with NOM-004-SEMARNAT-2002 and NOM-003-SEMARNAT-1997 standards.

General Objective

To carry out stabilisation treatment of sludge from the Intermunicipal System for Wastewater Treatment and Disposal Services for the Municipalities of El Rincón (SITRATA) in order to use it as fertiliser for vegetable crops.

Specific objectives

- To sample sludge from the Intermunicipal System for Wastewater Treatment and Disposal Services for the Municipalities of El Rincón.
- Determine the physicochemical and microbiological characteristics of the waste sludge.
- Treat the sludge in order to reduce the microbial load.
- Choose the best treatment according to the maximum permissible limits of NOM-004-SEMARNAT-2002 and NOM-003-SEMARNAT-1997.
- Evaluate the effect of the application of biosolids on vegetables.

Theoretical framework

Currently, different productive and domestic activities generate large quantities of wastewater, which contain a wide range of pollutants. These waters must be processed in Wastewater Treatment Plants (WWTP) for reuse or disposal with a higher quality (Guzmán & Campos, 2004).

In 2009, the generation of municipal wastewater was 237.5 ^{m3}/s, of which 209.1 ^{m3}/s was collected or drained and treated and 88.1 ^{m3}/s was collected. In 2012, Mexico had a total of 2289 WWTPs, with a capacity of 137082.13 L/s and a treated flow of 97640.22 L/s, reaching a municipal wastewater coverage of 46.5 % (Conagua, 2012).

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Wastewater is basically made up of 99% water and 1% dissolved, suspended or colloidal solids (UNESCO, 2017), in the treatment and purification process of water, by-products such as sludge are generated, obtained during the mechanical, biological and chemical steps, these can be found in liquid, solid and semi-solid consistency (Amador-Díaz *et al*., 2015).

The treatment and disposal of sewage sludge resulting from municipal wastewater treatment is a very important part to consider in WWTPs, as it represents up to 50% of the infrastructure and cost as it must undergo treatment, just like water, for its final use or disposal (IMTA, 2016). Municipal wastewater sludge production was estimated at 640 million ton/year on a dry basis (Mantilla-Morales *et al*., 2017).

In Wastewater Treatment (WWT) waste sludge is produced at approximately 0.5 to 1.2 kg SSV/kg BOD removed, whose composition and production depends on the degree of treatment (primary, secondary or tertiary) (Terreros-Mecalco *et al*., 2009).

Methodology

To initiate the project, solid sludge samples were taken from the Intermunicipal System for Wastewater Treatment and Disposal Services for the Municipalities of El Rincón, according to the sampling methods for sludge and biosolids of NOM-004-SEMARNAT-2002, labelling them with the letters A, B, C, D, E and F.

For the sludge (B), 20 kg of sludge was placed in a plastic box with the top uncovered so that it received direct sunlight, kept homogenised, and removed with a shovel twice a day. It was kept under these conditions for a period of 11 days.

For sludge (C), 20 kg of sludge was again mixed with 500 g of lime and kept under the same conditions as sludge (B).

The sludge (D) was placed in an Armfield UOP8-A tray dryer, in which 400 g of sludge was evenly distributed on four trays, at a temperature of 42 °C for 6 hours (Soto-Alcocer *et al*., 2018).

Microbiological tests were performed on untreated biosolids (A) and biosolids B, C and D after treatment, for the quantification of faecal coliforms, *Salmonella spp.* and helminth eggs, according to the regulations (NMX-AA-042-SCFI-2015, NMX-AA-113-SCFI-2012 and NOM-210-SSA1-2014).

Finally, biosolids A, B, C and D were compared according to the maximum permissible limits of the NOM-004-SEMARNAT-2002 for viable helminth eggs and most probable number (MPN).

For other solid sludge samples taken at later dates from the same water treatment plant (SITRATA), tests were performed using the C-214 benchtop multi-parameter photometer kit to determine the concentration of phosphorus and nitrogen in the biosolid (Hanna-Instruments, 2001).

Next, the amount of sludge and inoculum (algae consortium), as well as the litres of water to be used in a 100 L photo-bioreactor was determined according to the concentration of nitrogen and phosphorus obtained from the sludge in relation to the concentration necessary for microalgae growth.

The concentrations of the compounds ($_{NaNO3}$, $_{KH2PO4}$) of the BBM medium used as a substrate for microalgae growth were taken as a reference.

The compound $_{NaNO3}$ corresponds to $2.^{94X10-3}$ mol/l with respect to the medium, so the calculation was made to obtain the concentration of 1 molecule of N = 14 g/1mol N, for the compound $_{KH2PO4}$ the same calculation was made.

It was considered that for the growth of microalgae it is necessary to take double concentration of these compounds.

Calculations were then carried out to obtain the ideal amount of nitrogen and phosphorus for 50 L of water, with the double concentration of N and P.

Calculations were then made to obtain the kg of sludge to be used from the ideal concentration and the concentration of N in 1 kg of sludge.

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Once the photo-bioreactor was assembled as shown in Figure 1, microbiological tests were carried out on the water with the percentage of biosolid E (untreated), for the quantification of faecal coliforms according to NMX-AA-042-SCFI-2015, helminth eggs according to NMX-AA-113-SCFI-2012, as well as confirmation of the presence or absence of *Salmonella spp.* using the methods of the NOM-210-SSA1-2014 standard.

Box 1

Figure 1
Photo-bioreactor for algae treatment
Source: Own elaboration

After a period of 42 days, the same microbiological tests were carried out again on the water with the percentage of biosolid F (after treatment with algae).

Finally, the water was compared with the percentage of biosolids E and F according to the maximum permissible limits for public services with direct contact of NOM-003-SEMARNAT-1997 for viable helminth eggs and most probable number (MPN) of faecal coliforms, as well as confirmation of the presence and absence of *Salmonella spp* .

Samples of maize and sorghum that were treated with biosolids for germination were collected from a crop located in San Bernardo, near Purísima del Rincón Gto., sampled at different points of the crop; 5 bunches of sorghum and maize from each corner and 5 from the centre.

Samples were collected in polypropylene bags, each one was shelled and mixed to obtain a homogeneous and representative sample.

Microbiological characterisation was carried out according to the methods of the Mexican Official Standard NOM-113-SSA1-1994, goods and services. Method for counting total coliform microorganisms on a plate and the Official Mexican Standard NOM-114-SSA1-1994, goods and services. Method for the determination of Salmonella in food.

Results

The results obtained in the microbiological determination of the samples are shown in Table 1, each of them underwent a drying treatment to reduce the load of microorganisms, where they are identified according to the labels: (A) biosolid without drying treatment, (B) biosolid dried in the open air, (C) biosolid dried with lime, (D) biosolid dried in tray dryer.

Box 2

Table 1
Microbiological determination of biosolids (A, B, C and D), with their L.M.P. according to NOM-004-SEMARNAT-2002

Biosolid	Bacteriological indicator of contamination	Pathogens	Parasites
	Faecal coliforms MPN/g dry basis	*Salmonella spp. MPN/g dry basis*	Helminth eggs/g on dry basis
A	1 100 000	1 100 000	1
B	46 000	240	2
C	< 3 000	240	4
D	4 300	240	8
CLASS	**L.M.P (NOM-004-SEMARNAT-2002)**		
C	< 2 000 000	<300	<35

On the other hand, for sludge treatment using an algae consortium it is necessary to know the concentration of Nitrogen and Phosphorus obtained in the biosolid E (untreated) which can be seen in Table 2.

Box 3

Table 2
Determination of Nitrogen and Phosphorus in biosolids

Determination	Water with biosolids (E)
Nitrogen	17.3 mg/L
Phosphorus	46.3 mg/L

Source: Own elaboration

Table 3 below shows the amounts of inoculum, sludge and water for the photobioreactor.

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Box 4

Table 3

Amounts of inoculum, sludge and water in the photobioreactor

Inoculum (L)	Lodo (kg)	Water (L)
5.319	0.6	44.680

Source: Own elaboration

The results obtained in the microbiological determination of the photo-bioreactor (before and after treatment) are shown in Table 4, where the water with the percentage of biosolids is reported with the letter (E) and the water with the percentage of biosolids after a period of treatment with algae is reported with the letter (F).

The microbiological analyses carried out on sorghum and maize samples treated with biosolids for growth in the community of San

Bernardo, municipality of Purísima del Rincón, Gto. gave the results shown in Table 5.

According to the results obtained, considering what is established in NOM-004-SEMARNAT-2002, class C biosolids are those that are susceptible to use in agriculture, as well as in forestry areas and soil improvement.

Regarding the microbiological determination of the different biosolids labelled as (A, B, C and D), considering the L.M.P of faecal coliforms NMP/g on dry basis, all samples of treated biosolids comply, as they do not exceed the value established by the standard, which is <2 000 000.

On the other hand, for *Salmonella spp.*, NMP/g on dry basis, sample A exceeds the L.M.P of the standard which corresponds to <300 and taking into account the helminth eggs all the samples comply being <35 which is established by the standard; which can be seen in Table 1.

Based on the microbiological analysis of the photo-bioreactor, the water with the biosolid (E) is not within the L.M.P (240 NMP/100ml) of the standard, having a value of ≥2400 NMP/100 ml. However, it is important to consider the month and climatic conditions in which the sample was taken as this is a variable factor that influences the results, as can be seen in Table 4.

Box 5

Table 4

Microbiological determination of water with a percentage of biosolids (E and F), with its L.M.P. according to NOM-004-SEMARNAT-2002 (faecal coliforms and helminth eggs) and the presence and absence of Salmonella spp.

Water with biosolids	Bacteriological indicator of contamination	Parasites	Pathogens
	Faecal coliforms NMP/100ml	Helminth eggs/l	*Salmonella spp.*
E	≥2400	3	presencia
F	11	12	presencia
Public services with direct contact	L.M.P Norma Oficial Mexicana NOM-003-ECOL-1997		
	240	≤1	-

L.M.P Official Mexican Standard NOM-003-ECOL-1997

Source: Own elaboration

Box 6

Table 5

Microbiological determination of maize and sorghum

Grain sample	Total coliforms on plate (** and **.*)			Presence or absence of Salmonella spp. in 1 g sample) ***
	Representative dilution	Colonies counted on plate	UFC/g o ml	
Sorgo	1: 100 1: 1000 1: 10000	>250* 254* Extended growth	250000*	presence of Salmonella spp. in 1 g sample
Maize	1:100 1:1000 1:10000	Extended growth >250* 219	2200000	presence of Salmonella *spp.* in 1 g sample

Source: Own elaboration.

NOTE: The results that show the following sign (*) are of the "estimated value" because the values are outside the range of 25 to 250.

** Official Mexican Standard NOM-113-SSA1-1994, goods and services. Method to count total coliform microorganisms on plate.

**Mexican Official Standard NOM-092-SSA1-1994, goods and services. Method for aerobic bacteria count on plate (aerobic mesophilic* 35 ± 2ºC 48 ± 2 h).

*** Official Mexican Standard NOM-114-SSA1-1994, goods and services. Method for the determination of salmonella in food.

In the same table it can be seen that the number of pathogens in the water with the biosolid (F) with the treatment did not decrease, having a value of 12 helminth eggs/l, not falling within the L.M.P (≤1 helminth eggs/l) of the mentioned standard. In both sludges the confirmation of *Salmonella spp.* was positive.

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The sorghum and maize samples that had a germination and growth process with the help of the SITRATA biosolid, obtained a result of 250000 CFU/g as shown in Table 5, which represents an estimated value for sorghum, while for maize 2200000 CFU/g were obtained, as well as the presence of *Salmonella spp.* in 1 g of sample in both cases, which represents a not very favourable result.

Conclusions

The treatments of open air drying, treatment with lime and constant temperature drying, are able to reduce faecal coliforms and *Salmonella spp.*, biosolids B, C and D analysed complying with the L.M.P. of class C, being important indicators for biosolids to be implemented as fertiliser in short stalk crops. It is considered that drying the sludge for a longer period of time can further reduce the content of microorganisms.

As well as the stabilisation treatment using algae (consortium), according to the maximum permissible limits for public services with direct contact established by NOM-003-SEMARNAT-1997, it is considered to be suitable for reducing the load of faecal coliforms (NMP/100 ml). of *Salmonella spp.* in both sludges, considering that they are important indicators for the biosolids treated for the treated biosolids to be implemented in short-stemmed crops, it can be considered that it is not advisable to implement them as fertiliser.

In order to use the biosolids as fertiliser in crops, according to the results obtained in Table 5, it is considered necessary to carry out a stabilisation treatment in order to avoid the presence of *Salmonella spp.* which is an important parameter in foodstuffs.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest. They have no known competing financial interests or personal relationships that might have appeared to influence the article reported in this paper.

Authors' contribution

Rivera-Mosqueda, Ma. Cruz: Development of the original Project idea and sample collection.

Soto-Alcocer, José Luis: Assistance in sample collection, transport and administration of the project.

Arellano-Elizarraraz, Roberto: Experimental analysis and editing.

Ayala-Islas, Alberto: Experimental analysis and editing.

Availability of data and materials

All data and procedures resulting from the research can be obtained from the lead author.

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Abbreviations

M.P.L: Maximum permissible limit.
M.P.N.: Most Probable Number.
NMX: Mexican Standard.
NOM: Official Mexican Standard.
PTAR: Wastewater treatment plant.
SITRATA: Intermunicipal System for Wastewater Treatment and Disposal Services for the Municipalities of El Rincón.
spp.: Species.
CFU: Colony Forming Units.
WWTP: Wastewater Treatment Plant.

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Support

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