Colegio de Postgraduados

Evaluation of microbiological safety in bioinputs produced in Mexico

Gómez-Godínez Lorena J.[1]; **Arteaga Garibay Ramón, I.**[1]; **Ruvalcaba-Gómez José, M.**[2]; **Cadena Iñiguez, Pedro**[2*]; **Ariza-Flores, Rafael**[1]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Recursos Genéticos. Boulevard de la Biodiversidad 400, Rancho las Cruces. Tepatitlán de Morelos, Jalisco, México. C.P. 47600.

[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Centro de Chiapas. Carretera Ocozocoautla-Cintalapa km 3, Ocozocoautla, Chiapas, México.

[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Valles Centrales de Oaxaca. Melchor Ocampo núm. 7, Santo Domingo Barrio Bajo, Villa de Etla, Oaxaca, México. CP. 68200.

* Correspondence: cadena.pedro@inifap.gob.mx

ABSTRACT

Objective: This work aimed to evaluate the microbiological safety of bioinputs produced in Mexico. The main reason for this evaluation is that bioinputs are products made from the region's manures, plant residues and raw materials. The transformation of these raw materials is carried out by microorganisms present. The process goes through three stages: initial, thermophilic and final. The thermophilic stage is critical because weeds and microorganisms with pathogenic potential disappear in processes under optimal conditions.

Methodology: 1345 bioinputs samples were received from different states of Mexico. The samples were evaluated for the presence of total and fecal coliforms and *Escherichia coli* under the provisions of the Official Mexican STANDARDS, NOM-210-SSA1-2014 and NOM-114-SSA1-1994.

Results: It was possible to identify 79% of the samples with Most Probable Number values <3 of total coliforms, fecal coliforms and *Escherichia coli*, the minimum permissible by the Official Mexican STANDARD NOM-210-SSA1-2014, also identified 99% of samples free of *Salmonella*.

Conclusions: The results obtained allow us to conclude that the bioinputs produced in Mexico are free of pathogens for humans, which can also be represented as innocuous bioinputs.

Keywords: Bioinputs, organic fertilizers, safety, fecal coliforms, *Salmonella*.

Citation: Gómez-Godínez L. J., Arteaga- Garibay, R. I., Ruvalcaba-Gómez, J. M., Cadena-Iñiguez, P. & Ariza-Flores, R. (2023). Evaluation of microbiological safety in bioinputs produced in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i9.2681

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: September 14, 2023.
Accepted: September 29, 2023.
Published on-line: November 27, 2023.

Agro Productividad, 16(10). October. 2023. pp: 129-135.

INTRODUCTION

Sustainable agriculture has various strategies that propose reducing the use of agrochemicals (Carlise *et al.*, 2019), complementing their application with products of biological origin, such as bioinputs or organic fertilizers, whose production depends on renewable sources of raw materials present in the region of elaboration (Castro & Bertsch, 2009). Organic fertilizers and bioinputs are products made from cattle manure, stubble, plants, and microorganisms. We can find compost, bokashi, sulfocalcium broth, leachate, and others (Cabanillas *et al.*, 2017; Phooi *et al.*, 2022; Geisseler *et al.*, 2021). The main action of these bioinputs is to improve productivity, yield and health when applied to plants (Cabanillas *et al.*, 2017; Goulet *et al.*, 2021). Recycling organic waste products in agricultural soils is one of the most economical strategies and beneficial to the environment (Alvarenga *et al.*, 2017; Sayara *et al.*, 2020).

Bioinputs represent a beneficial natural fertilizer for agriculture. However, most are made from the fecal matter of farm animals (Yun *et al.*, 2007). Within the intestinal tract of farm animals, many microorganisms with pathogenic potential are deposited in the fecal matter when discarded by the animals (Lefebvre *et al.*, 2006). The microbiological safety of bioinputs or organic fertilizers can be evaluated based on some microbiological indicators.

There are various reports in which *Salmonella* and *Escherichia coli* were identified from bioinputs (Goldstein *et al.*, 1988; Yun *et al.*, 2007). This indicates that bioinputs can potentially have pathogenic microorganisms, which can be transmitted to food.

For this reason, it is essential to detect potentially pathogenic microorganisms, such as total and fecal coliforms *Escherichia coli*, which are known to cause stomach infections, diarrhea, fever, abdominal cramps, and hemolytic uremic syndrome. In Mexico in 2017, there were more than eight thousand cases of bacterial food poisoning due to the consumption of raw vegetables and fecal coliforms (Martinez *et al.*, 2020). Due to this, this work aimed to evaluate the microbiological safety of bioinputs produced in Mexico.

MATERIALS AND METHODS

Collection and storage of samples

One thousand three hundred forty-five bio-input samples were collected in different states of the Mexican Republic; they were transported to the National Center for Genetic Resources of INIFAP (CNRG-INIFAP) and stored at 4 °C.

Preparation of serial dilutions

From the bioinputs, serial dilutions were made. The samples were homogenized; 10 g or 10 mL of each one was taken, depending on whether they were in a solid or liquid state, and they were diluted in 90 mL of sterile peptone water from here. Serial dilutions were made up to 10-7. This procedure was carried out by the Official Mexican STANDARD NOM-110-SSA1-1994.

Determination of total and fecal coliforms and *Escherichia coli*

The determination of total coliforms (OCT), fecal coliforms (OCF), as well as *E. coli* was carried out according to the Official Mexican Standard NOM-210-SSA1-2014, following the method approved for the estimation. Of the density of OCT, OCF and *E. coli* by the Most Probable Number technique.

Determination of *Salmonella* in bioinputs

The determination of *Salmonella* was carried out according to the Official Mexican Standard NOM-210-SSA1-2014, which consists of taking 25 g of the sample and transferring it to 225 mL of selective pre-enrichment medium (medium of selenite cystine), this was incubated for 24 h at 37 °C, and later it was streaked on Petri dishes with Brilliant Green Agar and XLD xylose lysine deoxycholate agar, they will be incubated at 37 °C and typical or atypical Salmonella colonies will be selected.

RESULTS AND DISCUSSION

One thousand three hundred forty-five bioinputs from different regions of the country were received (Figure 1). Region 4, made up of Chiapas, had the highest number of samples for analysis, followed by Region 27, which belongs to San Luis Potosi, with 86 bioinputs, region 11 and region 23 with 80 bio inputs (Figure 1).

The bioinputs that were received were made up mainly of leachate (28%), Mountain Microorganisms (MM) (14%), bioles (10%), compost, humus, bocashi (8%), among others (Figure 2). The leachates were the bioinputs that were mainly produced and collected for the safety analysis, and this is due to their easy obtaining because they are the result of the application of water in the vermicomposting and compost piles; this allows to maintain the humidity of these organic fertilizers (Tejada-Gonzalez *et al.*, 2008), it is known that the application of leachates can be from a foliar application generating positive effects, such as the increase in chlorophyll, macro and micronutrients in tomato, rice and corn (Tejada and González 2004, 2006). The MM, for their part, are made up of colonies of fungi, bacteria and beneficial yeasts that are found in different ecosystems, such as forests, coffee plantations, and bamboo, among others (Suchini-Ramirez, 2012); these are extracted from the ecosystems and later reproduced in liquid cultures. It is known that applying these can increase the nutritional value and inhibit pathogens in plants of agricultural interest

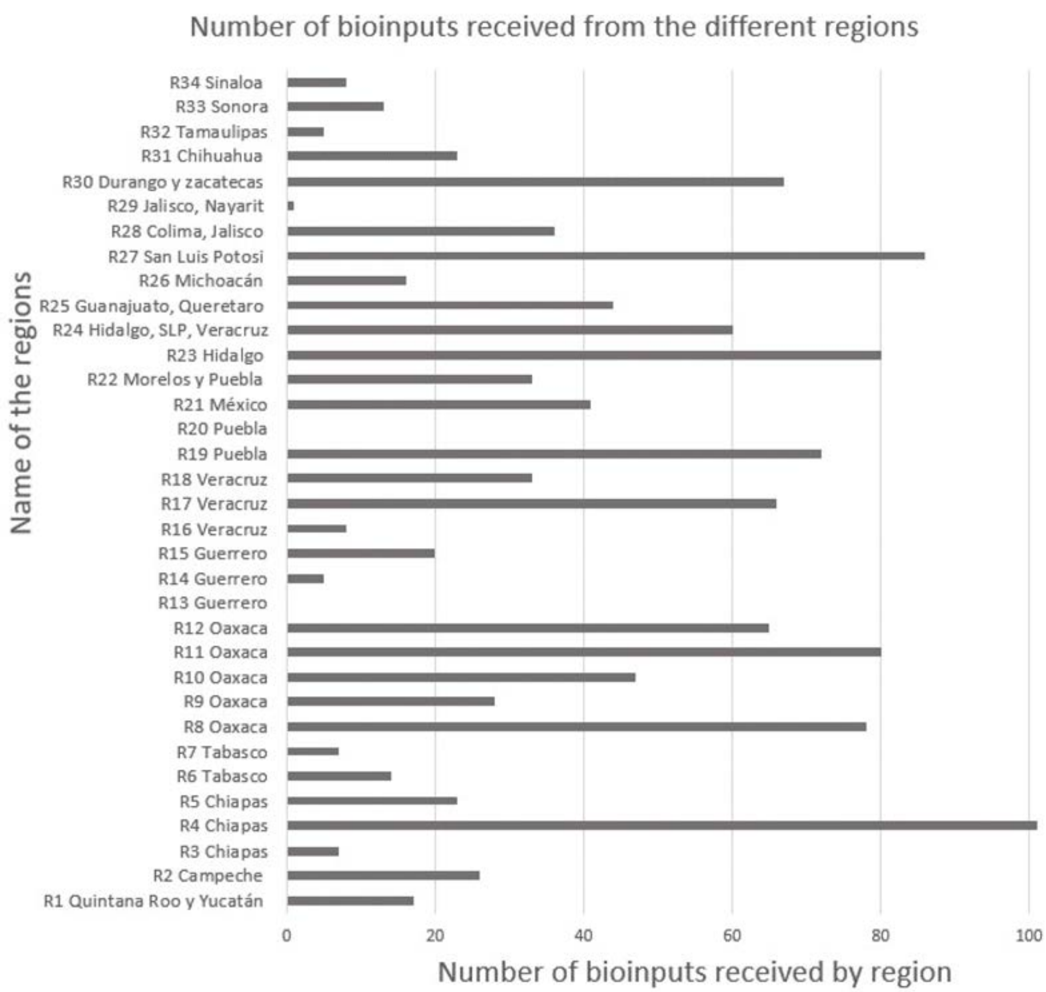

Figure 1. Number of bioinputs received by region of Mexico.

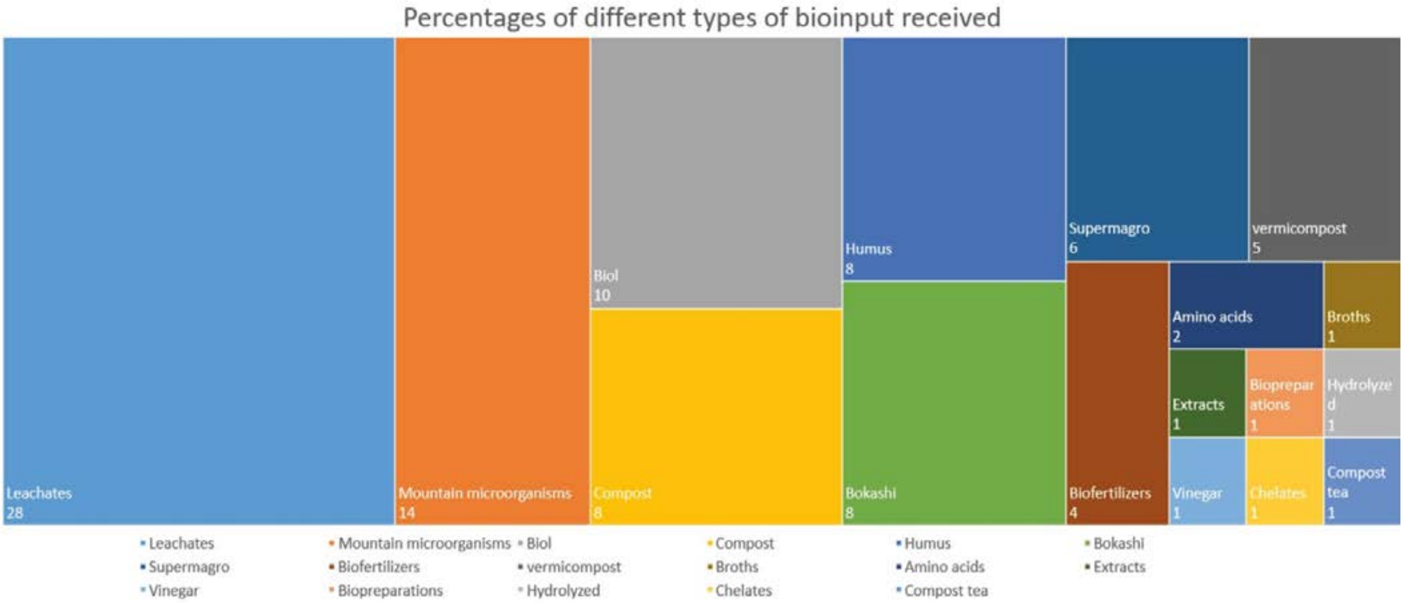

Figure 2. Percentages of bioinputs received from the Mexican Republic.

(Campo-Martínez *et al*., 2014). One of the by-products of the anaerobic fermentation of manures is bioles (Cano-Hernández *et al*., 2016), which are known to favor the growth of some crops such as spinach (*Spinacea oleracea*) (Siura *et al*., 2009). These were the third bioinputs that were received in greater quantity.

Analysis of total coliforms, fecal and *E. coli*

One thousand three hundred forty-five samples were analyzed to identify the presence of total coliforms, considered bacterial indicators in samples that present fecal contamination. According to NOM-210-SSA1-2014, with the Most Probable Numbers (MPN) technique, MPN /g or mL <1100 means that the microorganisms present are outside the permissible limit and represent a human-use limitation. Of the 1345 samples, it was found that 78% and 79% of MPN/g or mL <3 of total coliforms and fecal coliforms, respectively. *E. coli*, for its part, is considered an indicator of fecal contamination, which can be found in animal waste and food waste (Lalander *et al*., 2013; Mainoo *et al*., 2009); in this study, we found 79% of bioinputs with <3 NMP/mL or g, 5% <23 NMP/mL or g, 4% <3.6 NMP/mL or g (Figure 3), which means a minimum load of pathogenic microorganisms, permissible by the standard (Figure 3). There are various reports on the high efficiency of removing coliforms present in bioinputs at the end of the process. For example, a bovine manure composting process was evaluated for two years, where the numbers of total coliforms and *E. coli* decreased as the process progressed; this was achieved because the thermophilic stage of the composting remained above 55 °C for 15 days (Larney *et al*., 2003). On the other hand, fecal coliforms, *E. coli* and *Salmonella* were evaluated from composts obtained from residual water sludge, finding that temperatures of 57 and 61 °C, reached during composting, eliminated most of the pathogens present. At the beginning of the process (Banegas *et al*., 2007).

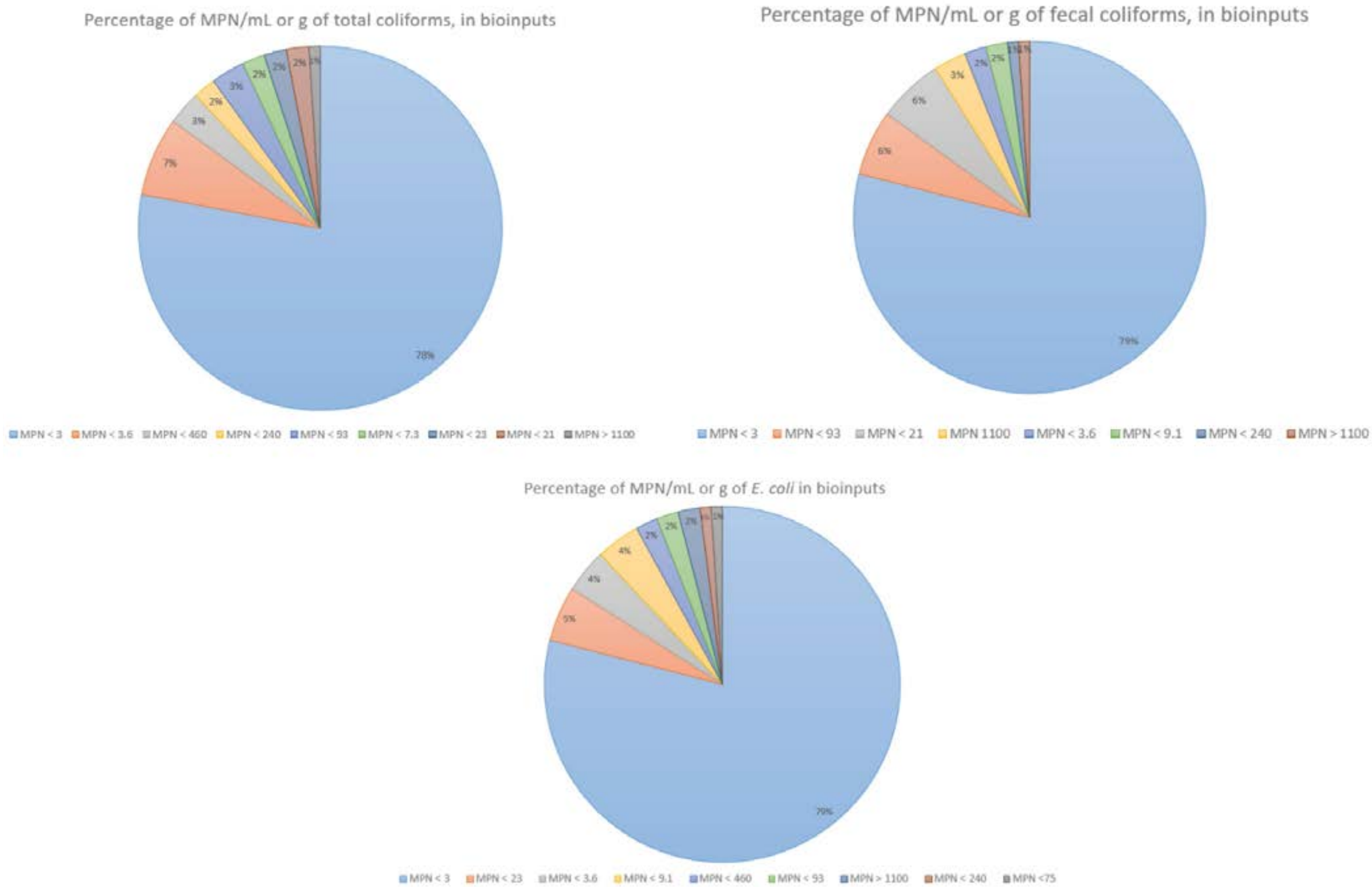

Figure 3. Percentages of MPN/mL or g of total, fecal coliforms, and *Escherichia coli*, present in the bioinputs.

Regarding the maximum allowable by the standard, 1% NMP/g or mL >1100 of total fecal coliforms and 2% NMP/g or mL >1100 of fecal coliforms and *E. coli* were identified in all the bio inputs analyzed (Figure 3), which indicates that this could have a potential risk to human health. As in this report, NMP sludge composting of >1100 g^{-1} has been identified in other works; these results are explained by some factors that contributed to the high levels of pathogens, such as the large size of facilities and waste piles. Composting and immaturity of the compost (Brinton *et al.*, 2000) probably also intervened in the 1% of the bio inputs we found with values >1100 NMP/mL or g.

Salmonella is considered a severe problem of the hygienic quality of compost and bioinputs; in this work, it was found that 99% of the samples were free of it. Domínguez and Edwards 2004 indicated that temperatures above 30 °C prevent the presence of *Salmonella* in composting processes; it has also been reported that the use of worms such as *Eisenia fetida* in vermicomposting processes decreases the presence of *Salmonella* by up to 99% (Brown & Mitchell, 1981), this is probably due to the antimicrobial response of gram-negative bacteria from the gizzard to the intestinal tract of earthworms (Soobhany *et al.*, 2018).

CONCLUSIONS

This research's analysis indicates that the fermentation process in the different processes of leachate, biol, compost, and vermicompost was efficient, reaching temperatures above 55 °C in the thermophilic stage, which is related to the decrease in total coliforms. Fecal coliforms, *E. coli* and *Salmonella* obtained values less than 1100 NMP/mL or g, which is allowed by NOM-210-SSA1-2014.

ACKNOWLEDGMENTS

Al Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias y al proyecto de programa para el bienestar

REFERENCES

Banegas, V., Moreno, J. L., Moreno, J. I., Garcia, C., Leon, G., & Hernandez, T. (2007). Composting anaerobic and aerobic sewage sludges using two proportions of sawdust. *Waste Management, 27*(10), 1317-1327.

Brown, B. A., & MJ, M. (1981). Role of the earthworm, *Eisenia foetida*, in affecting survival of *Salmonella enteritidis* ser. *typhimurium*.

Campo-Martínez, A. D. P., Acosta-Sanchez, R. L., Morales-Velasco, S. A. N. D. R. A., & Prado, F. A. (2014). Evaluation of microorganisms of mountain (mm) in the production of chard on the plateau of Popayán. *Biotecnología en el Sector Agropecuario y Agroindustrial, 12*(1), 79-87.

Cano-Hernández, M., Bennet-Eaton, A., Silva-Guerrero, E., Robles-González, S., Sainos-Aguirre, U., & Castorena-García, H. (2016). Caracterización de bioles de la fermentación anaeróbica de excretas bovinas y porcinas. *Agrociencia, 50*(4), 471-479.

Carlisle, L., Montenegro de Wit, M., DeLonge, M. S., Iles, A., Calo, A., Getz, C., ... & Press, D. (2019). Transitioning to sustainable agriculture requires growing and sustaining an ecologically skilled workforce. *Frontiers in Sustainable Food Systems,* 3, 96.

Castro, A.; Henríquez, C. y Bertsch, F. Capacidad de suministro de N, P y K de cuatro abonos orgánicos. *Agronomía Costarricense Revista de Ciencias Agrícolas*, 2009, vol. 33, no. 1, p. 31-43.

Domínguez, J., & Edwards, C. A. 17. Vermicomposting organic wastes: A review.

Goldstein N, Yanko WA, Walker JM, Jakubowski W (1988) Determining pathogen levels in sluge products. *Biocycle* 29:44–47

Lalander, C. H., Hill, G. B., & Vinnerås, B. (2013). Hygienic quality of faeces treated in urine diverting vermicomposting toilets. *Waste Management, 33*(11), 2204-2210.

Larney, F. J., Yanke, L. J., Miller, J. J., & McAllister, T. A. (2003). Fate of coliform bacteria in composted beef cattle feedlot manure. *Journal of environmental quality, 32*(4), 1508-1515.

Mainoo, N. O., Barrington, S., Whalen, J. K., & Sampedro, L. (2009). Pilot-scale vermicomposting of pineapple wastes with earthworms native to Accra, Ghana. *Bioresource Technology, 100*(23), 5872-5875.

Siura, S., Yarasca, I. M., & Dávila, S. (2009). Efecto del biol y la rotación con abono verde (*Crotalaria juncea*) en la producción de espinaca (*Spinacea oleracea*) bajo cultivo orgánico. In Anales científicos (Vol. 70, No. 1, pp. 1-8). Universidad Nacional Agraria La Molina.

Soobhany, N. (2018). Preliminary evaluation of pathogenic bacteria loading on organic Municipal Solid Waste compost and vermicompost. *Journal of Environmental Management,* 206, 763-767.

Suchini Ramírez, J. G. (2012). Innovaciones agroecológicas para una producción agropecuaria sostenible en la región del Trifinio. Serie Técnica. Manual Técnico.

Tejada, M., & Gonzalez, J. L. (2004). Effects of application of a by product of the two step olive oil mill process on maize yield. *Agronomy journal, 96*(3), 692-699.

Tejada, M., & Gonzalez, J. L. (2006). Effect of foliar application of beet vinasse on maize yield. *Biological agriculture & horticulture, 24*(2), 197-214.

Tejada, M., Gonzalez, J. L., Hernandez, M. T., & Garcia, C. (2008). Agricultural use of leachates obtained from two different vermicomposting processes. *Bioresource technology, 99*(14), 6228-6232.

Yun H-J, Lim S-Y, Song H-P, Kim B-K, Chung B-Y, Kim D-H (2007) Reduction of pathogenic bacteria in organic compost using gamma irradiation. *Radiat Phys Chem* 76:1843–1846. doi:10.1016/j.radphyschem.2007.02.093

Phooi, C. L., Azman, E. A., & Ismail, R. (2022). Role of organic manure Bokashi improving plant growth and nutrition: A review. *Sarhad Journal of Agriculture, 38*(4), 1478-1484.

Cabanillas, C., Tablada, M., Ferreyra, L., Pérez, A., & Sucani, G. (2017). Sustainable management strategies focused on native bio-inputs in *Amaranthus cruentus* L. in agro-ecological farms in transition. *Journal of Cleaner Production*, 142, 343-350.

Geisseler, D., Smith, R., Cahn, M., & Muramoto, J. (2021). Nitrogen mineralization from organic fertilizers and composts: Literature survey and model fitting. *Journal of Environmental Quality, 50*(6), 1325-1338.

Goulet, F. (2021). Biological inputs and agricultural policies in South America: Between disruptive innovation and continuity. *Perspective*, (55), 1-4.

Alvarenga, P., Palma, P., Mourinha, C., Farto, M., Dôres, J., Patanita, M., ... & Sousa, J. P. (2017). Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. *Waste Management*, 61, 582-592.

Sayara, T., Basheer-Salimia, R., Hawamde, F., & Sánchez, A. (2020). Recycling of organic wastes through composting: Process performance and compost application in agriculture. *Agronomy, 10*(11), 1838.

Lefebvre, B., Malouin, F., Roy, G., Giguère, K., & Diarra, M. S. (2006). Growth performance and shedding of some pathogenic bacteria in feedlot cattle treated with different growth-promoting agents. *Journal of food protection, 69*(6), 1256-1264.

Martínez, Y. E., Pimienta, F. A., García, J. O., De la Rosa López, R., & Rivera, D. E. V. (2020). Coliformes totales y fecales en lechuga iceberg de mercados de H. Caborca, Sonora. INVURNUS, 3-6.

Norma Oficial Mexicana (1994). NOM-110-SSA1-1994, Bienes y servicios. Preparación y dilución de muestras de alimentos para su análisis microbiológico. México, D.F., Diario Oficial de la Federación. 9 p.

NORMA Oficial Mexicana NOM-210-SSA1-2014, Productos y servicios. Métodos de prueba microbiológicos. Determinación de microorganismos indicadores.

Brinton, W. F. (2000). Compost quality standards and guidelines: an international view. Woods End Research Laboratory Inc., ME, 10.