

## Analysis by Pfeiffer chromatography on soil improved with bocashi and native microorganisms

## Análisis mediante cromatografía de Pfeiffer en suelo mejorado con bocashi y microorganismos nativos

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### Abstract

Ehrenfried Pfeiffer (1899-1961), created a technique with which one can observe the qualitative characteristics of soils, composts and biofertilizers. The research seeks to evaluate soil improvement by applying native microorganisms by Pfeiffer Chromatography as a way to remedy it. Representative samples of a soil worked with conventional agriculture and improved with a compost were taken, the first sample at the beginning of the test and the following three every week after applying a Bocashi-type organic fertilizer made from native microorganisms; the soil samples were analyzed separately by the Pfeiffer chromatography technique. The control sample revealed a compacted, mineralized soil, with a small amount of organic matter and without biological activity, while the other samples with the soil already improved revealed the presence of organic matter with minerals integrated in the soil, also showing the presence of Enzymatic factors and the good biological and chemical activity manifested by the harmony between their zones. It is possible to conclude that Pfeiffer chromatography represents a simple tool to know the evolution of a soil by improving it with organic matter and efficient microorganisms manifesting some qualitative characteristics that indicate its progress.

**Organic fertilizer, Bocashi, Mountain microorganisms**

### Resumen

Ehrenfried Pfeiffer (1899-1961), creó una técnica con la que se puede observar las características cualitativas de suelos, compostas y biofertilizantes. La investigación busca evaluar mediante la Cromatografía de Pfeiffer la mejora del suelo al aplicar microorganismos nativos como forma de remediarlo. Se tomaron muestras representativas de un suelo trabajado con agricultura convencional y mejorado con una composta, la primera muestra al inicio de la prueba y las tres siguientes cada semana después de haber aplicado un abono orgánico tipo bocashi elaborado a base de microorganismos nativos; las muestras de suelo fueron analizadas por separado mediante la técnica de cromatografía de Pfeiffer. La muestra testigo se reveló un suelo compactado, mineralizado, con poca cantidad de materia orgánica y sin actividad biológica, mientras que las otras muestras con el suelo ya mejorado revelaron la presencia de materia orgánica con minerales integrados en el suelo, mostrando además la presencia de factores enzimáticos y la buena actividad biológica y química manifestada por la armonía entre sus zonas. Es posible concluir que la cromatografía de Pfeiffer representa una herramienta sencilla para conocer la evolución de un suelo al mejorarlo con materia orgánica y microorganismos eficientes manifestando algunas características cualitativas que indican su progreso.

**Abono orgánico, Bocashi, Microorganismos de montaña**

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## Introduction

Chromatography is a physical method of separation in which the components to be isolated are distributed in two phases: stationary phase (at rest) and mobile phase (defined direction) (Banegas, 2019), which for Restrepo and Pinheiro (2011) is a set of techniques based on the principle of selective retention whose objective is to separate the different components of a mixture to identify and in many cases determine the amounts of said components.

Pfeiffer chromatography is a qualitative analysis technique that can be used in soils, composts and biofertilizers, being able to quickly observe the relationship between microorganisms, organic matter and minerals, as elements that compose them (Medina et al., 2018). In agriculture, chromatography is used as a self-certifier and qualitative verifier of the soil structure, you can also check the protein and enzymatic quality of roots, leaves, stem and animal fluids. (Banegas, 2019).

For Ehrenfried Pfeiffer (1899-1961), it was important to evaluate the quality of soil fertility and the food they produce, in the context of biodynamic agriculture, found that a solution of sodium hydroxide (NaOH), prepared at 1% , in a sample of living soil it was sufficient to solubilize the nitrogen substances of the metabolism of the microorganisms present in it, which reacted when exposed on a special filter paper impregnated with silver nitrate, and then revealed a series of specific colors and distances . (Restrepo and Pinheiro, 2011).

The soil is not only the support of plants, it is also a living and dynamic organism with three-dimensional and three-phase form (Sánchez, 2012; Medina et al., 2013), which as a natural resource has provided sustenance to the human population; However, the pressure on this resource to increase food production has had a serious impact on its quality (Sánchez, Hernández and Ruz 2011), when receiving fertilizers, pesticides and other chemical products periodically (Sandoval et al., 2017). Therefore, it is important to adopt agroecological alternatives that minimize their deterioration and provide solutions in the short, medium and long term, because 69.6% of the soils have low organic matter content (MO) and 43.3% have a erosion from strong to medium, which limits its productivity. (Sánchez Hernández and Ruz 2011).

Likewise, it is important to recover the soil microbiology and an alternative is the use of mountain microorganisms (MM), beneficial organisms that contain yeasts, photosynthetic bacteria and lactic acid bacteria. This type of microorganisms helps to improve the quality of the soil, by providing the nutrients that come from organic matter (Sandoval et al., 2017), so it is important to apply organic fertilizers, since organic matter, and particularly humus is the basic support for life in this environment, being able to define its productive potential. (Otiniano et al, 2006; Cabrera et al., 2018). The objective of the research was to perform an analysis by Pfeiffer chromatography in a soil that was improved by using bocashi and native microorganisms.

## Materials and method

*Sample collection.* Representative soil samples were taken in the greenhouse of the University of Guanajuato, Celaya-Salvatierra campus, Salvatierra headquarters. The collections were made at four different times, the first at the beginning of the test and before applying any improvement to the soil, and the next three every week after applying the fermented organic fertilizer based on native microorganisms (bocashi). They were taken from five points marked in zig zag and from three different depths (5, 15 and 30 cm), which were mixed to obtain only a representative sample of approximately 100 g and allowed to dry in a ventilated place not exposed to the rays of the sun and were labeled.

*Bocashi elaboration.* The bocashi was made in a place sheltered from rain, wind and sun rays in order not to affect fermentation. The elaboration process was carried out by collecting decaying litter from a site with the least anthropogenic disturbance. In addition, native microorganisms (mountain microorganisms), charcoal, cattle manure, molasses, yeast, ground stubble, sifted clay, stove ash, lime and water were used. The methodology used was that described by Restrepo, (2007) with some modifications. During its preparation, it was ensured that the temperature was maintained between 50 and 60 ° C for mesophilic microorganisms to develop.

**Chromatographic analysis.** MN 615 filter paper was used, with 150 mm diameter of number 5, the exact point was determined with a punch, from the center it was marked at 4 cm and 6 cm. Subsequently, the filter paper was impregnated in a 0.5% silver nitrate solution ( $\text{AgNO}_3$ ) up to 4 cm, to then protect it from light. The soil samples were ground in a mortar until pulverized, 5 g of each sample was weighed and mixed with 50 ml of a 1% sodium hydroxide solution in a beaker, then stirred 7 times to the left and 7 times to the right until 49 turns were counted, the operation was repeated for each sample at 15 and 60 minutes, finally the solutions were left at rest for 6 hours, then proceed to the analysis. 10 ml of the sample supernatant from the soil solution was taken and placed in a Petri dish, then a wipe (straw) was placed in the center hole of each filter paper impregnated with 0.5%  $\text{AgNO}_3$  solution and He let it run to the second mark (6 cm), the wipe was removed and allowed to dry exposing the chromatograms indirectly to the sun to begin its development. The interpretation was carried out in accordance with what Restrepo and Pinheiro (2011) propose.

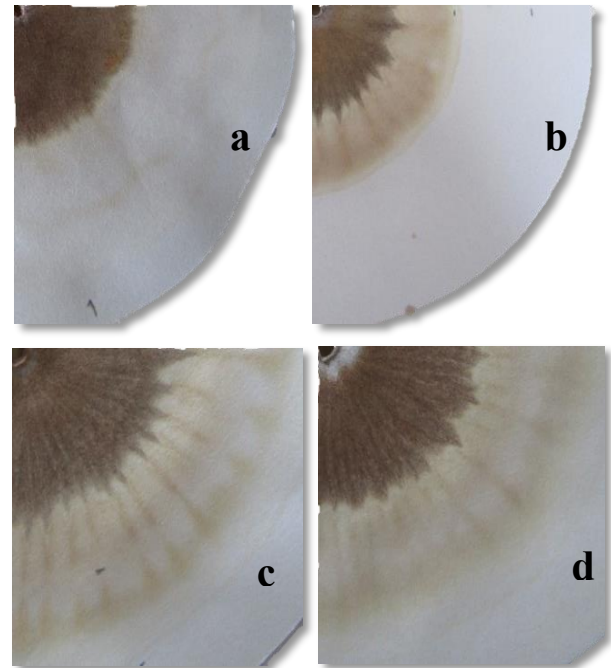
## Results and Discussion

The interpretation of the results was carried out in accordance with that indicated by Restrepo, J. and Pinheiro, S. (2011). Taking into account that the description is made based on the areas that compose it, its size, shape and the colors revealed. The zones are five, from the center outwards: central zone, internal zone (mineral), intermediate zone (organic matter), external zone (enzymatic) and management or peripheral zone (figure 1).



**Figure 1** Main areas of a chromatogram (Restrepo and Pinheiro 2011)

Figure 2 shows the chromatograms obtained after resting for 6 hours and having run the soil samples on the filter paper impregnated with silver nitrate.



**Figure 2** Chromatograms of soil analysis: (a) before applying a compost; (b), (c) and (d) during the 1st, 2nd and 3rd week of the application of Bocashi respectively

Figures 2a and 3 describe the soil chromatogram before improving it, it indicates the poor quality of the soil, because there is no harmony in the coloring of the areas that compose it. The central zone practically does not exist, which indicates that the soil is compacted; the internal zone is uniform and very large in relation to the other zones, this reveals that the soil is mineralized, with little amount of organic matter and biological activity.

The intermediate zone indicates that organic matter is scarce, raw and without integration. Finally, the enzyme zone is not distinguished, so there is little capacity to develop a crop, which is what Restrepo and Piñero (2009) interprets as a setback in the health, condition and structure of soils treated in a conventional manner.



**Figure 3** Soil analysis before applying any amendment

In figures 2b and 4 the state of the soil can be seen in the first week after applying the organic fertilizer (bocashi), in relation to the central area there is a very scarce white creamy color that fades to integrate with the following zones, likewise, there is presence of organic matter with minerals integrated in the soil, it can be mentioned that because this sample corresponds to the first week after applying bocashi, there is little evidence of enzymatic factors resulting from biological activity, since it is related to the metabolic demand of microbial biomass (Defrieri et al., 2005).



**Figure 4** Soil in the first week after applying bocashi based on native microorganisms

Figures 2c and 5 represent the situation of the soil at the second week after having applied the organic fertilizer, where the improvement obtained therein is observed, firstly, because the central area has a creamy white coloration indicative of greater oxygenation.

It fades and integrates with the following zones. It can be seen that minerals and organic matter are integrated in the soil, indicative of the biological activity, finally due to the very wavy clouds of the external zone, some enzymatic activity begins, it is well known that the decomposition of organic matter depends of the cellular production of microbial enzymes (Quintero, 2014)



**Figure 5** Soil in the second week after applying bocashi based on native microorganisms

Figures 2d and 6 show the structure of the soil in the third week after applying the organic fertilizer, it can be seen that there are few differences with respect to the previous chromatogram; the first one is the central zone because here the creamy white coloration is very scarce, but with respect to the other zones it is very similar.

With the above it can be said that there is a presence of organic matter, which is integrated in the soil and is involved in its microbiological activity, it is a good quality soil. According to Sánchez, Hernández and Ruz (2011) the effects of microorganisms on the soil are framed in the improvement of physical and biological characteristics and the establishment of a healthy soil.



**Figure 6** Soil in the third week after applying bocashi based on native microorganisms

On the other hand, Ramos, Terry, Soto and Cabrera (2014) mention that bocashi incorporates organic matter and essential nutrients into the soil, such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, copper and boron; which improve the physical and chemical conditions of the soil, which stimulates the microbial life of the soil and the nutrition of the plants and a chromatogram is a guarantee seal that records the quality of the soil's health and its relation to the biological value produced (Restrepo and Pinheiro, 2011).

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### Conclusion

Pfeiffer chromatography is a simple and very useful technique to know the qualitative characteristics of the soil, as well as the state in which organic fertilizers are found. The Pfeiffer technique is a useful tool that reveals how the incorporation of native microorganisms with bocashi intervenes in the improvement of soil structure and health.

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