Publisher: Sapienza Grupo Editorial
R. Santa Cruz, 2187, Vila Mariana
São Paulo, Brazil
editor@sapienzaeditorial.com

Sapienza
International Journal of Interdisciplinary Studies
ISSN: 2675-9780

Bioassay for bioremediation of degraded agricultural soil using bokashi compost with mountain microorganisms and bell pepper (*Capsicum annum*) indicator plants

Bioensayo para biorremediación de suelo agrícola degradado utilizando el abono bocashi con microorganismos de montaña y plantas indicadoras de pimiento (*Capsicum annum*)

Bioensaio para biorremediação de solo agrícola degradado utilizando fertilizante bocashi com microrganismos de montanha e plantas indicadoras de pimenta (*Capsicum annum*)

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ARTICLE HISTORY

Received: 02-01-2024
Revised Version: 28-01-2024
Accepted: 16-02-2024
Published: 23-02-2024

Manuscript type: Article

ARTICLE INFORMATIONS

Science-Metrix Classification (Domain):
Natural Sciences
Main topic:
Bioremediation of degraded agricultural soils
Main practical implications:
The findings of this study offer practical implications for soil restoration practices, suggesting that bokashi could serve as a viable alternative for farmers and land managers in rejuvenating degraded soils and improving agricultural productivity
Originality/value:
This research contributes to the existing literature by providing empirical evidence of the effectiveness of bokashi with mountain microorganisms in soil regeneration, thereby adding value to the field of sustainable agriculture and environmental restoration

ABSTRACT

The research aimed to regenerate degraded soils by obtaining samples in the commune of Los Bajos del Pechiche, canton Montecristo, Ecuador. Germination trays and samples of partially degraded and degraded soils as well as fertile soil were used as control for results comparison. Characterization of bokashi with mountain microorganisms was carried out in laboratories using physical, chemical, and microbiological analyses. In a bioassay, bell pepper seedlings were evaluated in treatments of degraded soil mixtures with different combinations of bokashi with mountain microorganisms through response variables: germination at 10 days, number of leaflets in seedlings at 15-30 days, plant height at 30 days and root diameter at 30 days. The doses of bokashi with mountain microorganisms were mixed with degraded soil in the following proportions 25%, 50%, and 75%, respectively. The best treatments were established as 75% degraded and partially degraded soil plus 25% bokashi with mountain microorganisms since the highest values were obtained in the different variables. The laboratory analyses presented bokashi organic fertilizer with mountain microorganisms as an excellent alternative for the regeneration of degraded agricultural soils. The results when compared with the analysis of degraded soil and the levels of FAO concerning the soils using a universal scale demonstrated its efficient alternative for bioremediation. Regarding the economic dimension, it has very high values unfeasible for producers or farmers. Then, the Ecuadorian State should establish promotion policies, including subsidies to make the regeneration of degraded soils a reality and not a utopia.

Keywords: organic fertilizers, mountain, microorganisms, soil.

RESUMO

A pesquisa teve como objetivo regenerar solos degradados por meio da obtenção de amostras na comuna de Los Bajos del Pechiche, cantão de Montecristo, Equador. Bandejas de germinação e amostras de solos parcialmente degradados e degradados, bem como solo fértil, foram usados como controle para comparação de resultados. A caracterização do bokashi com microrganismos da montanha foi realizada em laboratórios por meio de análises físicas, químicas e microbiológicas. Em um bioensaio, as mudas de pimentão foram avaliadas em tratamentos de misturas de solo degradado com diferentes combinações de bokashi com microrganismos da montanha por meio de variáveis de resposta: germinação aos 10 dias, número de folhetos em mudas aos 15-30 dias, altura da planta aos 30 dias e diâmetro da raiz aos 30 dias. As doses de bokashi com microrganismos da montanha foram misturadas ao solo degradado nas seguintes proporções: 25%, 50% e 75%, respectivamente. Os melhores tratamentos foram estabelecidos como 75% de solo degradado e parcialmente degradado mais 25% de bokashi com microrganismos da montanha, pois foram obtidos os maiores valores nas diferentes variáveis. As análises laboratoriais apresentaram o fertilizante orgânico bokashi com microrganismos da montanha como uma excelente alternativa para a regeneração de solos agrícolas degradados. Os resultados, quando comparados com a análise do solo degradado e com os níveis da FAO referentes aos solos, utilizando uma escala universal, demonstraram sua alternativa eficiente para a biorremediação. Quanto à dimensão econômica, tem valores muito altos e inviáveis para os produtores ou agricultores. Então, o Estado equatoriano deve estabelecer políticas de promoção, incluindo subsídios para tornar a regeneração de solos degradados uma realidade e não uma utopia.

Palavras-chave: fertilizantes orgânicos, montanha, micro-organismos, solo.

RESUMEN

La investigación tuvo como objetivo regenerar suelos degradados mediante la obtención de muestras en la comuna Los Bajos del Pechiche, cantón Montecristo, Ecuador. Como control para la comparación de resultados se utilizaron bandejas de germinación y muestras de suelos parcialmente degradados y degradados, así como suelo fértil. La caracterización del bokashi con microorganismos de montaña se realizó en laboratorios mediante análisis físicos, químicos y microbiológicos. En un bioensayo, se evaluaron plántulas de pimiento en tratamientos de mezclas de suelos degradados con diferentes combinaciones de bokashi con microorganismos de montaña a través de variables de respuesta: germinación a los 10 días, número de foliolos en plántulas a los 15-30 días, altura de la planta a los 30 días y diámetro de la raíz a los 30 días. Las dosis de bokashi con microorganismos de montaña se mezclaron con suelo degradado en las siguientes proporciones 25%, 50% y 75%, respectivamente. Los mejores tratamientos se establecieron como 75% de suelo degradado y parcialmente degradado más 25% de bokashi con microorganismos de montaña ya que se obtuvieron los valores más altos en las diferentes variables. Los análisis de laboratorio presentaron al abono orgánico bokashi con microorganismos de montaña como una excelente alternativa para la regeneración de suelos agrícolas degradados. Los resultados al ser comparados con los análisis de suelos degradados y los niveles de la FAO referentes a los suelos utilizando una escala universal demostraron su eficiente alternativa para la biorremediación. En cuanto a la dimensión económica, tiene valores muy altos inviables para los productores o agricultores. Entonces, el Estado ecuatoriano debería establecer políticas de fomento, incluyendo subsidios para que la regeneración de suelos degradados sea una realidad y no una utopía.

Palabras clave: abonos orgánicos, montaña, microorganismos, suelo.

INTRODUCTION

Bokashi compost is a Japanese word meaning fermented organic matter, it is a product that comes from an anaerobic process in which the degradation of organic matter, whether animal or vegetable, is accelerated and the temperature is raised to allow the elimination of pathogens.

Its elaboration consists mainly in the activation of aggregate yeasts and microorganisms found in the materials used for its production, this fertilizer allows to increase the microbiological biodiversity of soils to favor crops.

Thus, this research aims to provide specific data on the use of bokashi organic fertilizer with mountain microorganisms, implementing different mixtures in different percentages of both degraded soil and bokashi with mountain microorganisms in order to demonstrate which is the best performing treatment, in turn benefiting both the soil and plants as well as the farmer's economy by improving the productivity of their crops.

Nowadays it is necessary to apply fertilizers in order to achieve higher crop yields, therefore organic fertilizers are emerging as an alternative to reduce the use of chemical fertilizers which cause medium and long term repercussions not only on crops and soils where they are used, but also harm the environment by producing biological imbalances, degradation of ecosystems and pollution of air, soil and water.

The first consisted of the characterization by laboratory analysis of a sample of bokashi compost in its physical, chemical and microbiological components, and the second consisted of tests on plastic germination trays with different mixtures.

Every year a considerable amount of crop residues is produced, which in the future becomes not only a problem for the producer but also a source of environmental pollution. These problems are often due to the fact that there are no known alternatives to take advantage of crop residues and transform them to give them an appropriate use. Likewise, in certain cases, inadequate management and lack of environmental awareness end up generating even more repercussions (Ramos and Terry, 2014).

Therefore, taking advantage of organic residues is becoming increasingly interesting as an efficient alternative for the rational recycling of nutrients that can contribute to plant growth and return to the soil many of the elements extracted during cultivation.

Just as there are problems with crop residues, another conflict arises, and that is that as the world population has increased, there is a greater demand for food. This leads to intensive use of the natural resources around us, which produces negative impacts on the environment that affect the sustainability of production systems. Therefore, it is essential to have basic knowledge of resources to improve soil quality, such as the management of organic fertilizers (Ramos et al., 2014).

Organic fertilizers are used on soils to improve their physical, chemical and biological properties, which in turn improves fertility. The organic fertilizer that has gained popularity in Latin America is bokashi because it is a rich source of organic matter and microorganisms. This compost allows improving food production in quantity without harming the environment and promotes the use of local resources and techniques for its production and is already successfully used by many agricultural communities (Bertolí et al., 2015).

In Ecuador, after about five decades of applying the principles of the green revolution, part of the soil has deteriorated due to the use of inadequate technologies for the ecological, economic and socio-cultural reality of the country. As a result, the productivity of various crops has declined, agroecosystems have become unbalanced and environmental contamination has occurred, which in turn generates negative impacts on the health of farmers and consumers (Suquilanda, 2008). Also Suquilanda (2008) mentions that Ecuador, like most developing countries, has not been spared from the problem of soil degradation, estimating that about 48% of the national surface has erosion problems.

In addition to this problem, there is also the loss of nutrient base, acidification, salinization, sodification, increased toxicity due to the release or concentration of chemical elements, loss of humic base and therefore of microorganic activity, which increasingly accelerates the deterioration of soils dedicated to agricultural activities in the country. Therefore, since the agricultural sector plays an important role in Ecuador's economy, the soils used for this activity need to be kept in optimum condition. In this regard, the following objectives was settled to achieve in this research:

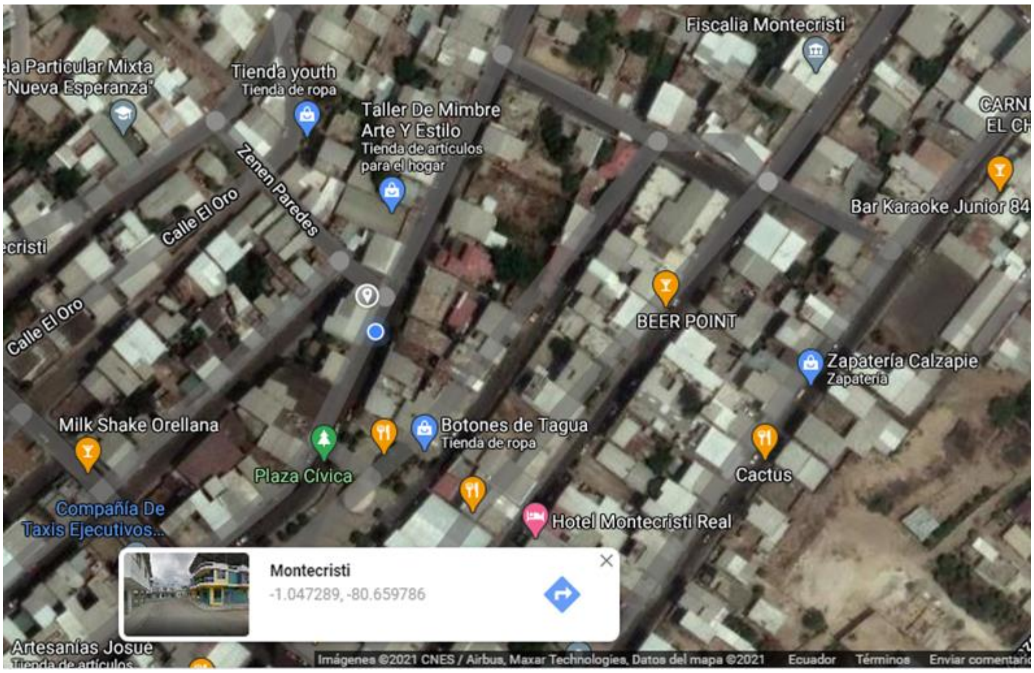
- To establish by laboratory analysis the physical, chemical and microbiological properties of bokashi with mountain microorganisms.
- To determine how bokashi with mountain microorganisms influences the improvement of fertility in degraded soil, using bell pepper (Capsicum annum) as an indicator plant.
- To make an economic estimate of the treatments being studied.

MATERIALS AND METHODS

The methodology employed involved obtaining soil samples from degraded areas, with specific coordinates. Germination trays and samples of partially degraded, degraded, and fertile soils were utilized as controls for comparison. Characterization of bokashi with mountain microorganisms was conducted through laboratory analyses encompassing physical, chemical, and microbiological parameters. A bioassay was performed, assessing bell pepper seedlings in various mixtures of degraded soil with different proportions of bokashi containing mountain microorganisms. It could be classified as a descriptive scientific investigation which allowed us to observe characteristics of the problem, identifying its causes and allowing us to verify the hypothesis that was raised in the investigation (Hernández Sampieri et al., 2018). In the second instance, bibliographic information and information collected in the field of study is described regarding the discussion of results.

This research was carried out in the city of Montecristi, which belongs to the province of Manabí, Ecuador, with geographical coordinates latitude south -1.047289 and longitude west -80.659786.

Figure 1. Location of the study site



Source: Google maps

The following materials were used: Bell pepper seeds (*Capsicum annum)*, Germination trays, Notebook, Pen, Shovel, Tachos, Phone, Balance, Rule, Cardboard, Computer, Zip lock bags.

Recovery of totally and partially degraded agricultural soils

Soil samples were collected from two sectors:

1. Partially degraded soil of a crop located in the *San José neighborhood of Montecristi,* Ecuador.
2. Totally degraded soil in the *Bajos de Pechiche* commune, *Montecristi* canton, Ecuador.

This research had two components: the first consisted of characterizing by laboratory analysis a sample of bokashi compost with mountain microorganisms in its physical, chemical and microbiological components; the second consisted of conducting a test in plastic germination trays using as indicator plant bell pepper seeds sown in different doses of bokashi compost and mountain microorganisms together with degraded soil, partially degraded soil and a control treatment of fertile soil for the comparison of the different levels of remediation.

The germination tray research was unifactorial, represented by the application of bokashi compost and mountain microorganisms in different percentages for bioremediation.

Table 1. Treatments with different percentages of bokashi and **mountain** microorganisms for bioremediation of agricultural soils, Montecristi 2021

Number of treatments	*Type of soil*	*+ bokashi with mountain microorganisms*
1	Partially degraded agricultural land	75%+25%
2	Partially degraded agricultural land	50%+50%
3	Partially degraded agricultural land	25%+75%
4	Degraded agricultural land	75%+25%
5	Degraded agricultural land	50%+50%
6	Degraded agricultural land	25%+75%
7	Fertile agricultural soil (T)	

Source: Own elaboration (2023)

Experimental design

A completely randomized block design (CRBD) was used in three replications. Below table 2 shows the scheme of variance analysis.

Table 2. Analysis of variance scheme (ADEVA)

Source of variation	GL
Total (t.r-1)	20
Treatments (t-1)	6
Repetitions (r-1)	2
Experimental error (t-1)(r-1)	12

*Significance test: Tukey at 5% probability.

$$C.V(\%) = \frac{\sqrt{CME}}{\bar{X}} * 100$$

Characteristics of the experimental units

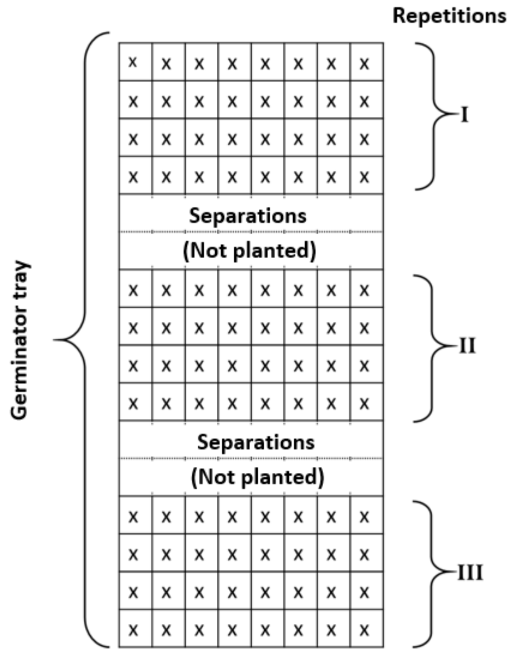
Total number= 20

Germination trays = 7

Seeds= Quetzal Hybrid Pepper

Outline for bioremediation testing

Figure 2. Diagram of germination trays



Source: Own elaboration (2023)

Experimental management: **laboratories for sample analysis**

Physicochemical: it was carried out in the laboratories of the "INIAP" complete type. Microbiological: it was held at "SEIDLABORATORY".

Response variables for bioremediation

Plastic germination trays were used where the respective substrate was placed and a seed of bell pepper (*Capsicum annum*) hybrid Quetzal was sown in each hole as an indicator plant of fertility or soil recovery. The main response variables were:

- *Percentage of seedling germination at 10 days*
- *Number of leaflets per seedling at 15 and 30 days*
- *Plant height at 15 and 30 days*
- *Root length at 30 days*
- *Root diameter at 30 days*

RESULTS AND DISCUSSION

Based on the data obtained, the following results and discussions were performed:

Test in plastic germination trays

Variable percentage of seedling germination at 10 days

According to this variable, 10 days after planting the bell pepper (C. annum) in the seedbed, seedling germination was evaluated in each of the substrates under study compared with the results of the control treatment, which consisted of using a fertile soil substrate.

According to the results shown in Table 3, there are highly significant differences (15.52**) between the germination averages for the different treatments, with a coefficient of variation of 4.87%, which is acceptable for the conditions of the type of study and provides confidence for the data obtained.

Table 3. Percentage germination of bell pepper (Capsicum annum) seedlings 10 days after planting in the different treatments

TREATMENTS (%)			%GERMINATION	
N°	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS		
1	S. Partially Degraded	25% + 75%	43.75	c
2	S. Partially Degraded	50% + 50%	57.29	B
3	S. Partially Degraded	75% + 25%	92.71	a
4	S. Degraded	25% + 75%	39.58	C
5	S. Degraded	50% + 50%	65.63	B
6	S. Degraded	75% + 25%	94.79	a
7	(T) Fertile Soil		93.75	a
C.V = 4.87%.				
Significance level 15.52 ** Significance level 15.52 ** Significance level 15.52 ** Significance level 15.52				
Tukey 5% probability 9.31				

Source: Own elaboration with the research results (2023)

Also in Table 3, it is indicated that Tukey at 5% or test of categorization of ranges of germination averages, whose value is (9.31), establishes as best percentages when 75% of degraded soil + 25% of bokashi with mountain microorganisms were mixed obtaining 94.79% germination of seedlings, mixture of 75% of partially degraded soil + 25% of bokashi with mountain microorganisms with 92.71% and the treatment with fertile soil substrate with 93.75%, and the treatment with fertile soil substrate with 93.75%.

In this context, the responses of the other treatments with respect to the germination percentage are considered not viable, so the germination levels are below 70%, results that are probably attributed to the high percentage of bokashi mixed with mountain microorganisms added to the substrates of degraded soils, which according to the literature consulted, the percentage of bokashi should not exceed 1% per ton.

Variable number of leaflets per seedling 15 and 30 days

At 15 and 30 days, the number of leaflets per seedling was recorded, and their averages are shown in Table 4. Thus, at 15 days the difference in numbers between the different treatments studied is significant (9.55*) with an acceptable coefficient of variation (7.49%), which determines the level of confidence or that the data dispersion fits the normal mean.

With the above indicators, the Tukey test was determined at 5% probability, whose value (0.38), categorized the averages of the treatments in three ranges, where the highest number of leaflets emission per seedling corresponded to the treatments with fertile soil, partially degraded soil with the mixture 50% + 50% and 75% + 25% of bokashi with mountain microorganisms and degraded soil with the mixture 50% + 50% and 75% + 25% of bokashi with mountain microorganisms with averages of 2 leaflets in each treatment.

Table 4. Average number of leaflets per bell pepper (C. annum) seedling at 15 and 30 days after sowing in the different treatments

TREATMENTS (%)			No. leaflets/plant	
NO.	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS	15 days	30 days
1	S. Partially Degraded	25% + 75%	1.80 **c**	2.47 **d**
2	S. Partially Degraded	50% + 50%	2.0 **a**	2.28 **d**
3	S. Partially Degraded	75% + 25%	2.0 **a**	5.03 **b**
4	S. Degraded	25% + 75%	1.33 **b**	2.44 **d**
5	S. Degraded	50% + 50%	2.0 **a**	2.36 **d**
6	S. Degraded	75% + 25%	2.0 **a**	5.36 **a**
7	(T) Fertile Soil		2.0 **a**	4.39 **c**
C.V			7.49%	2.88%
Level of significance			9.**5***	577**
Tukey 5% probability			0.38	0.28

Source: Own elaboration with the research results (2023)

In general terms, at 15 days with respect to the variable number of leaflets per seedling, there is no great difference in response to the treatments, noting (table A.2) that most of the treatments obtained the categorization a.

At 30 days, the difference in numbers between the different treatments studied is highly significant (577**) with an acceptable coefficient of variation (2.88%), which determines the level of confidence in the results.

With the above indicators, the Tukey test was determined at 5% probability, with a value of (0.28), categorized the averages of the treatments in four ranges, where the highest number of leaflets emission per seedling corresponded to the treatment of degraded soil with 75% + 25% mixture of bokashi with mountain microorganisms with an average of 5.36, in second place, to the treatment of partially degraded soil with 75% + 25% mixture of bokashi with mountain microorganisms with an average of 5.03 leaflets in the treatment.

Average seedling height in centimeters at 15 and 30 days

Table 5 shows the seedling height averages at 15 and 30 days in the seedbed, where at 15 days it can be seen that the averages of the different treatments are highly significant (224**) with a coefficient of variation of 4.13%, which is acceptable and provides levels of confidence. The Tukey test at 5% (0.28), establishes five ranges of difference between the averages of the treatments, highlighting that treatments six and seven have the highest average height of 3.11 and 3.84 respectively.

Table 5. Seedling height at 15 and 30 days of sowing in the different treatments

TREATMENTS (%)			HEIGHT OF PLANTS (cm)	
NO.	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS	15 days	30 days
1	S. Partially Degraded	25% + 75%	1.87 **d**	3.17 **c**
2	S. Partially Degraded	50% + 50%	1.87 **d**	3.14 **d**
3	S. Partially Degraded	75% + 25%	2.79 **c**	8.93 **a**
4	S. Degraded	25% + 75%	1.34 **e**	2.49 **d**
5	S. Degraded	50% + 50%	2.14 **d**	4.12 **c**
6	S. Degraded	75% + 25%	3.11 **b**	9.50 **a**
7	(T) Fertile Soil		3.84 **a**	7.09 **b**
C.V			4.13%	6.37%
Level of significance			**224****	217.83**
Tukey 5% probability			0.28	0.95

Source: Own elaboration with the research results (2023)

The plant height at 30 days highlights the trend of the values obtained in conjunction with other variables analyzed, highlighting the mixtures of partially degraded and degraded soils when mixed in proportion 75% + 25% of bokashi with mountain microorganisms, obtaining plants with greater height and phenotypically balanced. The lowest values were obtained when mixed with a proportion of 75% and 50% bokashi in partially degraded and degraded soils, which can be attributed to the fact that the higher the dose of fertilizer, the less favorable the results will be due to the condition indicated above that the appropriate dose of bokashi is in the order of 1% per ton.

Variable root length 30 days

Table 6 shows the root growth in length in centimeters evaluated at 30 days in the seedling stage, where the difference in numbers between the different treatments studied is highly significant (28.50**) with an acceptable coefficient of variation of 10.18%, which determines the confidence level for the data obtained.

Table 6. Root growth in length (cm) in bell pepper (C.annum) seedlings, 30 days after sowing in the different treatments

TREATMENTS (%)			ROOT LENGTH (cm)
NO.	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS	30 days
1	S. Partially Degraded	25% + 75%	1.91 **c**
2	S. Partially Degraded	50% + 50%	2.20 **b**
3	S. Partially Degraded	75% + 25%	3.75 **a**
4	S. Degraded	25% + 75%	2.04 **c**
5	S. Degraded	50% + 50%	2.35 **b**
6	S. Degraded	75% + 25%	4.11 **a**
7	(T) Fertile Soil		2.91 **b**
C.V			10.18%
Level of significance			28.50**
Tukey 5% probability			0.76

Source: Own elaboration with the research results (2023)

The Tukey average comparison test at 5% (0.76) determined that treatment 6 of 75% degraded soil + 25% bokashi with mountain microorganisms obtained the highest growth followed by treatment 3 of 75% partially degraded soil + 25% bokashi with mountain microorganisms, although without statistical differences, with the lowest values corresponding to treatments 1 and 4.

Variable diameter of the root system at 30 days

Samples were also taken 30 days after the final stage of the research to measure the growth in centimeter or thickness of the root system. Table 7 shows, by treatments, the average measurements in centimeters of root system diameter at 30 days, where the difference in numbers between the different treatments studied is highly significant (237.5**) with an acceptable coefficient of variation of 4.86%, which determines the level of confidence for the data obtained.

Table 7. Root system diameter of bell pepper seedlings 30 days after planting

TREATMENTS (%)			ROOT DIAMETER (cm)
NO.	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS	30 days
1	S. Partially Degraded	25% + 75%	1.83 **c**
2	S. Partially Degraded	50% + 50%	2.01 **c**
3	S. Partially Degraded	75% + 25%	4.40 **a**
4	S. Degraded	25% + 75%	1.82 **c**
5	S. Degraded	50% + 50%	1.99 **c**
6	S. Degraded	75% + 25%	4.46 **a**
7	(T) Fertile Soil		3.83 **b**
C.V			4.86%
Level of significance			237.5**
Tukey 5% probability			0.38

Source: Own elaboration with the research results (2023)

The Tukey comparison test at 5% (0.38) determined that there were two types of soil with better results, partially degraded soil and degraded soil with 75%+25% bokashi and mountain microorganisms with averages of 4.40 and 4.46, respectively. The lowest values were found in treatments 1 and 4.

Analysis of averages of studied variables

Table 8 shows, as a whole, all the variables studied, which made it possible to define the recovery of soil fertility using bell pepper (Capsicum annum) as an indicator plant. Here is a summary of all the variables analyzed, which compared with the performance of the control treatment or fertile soil, establishes as outstanding treatments at the seedbed or preliminary bioassay level, when mixed in partially degraded and degraded soil in proportions of 75% + 25% of bokashi with mountain microorganisms. These results in their respective mixtures could be considered as basic preliminary information for the design of larger experiments in open fields

Table 8. Analysis of averages of project variables

TREATMENTS			VARIABLES EVALUATED (AVERAGE NUMBER)						
			GERMINATION PERCENTAGE	Nª FOLIOLOS		PLANT HEIGHT (CM)		ROOTS (CM)	
N°	SOIL TYPES	+ BOKASHI WITH MOUNTAIN MICROORGANISMS		15 Days	30 Days	15 Days	30 Days	Length (30 Days)	Diameter (30 Days)
1	S. Partially Degraded	25% + 75%	43.75	1.80	2.47	1,87	3.17	1.91	1.83
2	S. Partially Degraded	50% + 50%	57.29	2	2.28	1.87	3.14	2.20	2.01
3	S. Partially Degraded	75% + 25%	92.71	2	5.03	2.79	8.93	3.75	4.40
4	S. Degraded	25% + 75%	39.58	1.33	2.44	1.34	2.49	2.04	1.82
5	S. Degraded	50% + 50%	65.63	2	2.36	2.14	4.12	2.35	1.99
6	S. Degraded	75% + 25%	94.79	2	5.36	3.11	9.50	4.11	4.46
7	(T) Fertile Soil		93.75	2	4.39	3.84	7.09	2.91	3.83
Level of significance			15.51 NS	9.5*	577**	224**	217.83**	28.50**	237.5**
Tukey 5% probability			9.31	0.38	0.28	0.28	0.95	0.76	0.38
C.V (%)			4.87	7.49	2.88	4.13	6.37	10.18	4.86

Source: Own elaboration with the research results (2023)

Economic cost of treatments

Table 9 shows the economic estimation of the treatments, considering the best dose of 75% + 25% mixture. Bioremediation per hectare requires high costs, so to reduce them it is necessary to create incentive policies by the State to facilitate the procurement of materials for the production of bokashi compost with mountain microorganisms and reduce economic costs, in addition to implementing subsidies so that bioremediation of degraded soils is implemented and is not a utopia.

Table 9. Cost estimate for bioremediation

VARIABLES	Treatments (cost 1lb. of bokashi with mountain microorganisms $0.15)						
	1	2	3	4	5	6	7 (Witness)
Dose of bokashi with mountain microorganisms/treatment	0.90	0.60	0.30	0.90	0.60	0.30	0
Labor /Transportation (1/$7)	7	7	7	7	7	7	7
Total cost / treatment	7.90	7.60	7.30	7.90	7.60	7.30	5
COST / Hectare (10,000m2)	79000	76000	73000	79000	76000	73000	50000

Source: Own elaboration with the research results (2023)

Physical-chemical and microbiological analysis of degraded and bokashi soil samples with mountain microorganisms

For the comparison and discussion of the physical, chemical and microbiological characterization of bokashi with mountain microorganisms, it is important to reference and know the ranges of values established as a worldwide reference by FAO (2008) regarding the characteristics for fertile soils, which are shown in the following table.

Table 10. Parameters and ranges of reference values for fertile soils (WRB). FAO (2008)

PARAMETERS	V. REFERENCE
pH	6.5 - 7.5
EC (electrical conductivity)	0.0 - 4.0
MO (organic matter)	1.5 - 2.5
H (humidity %)	30.0 - 45.0
N (nitrogen %)	0.1 - 0.2
P (phosphorus %)	35.0 - 70.0
K (potassium %)	0.5 - 1.2
Ca (calcium %)	9.0 - 10.5
Mg (magnesium %)	1.5 - 2.5
S (sulfur %)	5.0 - 35.0
B (boron %)	0.5 - 2.0
Zn (zinc %)	Microelements
Cu (copper %)	Microelements
Fe (iron %)	Microelements
Mn (manganese %)	Microelements

Source: Own elaboration with FAO (2008) criterium

Table 11 shows the results of the physical-chemical analysis of degraded soil samples obtained from the study site (Comuna Los Bajos) and carried out at the INIAP laboratories (Ecuador), Tropical Experimental Station "Pichilingue". These results of the different parameters were categorized with respect to the universal parameters in: low, medium, normal and high.

Regarding the analysis for the degraded soil sample (Table 12), the pH parameter was 7.7, which is slightly alkaline, although the ideal would be neutral pH or 7.0, which establishes that it is not suitable for many crop species. The nitrogen and magnesium elements are medium and the fundamental elements required for plant nutrition, such as P, K, Ca, Mg and S are high, a situation that reveals a disturbance that could affect the cation exchange capacity (CIA) and thus the absorption of nutrients, while the micronutrients that plants need are minimal, Cu, Fe and Mn are at high levels and as such are antagonistic substances in the solution or soil colloids.

Table 11. Laboratory test values

V. REFERENCE (FAO)	x̄	LABORATORY TEST VALUES									
		DEGRADED SOIL (Los Bajos, Montecristi)					BOKASHI SAMPLE				
		VALUE	UNDER	MIDDLE	NORMAL	HIGH	VALUE	UNDER	MIDDLE	NORMAL	HIGH
pH 6.5 - 7.5	7.0	7.7				x (L.Al)	6.1	x			
E.C. 0.0 - 4.0	2.0						14.5				x
MO 1.5 - 2.5	2.0						12.1				x
H 30.0 - 45.0	37.5										
N 0.1 - 0.2	0.15	21 ppm		x			1.4				x
P 35.0 - 70.0	52.5	24 ppm				x	0.57	x			
K 0.5 - 1.2	0.85	0.55				x	0.93		x		
Ca 9.0 - 10.5	9.75	12.0				x	1.09	x			
Mg 1.5 - 2.5	2.0	6.4				x	0.61	x			
S 5.0 - 35.5	20.0	65.0				x	0.15	x			
B 0.5 - 2.0	1.25	1.6				x	31				x
Zn microelements		1.6	x				60				x
Cu micro elements		4.8				x	25				x
Faith microelements		11.0	x				933				x
Mn micro elements		9.0		x			259				x

Results of physical-chemical laboratory analysis of degraded soil samples and bokashi.

Source: Own elaboration with the research results (2023)

Table 11 shows the values for the physical-chemical analysis of the bokashi sample and Table 12 shows the microbiological analyses of the sample, which reveal the presence of microorganisms very necessary for soil life, such as aerobes, molds, yeasts and ***S. aureus***; as well as total coliform pathogens ***E. coli*** and ***Enterobacteria***, but in minimal quantities that do not exceed the established permitted limits, which are 1000 CFU. Based on the above, it is established that bokashi organic fertilizer with mountain microorganisms is an excellent alternative for the regeneration of degraded agricultural soils and does not present any health problems in general.

Table 12. microbiological analyses of the sample

MICROORGANISMS DETECTED	METHOD (TECHNIQUE)	UNIT	RESULT
Aerobes	SEM-MI	CFU/g	$23/10^{1}$
Total coliforms	SEM-MI	CFU/g	<10
Escherichia coli	SEM-MI	CFU/g	<10
Enterobacteriaceae	SEM-MI	CFU/g	<10
Molds and yeasts	SEM-MI	UPML/g	<10
Sthaphylococcus aureus	SEM-MI	CFU/g	<10

Source: Own elaboration with the research results (2023)

CONCLUSIONS AND FINAL REMARKS

Based on the results and discussion of this research, the following conclusions were drawn:

At the bioassay level, it was determined at the seedling stage, using bell pepper (C. annum) as an indicator plant and interpreting the results of the different variables evaluated, that the optimum dose to achieve a better recovery of degraded soils is when proportions of this were mixed in 75% + 25% of bokashi with mountain microorganisms.

In the physical-chemical analysis of the degraded soil sample issued by the laboratory, in general, it has a slightly alkaline pH of 7.7, which exceeds the ideal parameter that should be neutral or 7.0, elements nitrogen and magnesium in average values, elements P, K, Ca, Mg and S with high values and micronutrients Cu, Fe and Mn in high values; with these results it is concluded that this soil is not suitable for many crops since it does not favor the absorption of nutrients.

In relation to the results issued by the laboratory of microbiological analysis of bokashi compost, these indicate that it is generally suitable for soil regeneration since it has microorganisms necessary for soil life such as aerobes, molds, yeasts and S. aureus and pathogenic microorganisms such as total coliforms, E. coli and Enterobacteria in minimal quantities that would not have an impact on health.

In the economic estimation of the costs of the recovery process, they are considered to be low because the mixture with the best results obtained is based on applying 25% of bokashi with mountain microorganisms to degraded soils on a small scale; however, applying bokashi organic fertilizer with mountain microorganisms per hectare requires a higher cost.

Bioremediation per hectare requires high costs, so to reduce them it is necessary to create incentive policies by the State to facilitate the procurement of materials for the production of bokashi compost with mountain microorganisms and reduce economic costs, in addition to implementing subsidies so that the bioremediation of degraded soils is implemented and is not a utopia.

Limitations of the study and future research agenda

The study's main limitations include a failure to account for specific environmental variables, potentially limiting the generalizability of results, and a relatively short follow-up period, which may constrain understanding of the long-term impact of bokashi on soil regeneration. To address the identified limitations and advance understanding of the efficacy and applicability of bokashi in regenerating degraded soils, the following areas of research are proposed in Table 13:

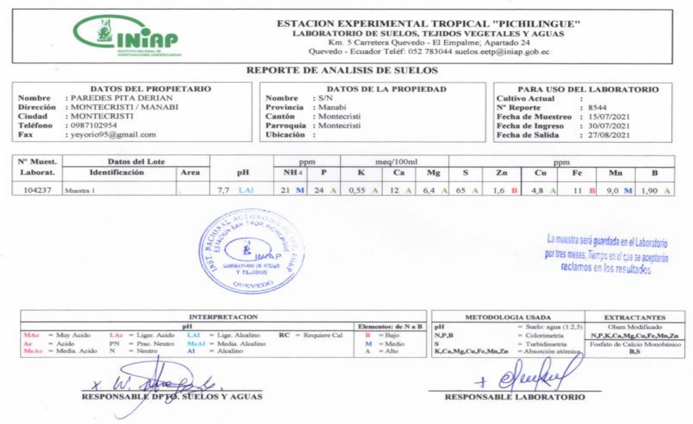
Table 13. Agenda for future research

Future Research Proposal	***Description***
Long-term Evaluation	Conduct long-term follow-up studies to better understand the effects of bokashi on soil quality and agricultural production under different environmental conditions.
Environmental Impact Assessment	Investigate the environmental impact of bokashi use, including effects on soil biodiversity, biogeochemical cycles, and greenhouse gas emissions.
Dosage Optimization	Perform experiments to determine optimal bokashi dosages and application frequency to maximize benefits in soil regeneration and agricultural productivity.

Source: Own elaboration with the research results (2023)

APPENDIX OF ILUSTRATIONS

1. Physical and chemical soil analysis



ESTACION EXPERIMENTAL TROPICAL "PICHILINGUE"
LABORATORIO DE SUELOS, TEJIDOS VEGETALES Y AGUAS
Km. 5 Carretera Quevedo - El Empalme; Apartado 24
Quevedo - Ecuador Teléf: 052 783044 suelos.eetp@iniap.gob.ec

REPORTE DE ANALISIS DE SUELOS

DATOS DEL PROPIETARIO
Nombre : PAREDES PITA DERIAN
Dirección : MONTECRISTI / MANABI
Ciudad : MONTECRISTI
Teléfono : 0987102954
Fax : yeyorio95@gmail.com

DATOS DE LA PROPIEDAD
Nombre : S/N
Provincia : Manabi
Cantón : Montecristi
Parroquia : Montecristi
Ubicación :

PARA USO DEL LABORATORIO
Cultivo Actual :
N° Reporte : 8544
Fecha de Muestreo : 15/07/2021
Fecha de Ingreso : 30/07/2021
Fecha de Salida : 27/08/2021

N° Muest. Laborat.	Datos del Lote		pH		ppm				meq/100ml						ppm											
	Identificación	Area			NH4		P		K		Ca		Mg		S		Zn		Cu		Fe		Mn		B	
104237	Muestra 1	.	7,7	LAl	21	M	24	A	0,55	A	12	A	6,4	A	65	A	1,6	B	4,8	A	11	B	9,0	M	1,90	A

La muestra será guardada en el Laboratorio por tres meses, Tiempo en el que se aceptarán reclamos en los resultados

INTERPRETACION
pH: MAc = Muy Acido; Ac = Acido; MeAc = Media. Acido; LAc = Liger. Acido; PN = Prac. Neutro; N = Neutro; LAl = Lige. Alcalino; MeAl = Media. Alcalino; Al = Alcalino; RC = Requiere Cal
Elementos: de N a B: B = Bajo; M = Medio; A = Alto

METODOLOGIA USADA
pH = Suelo: agua (1:2,5)
N,P,B = Colorimetria
S = Turbidimetria
K,Ca,Mg,Cu,Fe,Mn,Zn = Absorción atómica

EXTRACTANTES
Olsen Modificado: N,P,K,Ca,Mg,Cu,Fe,Mn,Zn
Fosfato de Calcio Monobásico: B,S

RESPONSABLE DPTO. SUELOS Y AGUAS
RESPONSABLE LABORATORIO

2. Physical and chemical analysis of Bokashi organic fertilizer

ESTACION EXPERIMENTAL TROPICAL "PICHILINGUE"
LABORATORIO DE SUELOS, TEJIDOS VEGETALES Y AGUAS
Km 5 Carretera Quevedo – El Empalme
Mocache – Ecuador Teléfono: 2783044 Ext. 201

Nombre del Propietario : PAREDES PITA DERIAN REYNALDO Telf 0987102954
Nombre de la Propiedad : S/N Cultivo: Abono
Localización : Parroquia — Cantón: Montecristi — Provincia: Manabi
Reporte N° : 8544-8566
Fecha de muestreo : 15-07-2021
Fecha de ingreso: 30-07-2021
Fecha salida resultados: 16/08/2021

RESULTADOS E INTERPRETACION DE ANÁLISIS ESPECIAL DE ABONOS ORGÁNICOS

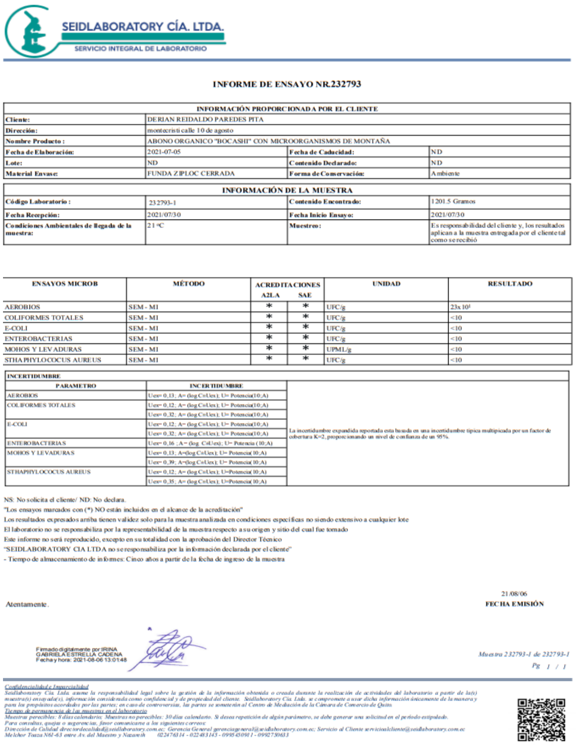
Número de Laboratorio	Identificación de las Muestras	pH	dS/m	Concentración %							ppm				
			CE	MO	N	P	K	Ca	Mg	S	B	Zn	Cu	Fe	Mn
76978	Muestra 2	6.1	14.5	12.1	1.4	0.57	0.93	1.09	0.61	0.15	31	60	25	933	259

Observaciones:

Dr. Manuel Carrillo Zenteno
RESPONSABLE DPTO

LABORATORISTA

3. Microbiological Bokashi organic fertilizer analysis



SEIDLABORATORY CÍA. LTDA.
SERVICIO INTEGRAL DE LABORATORIO

INFORME DE ENSAYO NR.232793

INFORMACIÓN PROPORCIONADA POR EL CLIENTE			
Cliente:	DERIAN REIDALDO PAREDES PITA		
Dirección:	montecristi calle 10 de agosto		
Nombre Producto :	ABONO ORGANICO "BOCASHI" CON MICROORGANISMOS DE MONTAÑA		
Fecha de Elaboración:	2021-07-05	**Fecha de Caducidad:**	ND
Lote:	ND	**Contenido Declarado:**	ND
Material Envase:	FUNDA ZIPLOC CERRADA	**Forma de Conservación:**	Ambiente

INFORMACIÓN DE LA MUESTRA			
Código Laboratorio :	232793-1	**Contenido Encontrado:**	1201.5 Gramos
Fecha Recepción:	2021/07/30	**Fecha Inicio Ensayo:**	2021/07/30
Condiciones Ambientales de llegada de la muestra:	21 ºC	**Muestreo:**	Es responsabilidad del cliente y, los resultados aplican a la muestra entregada por el cliente tal como se recibió

ENSAYOS MICROB	MÉTODO	ACREDITACIONES		UNIDAD	RESULTADO
		A2LA	SAE		
AEROBIOS	SEM - MI	*	*	UFC/g	$23x10^1$
COLIFORMES TOTALES	SEM - MI	*	*	UFC/g	<10
E-COLI	SEM - MI	*	*	UFC/g	<10
ENTEROBACTERIAS	SEM - MI	*	*	UFC/g	<10
MOHOS Y LEVADURAS	SEM - MI	*	*	UPML/g	<10
STHAPHYLOCOCUS AUREUS	SEM - MI	*	*	UFC/g	<10

INCERTIDUMBRE		
PARAMETRO	**INCERTIDUMBRE**	
AEROBIOS	Uex= 0,13 ; A= (log C±Uex); U= Potencia(10;A)	La incertidumbre expandida reportada esta basada en una incertidumbre típica multipicada por un factor de cobertura K=2, proporcionando un nivel de confianza de un 95%.
COLIFORMES TOTALES	Uex= 0,12; A= (log C±Uex); U= Potencia(10;A)	
	Uex= 0,32; A= (log C±Uex); U= Potencia(10;A)	
E-COLI	Uex= 0,12; A= (log C±Uex); U= Potencia(10;A)	
	Uex= 0,32; A= (log C±Uex); U= Potencia(10;A)	
ENTEROBACTERIAS	Uex= 0,16 ; A= (log C±Uex); U= Potencia (10;A)	
MOHOS Y LEVADURAS	Uex= 0,13; A=(log C±Uex); U= Potencia(10;A)	
	Uex= 0,39; A=(log C±Uex); U= Potencia(10;A)	
STHAPHYLOCOCUS AUREUS	Uex= 0,12; A= (log C±Uex); U=Potencia(10;A)	
	Uex= 0,35; A= (log C±Uex); U=Potencia(10;A)	

NS: No solicita el cliente/ ND: No declara.
"Los ensayos marcados con (*) NO están incluidos en el alcance de la acreditación"
Los resultados expresados arriba tienen validez solo para la muestra analizada en condiciones específicas no siendo extensivo a cualquier lote
El laboratorio no se responsabiliza por la representabilidad de la muestra respecto a su origen y sitio del cual fue tomado
Este informe no será reproducido, excepto en su totalidad con la aprobación del Director Técnico
"SEIDLABORATORY CIA LTDA no se responsabiliza por la información declarada por el cliente"
- Tiempo de almacenamiento de informes: Cinco años a partir de la fecha de ingreso de la muestra

21/08/06
FECHA EMISIÓN

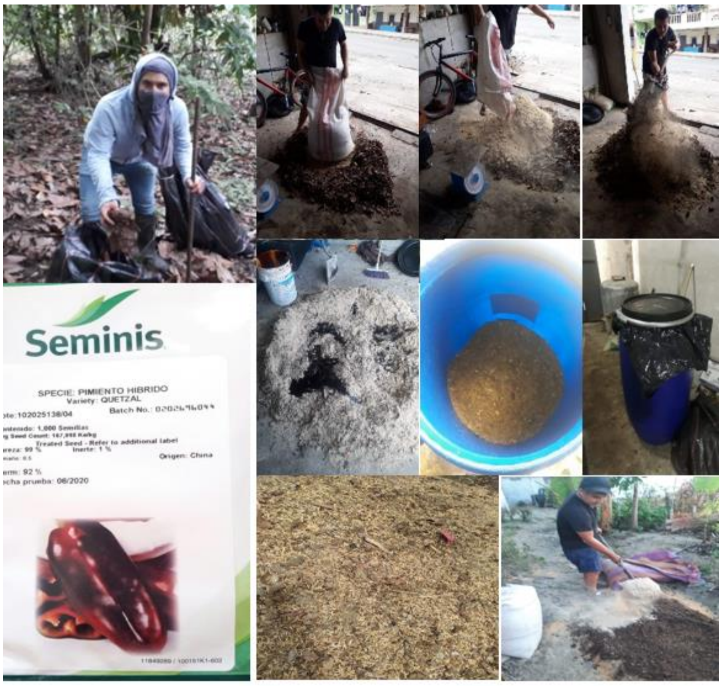
Atentamente.

Firmado digitalmente por IRINA GABRIELA ESTRELLA CADENA
Fecha y hora: 2021-08-06 13:01:48

Muestra 232793-1 de 232793-1
Pg 1 / 1

Confidencialidad e Imparcialidad
Seidlaboratory Cía. Ltda. asume la responsabilidad legal sobre la gestión de la información obtenida o creada durante la realización de actividades del laboratorio a partir de la(s) muestra(s) ensayada(s), información considerada como confidencial y de propiedad del cliente. Seidlaboratory Cía. Ltda. se compromete a usar dicha información únicamente de la manera y para los propósitos acordados por las partes; en caso de controversias, las partes se someterán al Centro de Mediación de la Cámara de Comercio de Quito.
Tiempo de permanencia de las muestras en el laboratorio
Muestras perecibles: 8 días calendario; Muestras no perecibles: 30 días calendario. Si desea repetición de algún parámetro, se debe generar una solicitud en el período estipulado.
Para consultas, quejas o sugerencias, favor comunicarse a los siguientes correos:
Dirección de Calidad directordecalidad@seidlaboratory.com.ec; Gerencia General gerenciageneral@seidlaboratory.com.ec; Servicio al Cliente servicioalcliente@seidlaboratory.com.ec
Melchor Toaza N61-63 entre Av. del Maestro y Nazareth 022476314 - 022483145 - 0995450911 - 0992750633

4. Collection of soil and microorganisms; preparation of microorganisms; pepper seeds; making bokashi



5. Materials for bioremediation; Soil mix + bokashi with microorganisms

6. Soil mix in germinating trays

7. Treatments after 15 and 30 days

8. Diameter of the root system

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Contribution of each author to the manuscript:

	% of contribution of each author					
Task	**A1**	**A2**	**A3**	**A4**	**A5**	**A6***
A. theoretical and conceptual foundations and problematization:	25%	25%	20%	5%	20%	5%
B. data research and statistical analysis:	25%	5%	20%	15%	20%	15%
C. elaboration of figures and tables:	25%	25%	5%	20%	20%	5%
D. drafting, reviewing and writing of the text:	20%	25%	25%	10%	10%	10%
E. selection of bibliographical references	25%	20%	20%	25%	5%	5%
F. Other (please indicate)	-	-	-	-	-	

***The inclusion of a sixth author was authorized under justification approved by the Editorial Board.**

Indication of conflict of interest:

There is no conflict of interest

Source of funding

There is no source of funding

Acknowledgments

There is no acknowledgments