Effect of treatment with *biochar* on the pH evolution in degraded forest soil

Rivoniony ANDRIANASOLO¹, Vonjison RAKOTOARIMANANA², Aimé Lala RAZAFINJARA³

¹Rivoniony ANDRIANASOLO, Faculty of Sciences, University of Antananarivo, Antananarivo, Madagascar

²Vonjison RAKOTOARIMANANA, Faculty of Sciences, University of Antananarivo, Antananarivo, Madagascar

³Aimé Lala RAZAFINJARA, Tsimbazaza Pedology Laboratory, National Center for Applied Research in Rural Development, Antananarivo, Madagascar

Abstract

As part of the Ambohimanga Rova natural forest restoration, classified as UNESCO (United Nations Educational Scientific and Cultural Organization) word heritage site in Madagascar, a research project to rehabilitate the degraded soil after fires in the western part of the forest is currently under way. This article focuses on the pH evolution of degraded soil treated with biochar combined with ox manure and useful microorganisms collected from healthy forest soil. The main objective was to increase soil pH after treatment. For that matter, the effect of dose and incubation period of biochar treatment in soil was studied during greenhouse semi-controlled condition. Phyllarthron madagascariense or Zahana, an endemic species of Madagascar was used as a test plant during the experiment.

The results show a significant effect of dose and incubation period of biochar in the substrate on the pH increase after treatment. Indeed, compared to the control, the pH value increases with the biochar at respectively 50 g, 100 g and 150 g. In addition, biochar incubated in the substrate one week before transplantation seems to influence the pH increase in soil. In contrast, incubation of biochar three weeks before transplantation has no significant effect on increasing soil pH.

The statistical test also shows a positive correlation between the pH evolution and the test plant development. **Key words:** *Biochar, pH, rehabilitation, degraded soil, Ambohimanga Rova, Madagascar.*

1. INTRODUCTION

Located 21 km north of Antananarivo, the capital city of Madagascar, the Royal Hill of Ambohimanga Rova is the former Palace of the Kings of Ambohimanga. It has been classified as a UNESCO World Heritage Site since December 2001 and occupies an area of about 59 ha. The natural forest formation consists of modified and original primary forest and currently covers an area of 16 ha (Ramanankierana and Randriambanona, 2012) [1].

Ambohimanga Rova natural forest is currently the only remnant of Madagascar Middle Altitude Evergreen Forest with *Weinmannia* and *Tambourissa*, closest to the capital, which characterises the Malagasy highlands.

Despite the persistence of various pressures and threats, notably illegal logging and fires, recent results of various studies and research have shown the ecological integrity of Ambohimanga natural forest. In 2007, a large part of the forest on the western slope of the hill was burnt, resulting in many losses of natural resources, including soil and vegetation (Kull, 2004) [2]. After the fire, invasive species such as *Lantana camara* (*Radriaka*) and *Phyllostachys aurea* (Bararata), colonise the soil, preventing the forest natural regeneration. Since 2014, the Cultural Site of Ambohimanga Rova Office (OSCAR), the current manager of this world heritage site, has been planting native forest species in the fire-damaged area. However, a slowdown in the growth and development of young plants has been noted and the mortality rate is quite high. Faced with this situation, a research project based on scientific studies is currently being carried out in the framework of the ecological restoration of the Ambohimanga Rova natural forest. Part of this research, which is the topic of this article, has focused on the greenhouse experimentation to study both the effect of treatment with *biochar* (charcoal powder) on the pH evolution of degraded soil and the growth and development of an endemic forest species *Phyllarthron madagascariense* (*Zahana*).

2. MATERIALS AND METHODS

2.1. Study site

The experiment was conducted at the Regional Directorate of Ecology, Environment and Forests Analamanga, Nanisana, Madagascar, in a transparent plastic greenhouse, during the hot period (summer), from November 2016 to April 2017.

2.2. Choice of test plant

According to Rafolo and Ravaonatoandro (2000), the abundance of *Zahana* in the Natural Forest of Ambohimanga Rova is considerable [3]. In addition, this plant also shows a high availability of seeds. *Phyllarthron* is an endemic genus of Madagascar belonging to the Bignoniaceae family [4]. Flowering begins in December and ends around February, whereas fruiting occurs between March and August. Diaspores dispersion is effected by barochory or zoochory [5].

Furthermore, the results of the floristic inventory carried out in 2012 and recently published by Randriambanona et *al.* in 2021 have confirmed the ubiquity of *Zahana* throughout the forest [6]. However, during forest native species plantation, suffering of young seedlings including *Zahana* was observed. This is the reason why *Zahana* is used as an indicator plant in this study.

Before experimentation, test plant production was carried out by direct sowing in the nursery at the *Silo National des Graines Forestières* (SNGF) in Ambatobe Antananarivo. The young plants were transplanted into the plastic pots five months after sowing and their transplantation for the experiment was realised five months after transplanting.

2.3. Treatment used in the experiment

2.3.1. Choice of treatment

Before the greenhouse experiment, an experiment in real conditions on the ground was carried out on the degraded part to the west of the natural forest of Ambohimanga Rova, between April and August 2016. Therefore, different treatments based on cattle manure, *biochar* and beneficial microorganisms from healthy forest soil were tested alone or in combination on the growth and development of the *Zahana*.

The best result was obtained from the combination of *biochar* with cattle manure and beneficial microorganisms. Greenhouse experimentation is nothing else than the optimization of the experimentation result obtained in the field.

2.3.2. Charcoal powder (biochar)

Biochar is a microporous carbon product resulting from pyrolysis [7]. The origin of *biochar* is from the agricultural practices of the inhabitants in Amazon who incorporated large quantities of charcoal into the soil as well as manures and other organic fertilizers to improve their crop yields [8]. The study by Biederman and Harpole in 2013 also showed the importance of burying vegetable charcoal in the soil to sustainably increase agricultural production [9].

Overall, soil fertility is improved by *biochar* addition explained by its buffering effect on soil pH and better nutrient retention in the soil resulting from the *biochar* ability to adsorb cations [10]. *Biochar* also influences the biological community of the soil [11].

In addition to improving soil fertility and carbon sequestration, two additional benefits can motivate the production of *biochar*: organic waste valorisation and recoverable energy production during pyrolysis [12], which contributes to reduce greenhouse gas emissions from fossil fuels [13].

In the case of Madagascar, charcoal, the main source of energy for the Malagasy household, is produced in a traditional technique by the same principle of thermo-degradation of wood without oxygen (pyrolysis).

The *biochar* used for the experiment was collected from a charcoal seller in the capital. It was a unsold scrap of *Eucalyptus robusta (Kininia)* charcoal powder.

2.3.3. Cattle manure

According to Rodriguez et *al.*, (2014), mixing *biochar* with organic fertilizers such as compost increases the pH and total C and N content of the soil [14].

The cattle manure used for the experiment was collected from the Rural Commune of Mahitsy, in Analamanga Region. It is an organic fertilizer composed of cow dung and decomposed rice straw.

In addition to its role as a biological fertiliser, cattle manure has been used as a source of carbon that serves as a substrate for micro-organisms.

2.3.4. Healthy soil

Healthy soil serves as a source of useful microorganisms for experimentation. It is a soil taken from the first twenty centimeters of the natural forest of Ambohimanga Rova, where there is high population of *Zahana*.

To avoid any risk of contamination, the healthy soil was directly stored in a previously disinfected plastic bag before its use for the experiment.

2.3.5. Degraded soil

The substrate used for the experiment is a degraded soil after the passage of fires, collected in the western part of the natural forest of Ambohimanga Rova where the real experimentation in the field was previously carried out.

By walking and following the diagonals of a previously determined surface, five samples of soil were taken approximately every 5 m. To obtain a homogeneous substrate, the soil samples were mixed beforehand and then stored in a clean plastic bag before being used for the experiment.

2.4. Experimental design

The randomised complete block design (Fisher blocks) was used as the experimental design. The allocation of treatments to each block was done randomly.

Two factors with 8 treatments and 3 repetitions were studied during the experiment, including :

<u>Factor 1</u>: Incubation period of treatment with *biochar* in the substrat before planting. This is incubation period of *biochar* combined with cattle manure and useful microorganisms in the soil before planting the test plant. This factor has two modalities:

- Modality 1: Application of treatment with *biochar* one week before planting (P1);
- Modality 2: Application of treatment with *biochar* three weeks before planting (P2).
- Factor 2: biochar dose in the treatment, with four modalities:
 - Modality 3: 50 g of *biochar* (D1);
 - Modality 4: 100 g of *biochar* (D2);
 - Modality 5: 150 g of *biochar* (D3);
 - Modality 6: Control without biochar (D0).

The treatments distribution in each block was done randomly.

The combination and dose of the treatment used for the experiment are as follows:

P1D0: 5000 g of degraded forest soil without treatment, incubated 1 week before planting (control 1);

P1D1: 5000 g of degraded forest soil with 50 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 1 week before planting;

P1D2: 5000 g of degraded forest soil with 100 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 1 week before planting;

P1D3: 5000 g of degraded forest soil with 150 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 1 week before planting;

P2D0: 5000 g of degraded forest soil without treatment, incubated 3 weeks before planting (control 2);

P2D1: 5000 g of degraded forest soil with 50 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 3 weeks before planting;

P2D2: 5000 g of degraded forest soil with 100 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 3 weeks before planting;

P2D3: 5000 g of degraded forest soil with 150 g of *biochar*, 250 g of cattle manure and 250 g of healthy forest soil containing useful microorganisms, incubated 3 weeks before planting.

24 plastics buckets (6 liter) were used in the experiment for the distribution of each modality.

2.5. Experiment Follow-up

Two types of monitoring were carried out during the experiment: monitoring of the test plant response and monitoring of the dynamics of the chemical elements in the soil, including the pH.

2.5.1. Test plant monitoring

The test was carried out for five months. Each month, the height of the plant was measured and at the same time the number of leaves was counted.

The number of the small leaf newly formed was counted separetely and adopted as the main indicator of the test plant growth and development.

If necessary, each plant was watered with an equal quantity of water.

In the greenhouse, the daily temperature and humidity were monitored.

2.5.2. Chemical elements dynamics monitoring

The monitoring of soil chemical properties was carried out monthly by analysis at the soil laboratory of the National Center for Applied Research in Rural Development Tsimbazaza for five months.

The two types of monitoring are realised in the same time and the data obtained during the experiment were processed with R and Excel software.

2.6. Data processing and analysis

The data obtained during the experiment were recorded manually on a monitoring sheet and then stored in an Excel file so that they could be processed and analyzed.

Excel software was used to obtain interpretable figures and R software for statistical data processing.

2.6.1. Normality Test

The Shapiro & Wilk test was used to verify normality during statistical testing.

2.6.2. Statistical comparison test

If normality is respected, the variants studied are compared by an analysis of variance (ANOVA). To compare the variants with each other, the parametric test is used.

In the case where normality is not respected, the non-parametric test will be used.

A correlation test was also carried out in order to measure the statistical dependence between pH and other variables, in particular height and number of leaves of the test plant.

2.6.3. Current acidity (soil pH)

The current acidity of a soil is expressed by the pH of the soil solution. It is measured by placing 25 g of airdried soil (2 mm diameter) in an amount of 25 ml of distilled water. After stirring for 30 minutes, the pH is measured using a combined electrode, connected to an ORION model 410 pH meter.

The pH value obtained in this method characterizes the overall activity of the free H⁺ ion in solution, in a system comparable to the natural environment.

3. RESULTS AND INTERPRETATION

3.1. Effects of the two variables (Period and Dose) on pH

Because normality is not respected in the test, the non-parametric 2-factor analysis of variance test: "Scheirer-Ray-Hare test" was used to determine the effects of the two variables (Period and Dose) on the pH and the following table shows the "P-value" of each variable

Table 1:	Scheirer-Ray-Hare test
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Variables	P-value
Period	0.02435
Dose	0.00802
Period/Dose	0.81993

The P-value is less than 0.05 for the two variables tested, thus, their effect on pH is statistically significant. On the other hand, the interaction between the two variables does not show a significant effect on pH (P-value=0.81993 > 0.05).

To find out which of the two variables tested influences the pH, the post-hoc test was used.

3.1.1. Factor Period

The following table shows the result of the permutation Student's test.

Table 2: Average	pH for P1	and P2 after	experimentation

Modality	Period 1	Period2
Average pH	6.22	5.99

P-value = 0.01

After experimentation, compared to the initial pH (5.90), the average pH rises to 6.22 for P1 while it remains almost unchanged (5.99) for P2. The incubation of *biochar* one week before planting seems to accelerate the increase of pH in the soil.

The statistical test shows that P-value = 0.01 (less than 0.05) indicating that the difference in value between the two modalities is significant.

3.1.2. Dose Factor

Figures 1 and 2 below show the evolution of the average soil pH value, depending on the *biochar* dose used after the experiment.



Chart 2: Soil pH evolution corresponding to P2

In general, the pH value for P1 and P2 tends to increase with the *biochar* dose. For each treatment, this value is higher than 6 except for the two controls without *biochar* P1D0 (pH=5.96) and P2D0 (pH=5.75), a value almost identical to the initial pH of the soil before experimentation (5.9).

Dunn's test was used to determine the effect of the Dose on the soil pH. The results are as follows:

Biochar dose	Grouping	
D0 (Control without <i>biochar</i>)	a	
D1 (50 g biochar)	ab	
D2 (100 g biochar)	b	
D3 (150 g biochar)	b	

Groups sharing the same letters are not significantly different (alpha = 0.05)

According to this result, compared to the control, the effect of D1 corresponding to 50 g of *biochar* has no significant difference on the evolution of the pH of the soil. It is from the dose D2 corresponding to 100 g of *biochar* that the difference relative to the control without *biochar* becomes statistically significant. However, between D2 and D3, the effect of *biochar dose* is not statistically significant on the evolution of soil pH.

The following table shows the result of the correlation test between soil pH, number of leaves and height of the test plant

Correlation	Old leaf	Height	New leaf	pH
Old leaf	1	11		
Height	0.7710	1		
New leaf	0.7045	0.5426	1	
рН	0.3167	0.3414	0.2304	1

 Table 4: Spearman test

The statistical test shows a positive correlation between the evolution of soil pH and the growth and development of the test plant (height and number of leaves) during the experiment.

4. DISCUSSION

Depending on their nature and their action, human beings have a more or less influence on the morphology of soils, their physical qualities, their chemical characteristics, their dynamics and their fertility [15]. According to André and Marc (1991), plants take water and nutrients from the soil, similarly, the oxygen consumption and the carbon dioxide production during root respiration induces a dynamic of exchanges within the gas phase of the soil [16]. According to Alabouvette and Cordier (2018), the root of the plant greatly modifies certain soil characteristics: pH, water potential, oxidation-reduction potential, and brings, via root exudates, many elements, in particular sugars and organic acids, which stimulate microbial activities and their interactions [17].

The results obtained during this experiment confirm all these ideas mentioned by various authors on the dynamic soil-microorganism-plant system functioning. Indeed, the beneficial effect of treatment with *biochar* in favor of the increase in pH in the soil is conditioned by the presence of the plant through its roots in the rhizosphere. Therefore, treatment application should be carried out during plantation in order to immediately stimulate the dynamism of the soil-microorganism-plant system in favor of plant growth and development.

With its alkalizing nature, *biochar* indirectly contributes to improving the pH of acidic soil. The results obtained by Rodriguez-Vila et *al.* (2014) confirms that the mixture of *biochar* and compost incorporated into a copper mine rejection increases the pH [14]. Moreover, pH being an important control factor of several soil biological processes, its modification should affect them [18]. According to Coppenet (1980), nutrient availability usually depends on soil pH. Between pH 6 and 7, most elements are at their maximum assimilability except magnesium,

molybdenum and iron. However, a closer examination indicates that many other factors than the pH participate evenly, like : humidity, light, temperature, richness in fertilizing element [19].

The results obtained during this experiment also show the importance of the dose of *biochar* on the soil pH evolution. At low doses, the effectiveness of *biochar* on increasing soil pH is not significant. Moreover, in their study, Fellet et *al.* (2011) characterized four substrates obtained after mixing mine residue at 0, 1, 5 and 10% of *biochar* and affirm that pH increased with *biochar* rate [20].

In general, the application of *biochar* in the soil requires a preliminary eco-pedological study.

CONCLUSION

The problems of the Malagasy natural forest degradation imply the search for techniques and strategies allowing their restoration while respecting the ecological processes of the whole ecosystem. This study showed the effect of treatment with *biochar* on the pH evolution of the degraded forest soil of Ambohimanga Rova after fire. The experimental results show that the dose and incubation period of *biochar* in the soil have a significant effect on the increase of pH after treatment with cattle manure and beneficial soil microorganisms. However, the pH dynamics in the soil were not significantly influenced by their interaction. Among the tested doses (D1: 50 g *biochar*, D2: 100 g *biochar*, D3: 150 g *biochar*) a respective increase of the pH value was observed compared to the control without *biochar*. Nevertheless, the statistical test showed that Dose1 (50 g *biochar*) has no significant effect on soil pH compared to the control. Furthermore, this experiment highlights the importance of the interaction existing between soil and plant system. The effect of the incubation time of the *biochar* in the soil on the pH increase seems to be conditioned by the presence of the root system of the test plant in the rhizosphere.

Statistical test show that there is a positive and significant correlation between the soil pH evolution and the growth and development of the test plant. However, *biochar* should be accompanied by a source of organic matter and useful microorganisms for its effects to be beneficial to the soil-plant system.

Before, *biochar* is considered as a solid waste dispersed in most regions in Madagascar. Because of its alkalizing character, it can currently replace the role of limestone amendments (more expensive), in order to raise soil pH. Nevertheless, much remains yet to be discovered concerning the roles and characteristics of *biochar* on soil fertilization. Thus, further studies are essential especially on the effect of the dose of *biochar* used, and its effect with the microbial activities.

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