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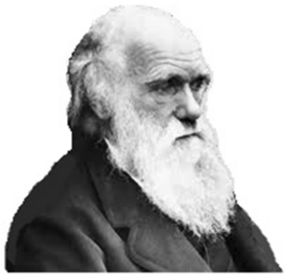

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Restoration of Charcoal-Site Soil Properties on Modified Land Models through Bioremediation Potential of Peanut (*Arachis hypogaea* L.)

Siegfred M. Mejico[1], Renier B. Garcia[2], Joanne A. Familara[3]

Bansud National High School, Regional Science High School for MIMAROPA,
Bansud, Oriental Mindoro, 5210, Philippines

[1-3]E-mail address: sieg.mejico@gmail.com , renierg76@gmail.com ,
joanne_familara@yahoo.com

ABSTRACT

Burning can substantially change vegetation status, and enhance the soil erosion of previously productive areas (Santin & Doerr, 2016). This is why bioremediation techniques have been developed to accelerate the recovery of soil properties. In this four month-long study, the bioremediation potential of peanut plants was tested in restoring charcoal-site soil properties. The experiment had three set-ups, a positive control and a control that had undergo pyrolysis for a week and then was planted with peanuts. The moisture content and pH, Nitrogen, Phosphorus and Potassium (NPK) of all soils were tested with a soil kit from the Department of Agriculture, before and after pyrolysis and after four months, which was also validated by the Bureau of Soils. In the experiment, plant morphology, mainly height, number of leaves and leaf area index (LAI) showed a linear increase all throughout the study, unlike the number of flowers. These were sporadic, with first appearance on week 4, and had a peak of 16 flowers at week 10 from 14 pods. With regard to soil properties, planting peanuts made the soil alkaline (7.3 - up from 5.8 pH after pyrolysis), while Nitrogen content increased from low to medium. In contrast, Phosphorus levels stayed high all throughout the study, while Potassium levels decreased after the pyrolysis and become deficient after four months. Moreover, the moisture content increased from 3.905 after pyrolysis, to 12.69. These results provide evidence that the peanut plant has bioremediation potential on charcoal-site soils after a four month long treatment period.

***Keywords*:** bioremediation potential, peanuts, fire-induced perturbation, charcoal site soil, physicochemical properties, peanut, *Arachis hypogaea*

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1. INTRODUCTION

Mankind has impacted on soils from its early days in many different ways, with burning being the first human perturbation at landscape scales; where fire was long been used as a tool to fertilize soils and control plant growth, but it can also substantially change vegetation, enhance soil erosion and even cause desertification of previously productive areas (Santin & Doerr, 2016).

In the Philippines, 426, 000 tons of wood charcoal is produced on the year of 2016, as reported by the United Nation – Food and Agriculture Organization. This shows that there is still a huge demand for wood fuels in our country, especially in the Southern region, where most charcoal producers use the traditional ground-pit method of production (ARECOP Team, 2006). The production of charcoal via a pyrolysis process is one of the human perturbations, as it generates a temperature of 400 ºC, unless external heat is provided (Food and Agriculture Organization of the United Nations, 1987). This is supported by the result of Hardy B., Cornelis J., Houben D., Lambert R., and Dufey J. on 2016, where they state that, regardless of soil type, the kiln topsoil is greatly depleted in exchangeable K^+ and available P. In addition, charcoal production is often perceived to have devastating ecological and environmental effects, making the public forestry institutions, government and non-government organizations to be particularly concerned about these charcoal-related impacts. Furthermore, emissions of greenhouse gases from charcoal production in tropical ecosystem in 2009 are estimated at 71.2 million t for carbon dioxide and 1.3 million t for methane.

Alongside with greenhouse gases, fire–induced perturbations involve changes in the physical, mineralogical, chemical and biological properties of soil (Certini, 2005). Studies have shown that severe burning has drastic effects on soil texture, color, mineralogy, and other soil properties (Sertsu and Sanchez, 1978; Ketterings et al., 2000; Oguntunde et al., 2004). There are many immediate effects of fire on soil in which one of these is the release of several pyrolytic substances as polycyclic aromatic hydrocarbons (PAHs) which are toxic and have a tendency to accumulate (Vila-Escale et al., 2007). Fires also have been reported to cause increased runoff and erosion losses due to removal of vegetation and water repellency resulting in reduced infiltration and increased sediment loads in rivers (Inbar et al., 1998).

With these effects of fire on soil, the soil itself can recover naturally, but due to its slow recovery, bioremediation techniques have been developed combining microorganisms with plants to accelerate the recovery of soil properties, increase microbial biomass and accelerate plant recolonization. The general process is referred to as phytoremediation, whereas the process is known as rhizoremediation when plants with root associated microorganisms. (Kuiper et al., 2001; 2004; Wood, 2008; Segura et al., 2009; Segura and Ramos, 2012). Many studies had used bioremediation and their common plant subject is a legume, because the ability of these plants to fix nitrogen makes it grow better on nitrogen-depleted soils and restores soil fertility of such soils, which is important in farming systems where the application of fertilizers is limited (Githae E., Gachene K., & Njoka J.,2011).

Arachis hypogaea (Peanut) plant is an annual herbaceous plant of the Fabaceae or Legume family that is drought tolerant and is considered one of the major field legumes grown by local farmers. It seeds are planted on hill or drill method that can grow for 20 inches tall on four months period. It develops parted flowers that are yellow and self-fertile, that elongates after pollination and pushes the developing pods into the ground. (Bureau of Plant Industry, n.d.)

Thus, the current study focused on bioremediation of burnt soil after a pyrolysis process with the use of Arachis hypogea (peanut) plant for four months and determine the physicochemical properties, specifically the pH and NPK content of soil before and after the pyrolysis process, the physicochemical properties of the treated soil compared to the pre-pyrolysis property of the soil and determine the difference on the physicochemical property of the experimental and control set-up after the four months period, in comparison to the pre-pyrolysis property of the soil. Moreover, the height, number of leaves, leaf dimension and number of flowers of the plants will be observed every week after the first three weeks and also at the end of the study as well as the number of pods.

The findings of the study will be beneficial on the agricultural sector, as this suggest a simple treatment on burnt soil that would also give income to our farmers as they treat the soils.

2. MATERIALS AND METHODS

2. 1. Preparation of Land Models

LM_1 and LM_2 in the earth pit with woods placed and set fire above, for pyrolysis

The pit was carefully covered with soil and banana leaves, ensuring there is no presence of smoke

The earth pit was opened after a week.

Twelve land models were made of iron sheets with a dimension of 10×10×20 inches with little holes at the base. A total of thirty-six (36) kg of loam soil was collected, were two (2) kg of each land model, and 34 kg of loam soil were added on the prepared land models.

From the prepared land models, three set-ups were made with four replications per set-up namely: LM1- experimental, the land model that undergoes pyrolysis and planted with peanuts; LM2 = controlled, the land model that undergo pyrolysis and LM3 = positive control, the land model that did not undergo pyrolysis Figure 1). A pit with a dimension of 1m × 1m × 0.5m was made and eight of the land models, LM1 and LM2 specifically, were placed in the pit and underwent pyrolysis for one week. After pyrolysis the pit were opened and the land models were removed and allowed to cool for three days before further test was done.

From the three prepared land models only LM1 was planted with peanuts following the method of Camilotti, F. Silva, A. R and Marques, M. O., (2012).

Nine peanut seeds (ps) was planted in each land model in LM1 using the “hill method “where the seeds were placed 2 inches beneath a hill while LM2 was left unplanted. All the three land models received equal amount of water and sunlight for the whole experimentation period. When the peanut plants reached a height of six (6) inches, soil around was cultivated for the pegs to penetrate easily. On the third week, each land model in LM1 was kept with only a seedling until the fourth- month period.

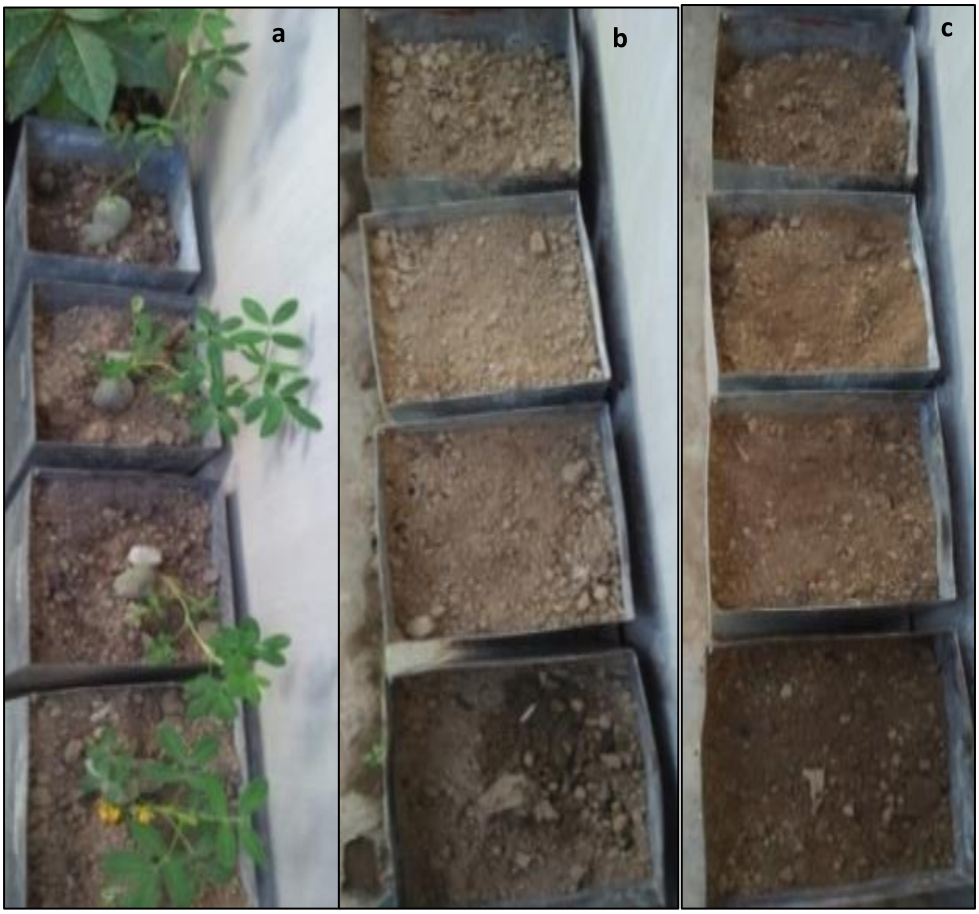


Figure 1. Three land model set-ups with four replications. (a) LM1; (b) LM2 and (c) LM3.

2. 2. Plant Morphology

Evaluation of the plant morphology such as height, leaf dimension, number of leaves and number of flowers, was based on the study of Pizarro-Tobias et al. (nd). All indicators for plant morphology were measured every week. A tape measure was used to measure the height of the peanut plant while ruler was utilized for the leaf dimension (length and width). The number of leaves and flower was also counted. After the four months periods, the plant was pulled out and the number of pods was counted.

2. 3. Soil Sampling

Soil samples were collected following the composite method as stated in the study of Githae E., Gachene K., and Njoka J. (2011). Soil samples from a depth of seven inches from each set-up were obtained using a trowel. Only the middle inch of the soil stuck on the trowel was kept. Composite sampling strategy was utilized, where four samples from one land model was randomly generated and combined on a plastic container to form a composite sample.

2. 4. Physicochemical Evaluation

Soil Test Kit was obtained from Integrated Laboratory of MIMAROPA, Department of Agriculture to determine the level of macronutrients (NPK) present and pH of the soil. Physicochemical screening was done before and after the land models underwent pyrolysis and after the four months period, and was then verified by the Provincial Bureau of Soil, in Calapan City.

2. 5. Nitrogen Content

Soil sample for each land model was placed on the designated test tube up to the scratch, and 24 drops of solution N was added on the soil sample. It was then swirled for 30 times and let it rest for five minutes and swirled again for 30 times and let it rest undisturbed for 30 minutes. The result color was compared to the color chart given by the Bureau of Soil.

2. 6. Phosphorus Content

Soil sample for each land model was put on the designated test tube up to the scratch and 24 drops of P was put and four drops of P1 was added. It was then swirled for a minute and rest for three minutes, and then swirled again for a minute. The test tube was then set aside undisturbed for five minutes. After five minutes, a foil wrapped on a stick was used to stir the solution, without disturbing the soil below the test tube, for a minute. The solution was then set aside for two minutes and stirred again with the used of the same plastic stick with foil, still not disturbing the soil. The resulting color was then matched to the given color chart form the Bureau of Soils.

2. 7. Potassium Content

Soil sample for each land model was put on the designated test tube up to the scratch and 24 drops of K solution was poured on the tube and eight drops of K1 solution was added. It was then swirled gently for a minute and set aside for three minutes. After three minutes, the test tube was then swirled again for a minute and then let it rest, undisturbed for five minutes. After five minutes, tilt the test tube partially, and carefully add 12 drops of K2 on the solution one at a time, and then set aside undisturbed for two minutes. The presence of cloudy yellow layer above the solution and its thickness was used to determine the level of Potassium on the soil, where a partial layer indicates a Sufficient, one-fourth layer indicates Sufficient+, and a layer that is almost one-half of the solution indicates a Sufficient++ of Potassium content. On the other hand, the absence of the cloudy yellow layer means that there is a deficient of Potassium on the soil.

2. 8. pH Level

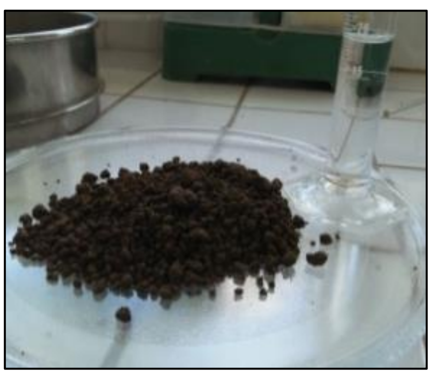
Soil sample for each land model was put on the designated test tube up to the scratch, and 12 drops of CPR was put on the soil. It was then swirl for 20 times and let it rest for two minutes. Then it was then swirled again for 20 times and let the test tube stand undisturbed for five minutes. The resulting color of the process was match to the given indicator the Bureau of Soil.

Materials like chemicals, beakers, test tubes, aluminum foils and gloves were then prepared.

Testing the pH and NPK of the sieved soil up to the designated mark, provided by the Bureau of Soil.

Soil Properties were verified by the Bureau of Soil in Calapan City

2. 9. Moisture Content



Wet sample was obtained with combining 100 g. of soil and 20 ml of water.

Samples were heated in a microwave oven for 20 minutes at high setting.

Sample was weight and reheated for 5 minutes, until the weight is constant.

Tavares M.H. and others in 2008 concluded that microwave oven may be used as an alternative to soil content measurement, and with that 100 grams of composite sample was mixed with 20 ml of water, in order to create a wet sample. It was then heated on a microwave oven for 20 minutes, with high as a setting. Then the heated sample was weighed and then stirred carefully. It was then reheated for five minutes. The sample was stirred to ensure that there will be no overheating. The weighting, stirring and reheating process was repeated until a constant weight was achieved and recorded as weight of dry material. The moisture content of the soil samples was determined using the formula below.

$$\text{Moisture content} = \frac{Weight\ of\ Wet\ Material - Weight\ of\ Dry\ Material}{Weight\ of\ Dry\ Material}\ x\ 100$$

3. RESULTS AND DISCUSSION

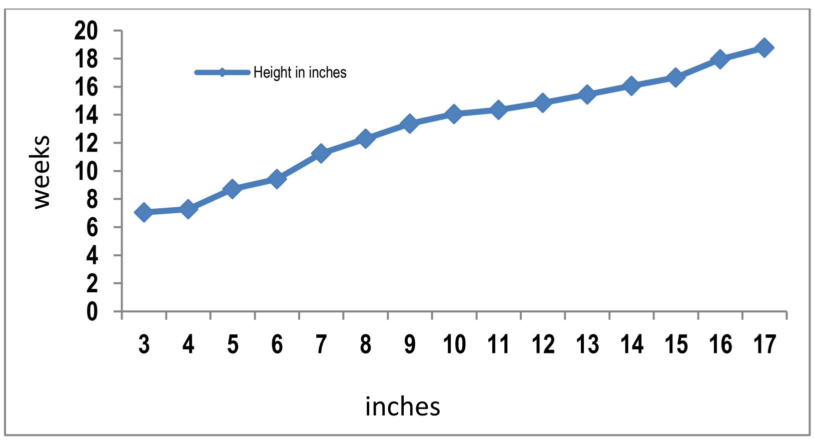


Figure 2. Shows the height of *Arachis hypogaea* (Peanut) on soil subjected to pyrolysis at 400 °C. Each data point corresponds to the mean of the four replicates in LM1 recorded weekly.

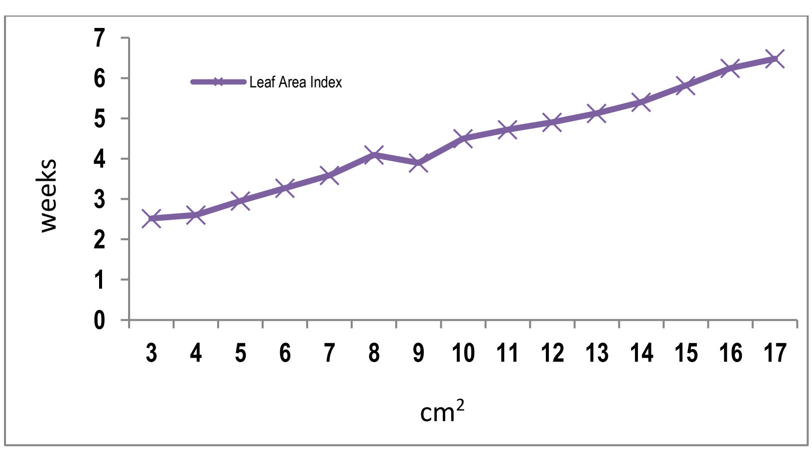


Figure 3. Shows the leaf area index of *Arachis hypogaea* (Peanut) on soil subjected to pyrolysis at 400 °C. Each data point corresponds to the mean of the four replicates in LM1 recorded weekly.

The morphology of the plant samples such as height, number of leaves, number of flowers and leaf area index was recorded every week and summarized as was shown below.

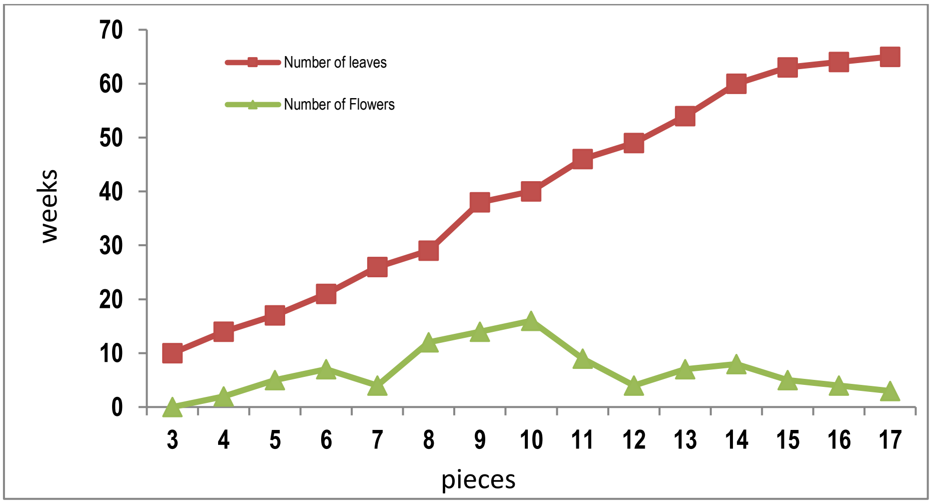


Figure 4. Shows the number of leaves and flowers of *Arachis hypogaea* (Peanut) on soil subjected to pyrolysis at 400 °C. Each data point corresponds to the mean of the four replicates in LM1 recorded weekly.

According to the study of Stancheva I., et.al, in 2014, the success of bioremediation depends on plant growth rates, as the significant increase in the parts of the plants, aboveground; suggest that the plant sample used could be successfully used for bioremediation.

The height of the peanuts had a linear growth without any significant decline, just like the number of leaves which also showcase a linear increase, throughout the four-month period. This linear growth of peanut plants signifies that the plant is developing normally. In terms of leaf area index (LAI) is an important structural property of vegetation since leaf surfaces are primary border of energy and mass exchange. The figure above shows a direct relationship between plant height and LAI. This result was affirmed by Davis and Mack (1991) stating that the plant height is a good predictor of leaf area, as supported by their result that shows no non-constant variances for the linear model of plant height and LAI.

The basic reproductive units of groundnuts are the flowers, and in this study, the first flower was seen on week 4 (28 days after planting). This behavior was also reported on the studies of Prasad, Craufurd and Summerfield (2000). Furthermore, Ramanatha Rao (1988) stated that groundnut plants started flowering about 30 - 40 days after planting and an average of 16 flowers was recorded to develop on the peanut plants within the testing period

Moreover, the weekly flower count showed a sporadic flowering pattern from first week to fourth week, where there was a low flower number at first week, with a gradual increase to a maximum at second and third week flower count. However, a decrease in flower number was observed in fourth week compared to second and third week. This observation was also reported in the study of Kaba et.al in 2014.

It was also observed in this study that the peak of flower development was recorded on week 8- week 10. The flower growth had decrease, but still a sporadic pattern was visible.

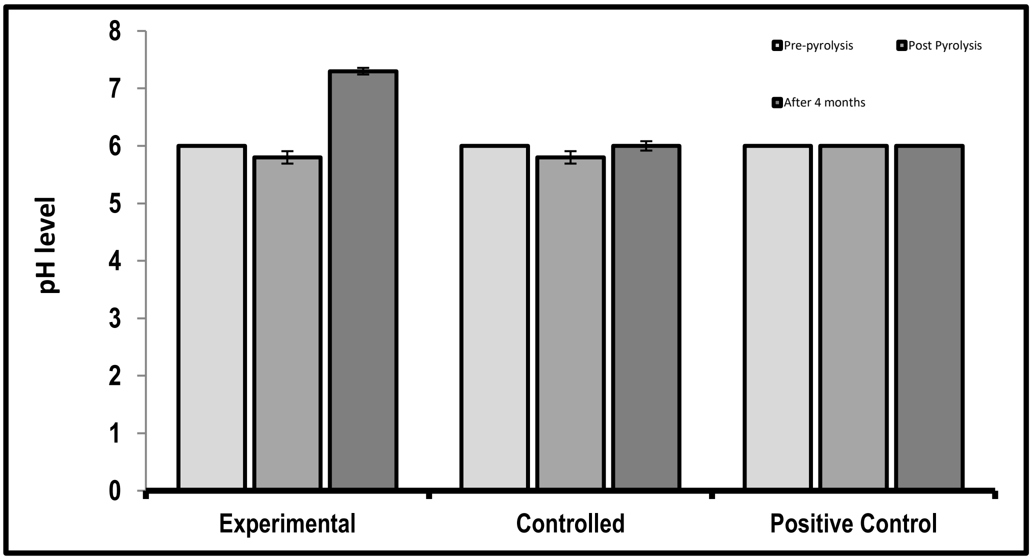


Figure 5. Soil pH level of the three set-ups recorded at specific interval. pH level was measured before the pyrolysis, after the pyrolysis and after the four – month experimentation period.

Soil pH or soil reaction is an indication of the acidity or alkalinity of soil and is measured in pH units. There is a slight decrease in the pH level of after the soil samples undergo pyrolysis. Some studies stated that after fire, soil pH has been found to increase, because of the release of alkaline ions from production of potassium (K) and sodium (Na) oxides, hydroxides and carbonates in the ash, but this was not shown on the study. This is because of the process of pyrolysis, an incomplete combustion which does not produce much ash, compared to an open combustion like forest fires, and as stated by Xue L., Li Q., & Chen H., in 2014, that the main reason for increased nutrient availability are the generation of ashes and increased mineralization. The soil pH can influence plant growth by its effect on activity of beneficial microorganisms. Bacteria that decompose soil organic matter are hindered in strong acid soils. This prevents organic matter from breaking down, resulting in an accumulation of organic matter and the tie up of nutrients, particularly nitrogen, that are held in the organic matter. Soil pH can also affect plant growth in several ways. Bacteria that change and release nitrogen from organic matter and some fertilizers operate best in the pH range of 5.5 to 7.0 making this the optimum pH range. Groundnut nodulation, growth and nitrogen fixation was optimal in the pH range from 5.7-6.3

It can be gleaned from the pH result (Figure 3) that the soil samples used in making the land models were generally acidic ranging from 5.8 to 7.3. The 7.3 pH level was recorded on land model planted with peanut after the fourth month period. This outcome can be attributed to the interaction of bacteria present in the peanut plant and the acidity of the soil. It was discussed in the study of Yan, Schubert & Mengel (1996) that during the cultivation of legumes, soil is acidified due to proton release from roots. As a consequence of proton release, plants accumulate organic anions which may, if returned and decomposed in the soil, neutralize the soil acid. A neutral pH now is ideal for microbial action that produces chemical changes in soil, making nitrogen, sulfur and phosphorus more available. In addition, accumulation of organic anions can be strongly affected by environmental conditions such as supplied nitrogen forms, solution pH, and carbonate concentration of soil solutions. Legumes accumulate higher amounts of organic anions than grasses.

Table 1. Level of Macronutrients Present in the three set-ups.

	Experimental			Controlled			Positive Control		
	Pre	Post	4th month	Pre	Post	4th month	Pre	Post	4th month
Nitrogen (N)	Low	Low	Medium	Low	Low	Low	Low	Low	Low
Phosphorus (P)	High	High	High	High	High	High	High	High	High
Potassium (K)	S+	S	D	S+	S	D	S+	S+	S+

These important nutrients are absorbed by plants at varying levels of effectiveness based upon the soil's acidity level. Nitrogen, potassium, sulfur, magnesium and iron are available along a broader range of acidity, while the availability of phosphorus, manganese, copper, boron and zinc lessens as alkalinity increases (Naples Daily News).

The table below shows the level of macronutrients present in the soil samples used in the three set-ups. Level of nitrogen is consistently low (<2.0%) on the three set-ups during pre and post pyrolysis but increases to medium (2-4.5%) on the fourth month in the experimental set-up where peanut plant was present. The low nitrogen content was validated by the Department of Agriculture, Integrated Laboratory of MIMAROPA (2018). The agency tested the soils on the provinces of Mindoro and the result of their study shows that all soils on the island have low Nitrogen content. Furthermore, the increases of nitrogen can be attributed to the presence of bacteria responsible for fixation. Nitrogen fixation, any natural or industrial process that causes free nitrogen (N2), which is a relatively inert gas plentiful in air, to combine chemically with other elements to form more-reactive nitrogen compounds such as ammonia, nitrates, or nitrites. Two kinds of nitrogen-fixing microorganisms are recognized: free-living (non-symbiotic) bacteria, including the cyanobacteria (or blue-green algae) Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia, and Clostridium; and mutualistic (symbiotic) bacteria such as Rhizobium, associated with leguminous plants, and various Azospirillum species, associated with cereal grasses (Encyclopedia Britannica).

In this study, high (> 10 ppm) phosphorus (P) content of the soil was not directly affected by heat or the presence of the peanut plant since it generally unresponsive to P. However, phosphorous is most critical essential element after nitrogen in influencing plant growth and production throughout the world. Amongst the more significant functions and qualities of plants on which phosphorous has an important effect include photosynthesis, nitrogen fixation, crop maturation – flowering and fruiting including seed formation, root development and protein synthesis.

In addition, it was observed that after the pyrolysis process, potassium content of the soil were decreased from sufficient (> 75 ppm) to deficient (25-50 ppm). Smith D. W. (1969), reported that potassium concentration showed a large decrease as early as 5 weeks after burning that had decrease continuously up to the 13th month of his study. This decrease on potassium content had no effect on the plant uptake, as according to Morgan J., in 2013, the potassium plant uptake is not significantly affected by the availability of this macronutrient, but shows symptoms on plant morphology like curling of leaf tips, as well as reduced in fertility. These symptoms were also reported on the study that further supports the decrease of potassium. Although variable effects of K fertilizer on groundnut pod yield have been reported in literature, the consensus is that there is no advantage in applying K fertilizer directly to the groundnut crop.

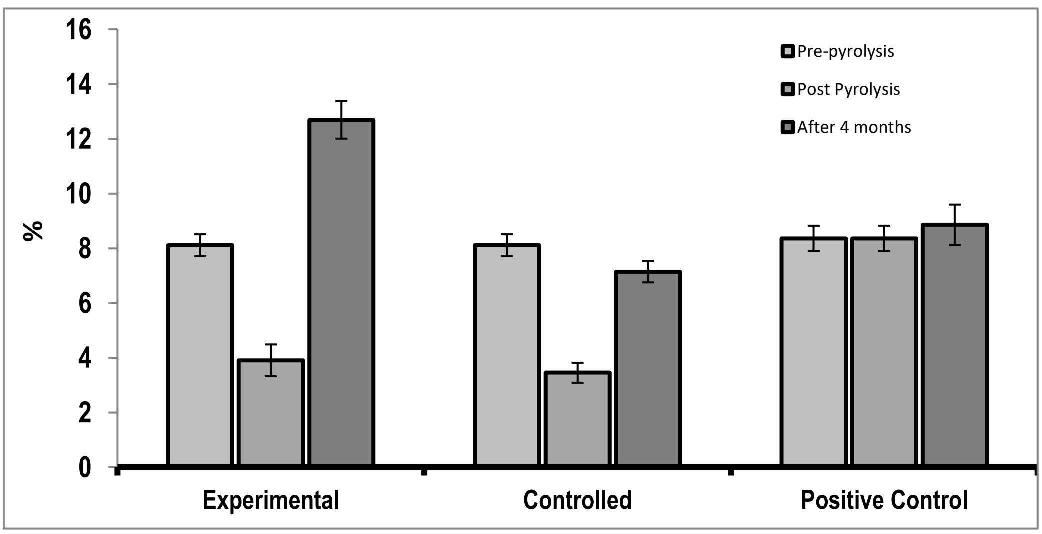


Figure 6. Moisture content of the three set-ups recorded at specific interval. Moisture content was measured before the pyrolysis, after the pyrolysis and after the four –month experimentation period

The soil moisture content of soil is the quantity of water it contains. It is due to their colloidal properties and aggregation qualities. Water content is used in a wide range of scientific and technical areas and is expressed as a ratio, which can range from 0 (completely dry) to the value of the materials' porosity at saturation. Soil water repellency is one of the properties most affected by combustion during forest fire, as the main effect of fire on soil physical properties is to eliminate the storage capacity of water in several centimeters (Verma S., & Jayakumar S., 2012). They also stated that on high surface temperature burn off organic material and create vapours that move downward in response to a temperature gradient and then condense on soil particles causing them to become water repellant.

Soil water content is expressed on a gravimetric or volumetric basis, where gravimetric water content (θg) is the mass of water per mass of dry soil (Bilskie J., n.d.), and according

Korenkova L.N. and Urik M., in 2012, the change in soil water content results to changes in bulk density and thus in porosity.

The moisture content of soils had decrease after the pyrolysis process. This result is supported by Xue, Li and Chen stated that with fire, stable organic matter-clay linkages are destroyed as clay minerology change to relatively inert clays by burning litter and organic matter, thus lessen the water content of soil, because according to them, organic matter and clay are crucial to the formation of structure in the surface, which provides the macro pores spaces needed for water movement and storage. However, the Peanut plant was able to restore this property of soil.

A study of Mulinge J.M., Saha H.M., Mounde L.G. and Wasilwa L.A., in 2017, had reported that, the use of legume cover crop, has been observed to increase soil organic matter, soil water holding capacity and the recharging of soil water from rainfall improved, where the varied increase in soil moisture from 2012-2014 in both soil depths 0-20 and 20-40 cm was because different legume crop cover have different capacities in the increasing soil biomass, and hence the increase in soil moisture content and water storage differed with treatment.

Table 2. Average number of Pods of Peanut Plant Planted in the Experimental Set-up.

	LM1a	LM1b	LM1c	LM1d	Mean
Number of pods	15	10	13	18	14

The fruits of groundnut develop underground, they are just as vulnerable to direct effects of soil acidity as the roots are, thereby necessitating an assessment of the effects of acidity both in the podding (0 – 10 cm soil depth) and rooting (20 – 30 cm soil depth) environments of groundnut and usually produces between 25 and 50 peanuts. However, only an average of 14 pods was counted in this study. This low number of pods is also the reason on the increase of nitrogen as it was transferred to the soil rather than for the development of pods. Nitrogen fixation is also affected by the level on nitrogen in the soil. High soil N level reduce N fixation because legumes will preferentially use most of the available soil N before they begin to fix atmospheric N.

4. CONCLUSIONS

The physicochemical analysis revealed that the soil pH became alkaline, N content was improved to medium, no evident change was recorded in P level, a K content was decreased to deficient, and moisture content of the soil samples was recovered.

In addition, it can be affirmed that the plant sample (peanut) can withstand the initial condition of the charcoal site soil through the evidences provided by the plant's morphology throughout the study. A linear increased was evident for the height, number of leaves and leaf area index all throughout the treatment period. First flowers were first seen 28 days after planting (week 4), with a peak on week 10 at an average of 16 flowers. However, only an average of 14 pods was counted in this study. This low number of pods is also the reason on the increase of nitrogen as it was transferred to the soil rather than for the development of pods.

The study provided reasonable data to conclude that Arachis hypogaea (Peanut) had a bioremediation potential as it had restored the charcoal-site soil properties after the four (4) months treatment period.

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