Practical Training Report

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2012/10/31

1. Introduction

Soil provides a variety of requirements for plants, and hence, animal lifeð it is one of the most important bases of terrestrial ecology. The principal requirements that soil provides for plants are anchorage, moisture storage in that soil is like a sponge storing water, and a supply of nutrients. Soil creates habitable habitats for animals by providing space for them to live and by determining the local chemistry; soil provides the proper type of habitat. Analyzing soil nutrition is essential for vegetation cultivation.

2. Methodology

2.1 soil nutrient test

The soil environment where plants grow can be divided into 3 characteristics: physical attributes, biological attributes and chemical attributes. Soil analysis is an effective way to check the chemical conditions .Because soil analysis provides objective figures on the chemical condition of soil, we can find both deficiency symptoms and excess symptoms. Analyzing equipment provided is Dr. Soil, digital indicator, pH meter, EC meter. Dr. Soil includes all the necessary tools in one box for soil nutrient test in the field. By take advantage of Dr. Soil, the fowling analyzing items are available:

Analyzing Item	Analyzing Element	Range	Unit
EC	-	0~20	mS
pH（H2O）	-	3.0~9.0	-
pH（KCl）	-	3.0~9.0	-
ammonia nitrogen	NH4+ī N	1~25	mg/100g(N)
nitrate nitrogen	NO3ī ī N	1~50	mg/100g(N)
phosphoric acid	P	5~150	mg/100g(P2O5)
potassium	K	1~150	mg/100g(P2O5)
calcium	Ca	50~1000	mg/100g(CaO)
magnesium	Mg	1~150	mg/100g(MgO)
iron	Fe	5~100	ppm(Fe)
manganese	Mn	5~100	ppm(Mn)
salt content	Cl	0.002~0.200	%(NaCl)

Table 1. Available analyzing items

We mainly did four soil related tests by using tools for extraction, filtration and color formation: Acidity (pH), nitrogen (N), phosphorus (P) and potassium (K). As being advised to do several tests from different locations and averaging the results, we took the soil sample from about 5-20 cm deep from soil at Pagilaran tea plantation and coconut plantation. After assembling the soil samples, we practice the test for pH and the extraction of soil nutrient. Hereby, we take the test for pH or detail experiment explanation.

In the first step, we place a sample of soil into the empty test tube, up to the 1st line.

Then we use the supplied measuring spoon place one spoonful of barium sulphate on top of the soil sample in the test tube. Barium sulphate is inert and helps to settle clay particles in the solution so that it does not become too cloudy. PH indicator solution was added into the test tube filling to the third mark. After placing the cap on top and then give the tube a good shake so that the contents are well mixed, leave to settle, this will take a few minutes. When the mixture has settled and the liquid clarifies we compared the solution against the chart.

Except for the pH test, where the soil is mixed directly together with the color changing chemical, all of the tests have two parts. The first part involves mixing the soil together with an extraction solution, then passing it through a filter in the plunger assembly to remove the soil. The color changing chemical is then mixed separately with the extraction solution and compared to the provided charts (Fig. 1).

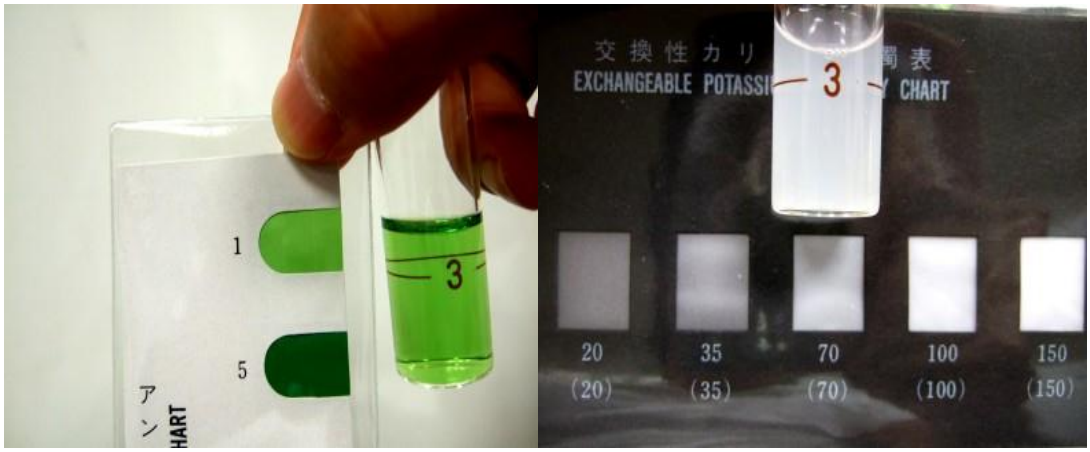

Fig. 1 Comparing the color to the chart, the left figure is NH3 scale, and the right one is CaO scale.

2.2 Soil temperature test

In order to measure the temperature environment of soil, we put one temperature sensor at each of the place that soil sample being taken. Then aggregate the measure results into the soil analysis.

3. Result and discussion

3.1 Soil temperature analysis

In Pagilaran tea plantation area, our practice was divided into 2 parts, high land and low land tea plantation areas. We found out that the temperature of high land is 1.7 ºC lower due to the temperature decreases with the mountain elevation increasing. We also found out that, at low land, the temperature of soil increased with the land

depth changed from 5cm to10 cm (Fig.2), which mainly due to the daily rainfall. Rainfall decreases the temperature of surface soil.

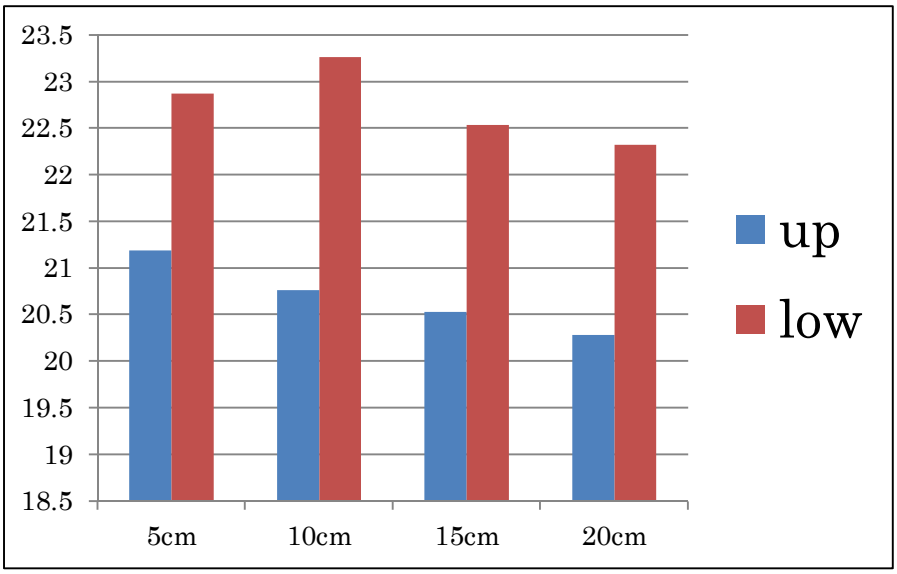

Fig.2 Soil temperature of 5-20cm at up and low land

There are 2 blocks in cocoa plantation area. We divided each block into sunshine area and shadow area for taking soil samples and measure their temperature. Block 2 is inorganic fertilizer area; on the contrary, Block 4 is organic fertilizer area (Fig.3).

Fig.3 Blocks in cocoa plantation

According to the assembled data of temperature, we found out that temperature of sunshine area is higher than shadow area as a result of absorbing more solar energy than shadow area; and temperature of block 2 is higher than block 4, which dominated by larger space between single tree in block2; both blocksôtemperature decreased with the soil depth change from 5 to 20 cm (Fig.4).

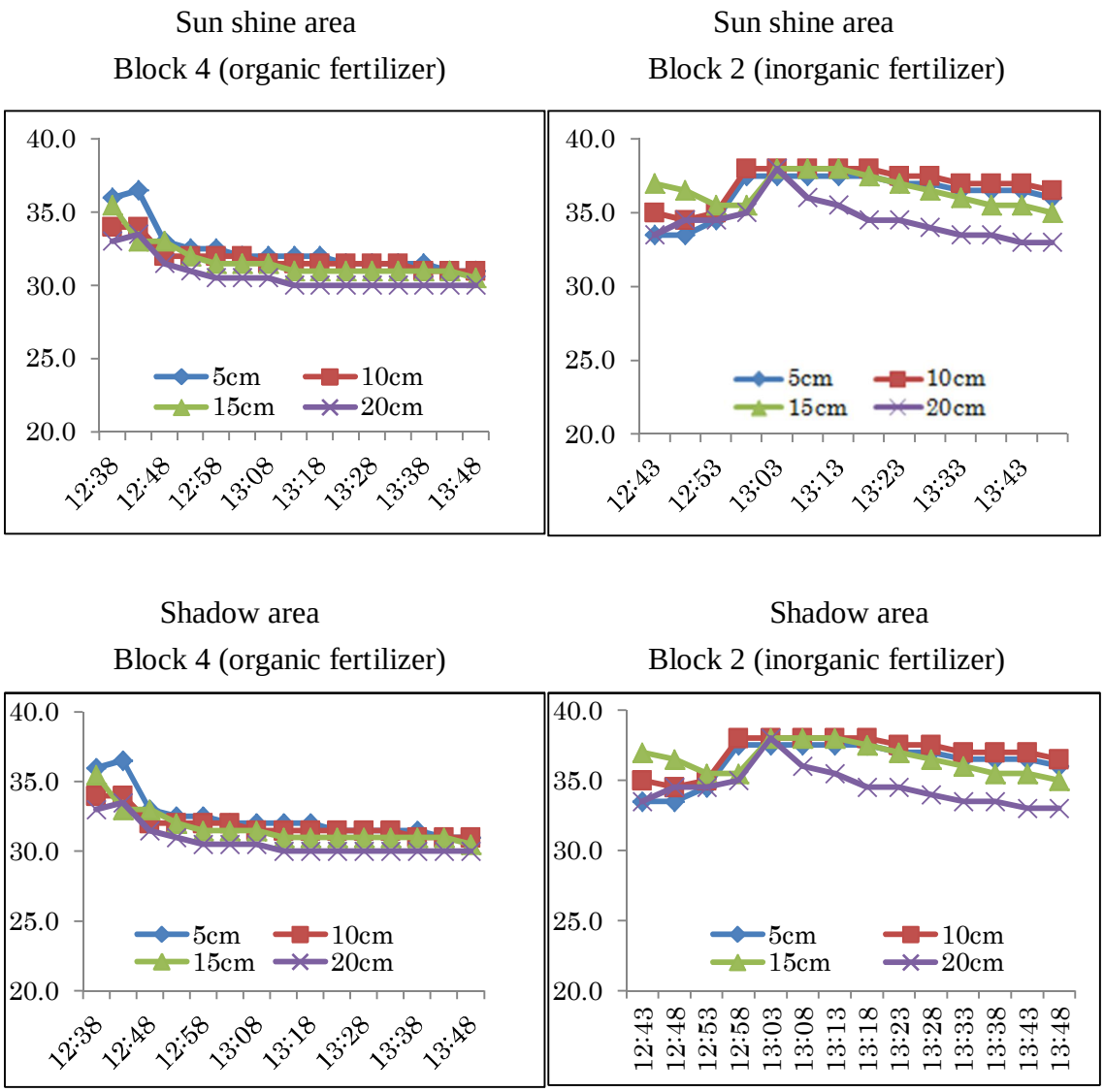

Fig.4 temporally soil temperature changes at different depth

3.2 Soil nutrition analysis

Since nitrogen is responsible for vegetative growth, managing its availability in tea soils directly reflects in crop yield. Tea being a leaf crop, in the flush shoot the nitrogen content is the highest followed by potassium (K), calcium (Ca), phosphorus (P), sulfur (S), magnesium (Mg) and zinc (Zn).

Tea is basically a rain-fed crop cultivated commercially in humid zones of tropical, sub-tropical and temperate regions with alternating wet and dry periods, on well-drained soils preferably with pH 4.5-5.0 although in practice ranging from 3.0 to 6.5. Both high location and low location shows proper range of pH value which consistent with what mentioned above (Fig.7). In soils with pH >5.0, accumulation of calcium interferes with synthesis of carbonaceous materials and uptake of K. In soils with pH <4.0, the H+ and Al3+ ions interfere with K uptake. Nitrogen efficiency is maximum in soils between pH 4.5 and 5.0 and hence liming is recommended to maintain the soil pH around 4.8. Therefore, the nitrogen efficiency of Pagilaran tea soil has reached the maximum efficiency. The reason why ph. has act such vital role are explained by two things, first certain plants prefer acidic soil conditions; including Rhododendrons, Camellia blueberry bushes and most heathers. Secondly, pH has a great influence on the availability of soil nutrients, certain nutrients become unavailable at a low pH-magnesium and calcium, while iron and manganese are restricted when the pH is high. All nutrients are affected by pH and the widest availability of nutrients is at a pH of 6.5. For most vegetables a pH of 6.5 is ideal.

Fig.5 shows that Potassium oxide (K_2O) is the major nutrient for tea ranging from 10% to 35%. Tea soils contain predominantly kaolinite as the clay mineral and the soils have no fixation sites for potassium. Therefore, the leaching loss of potassium is considerable and it increases with the decrease in soil pH. Potassium is required in large quantities and be involved in almost all biological reactions.

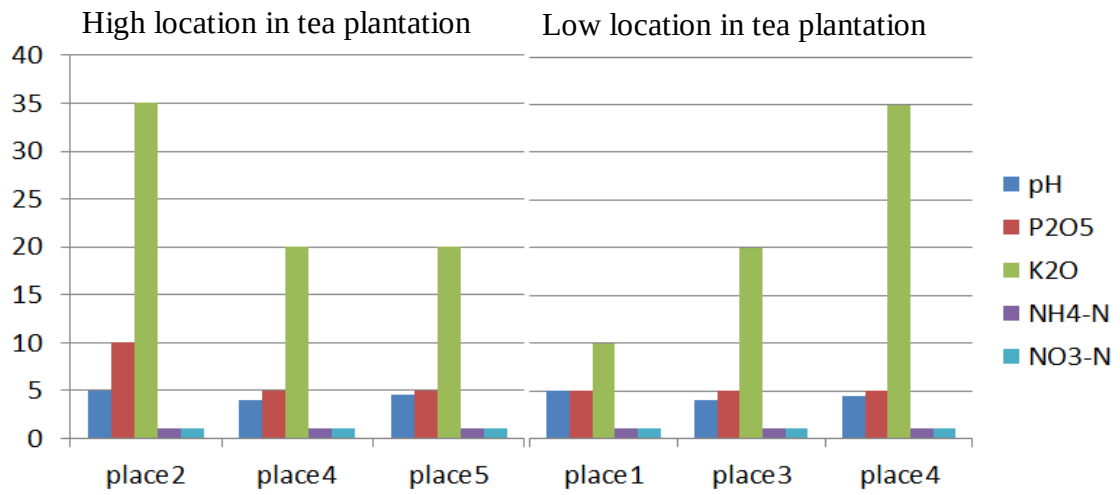

Fig. 5 constituent part of soil in high and low land of tea plantation

3.3 DNA Extraction

DNA Extraction is the removal of deoxyribonucleic acid (DNA) from the cells or viruses in which it normally resides. DNA isolation is a routine procedure to collect

DNA for subsequent molecular or forensic analysis. Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes Extraction of DNA is often an early step in many diagnostic processes used to detect bacteria and viruses in the environment as well as diagnosing disease and genetic disorders. We conduct DNA isolation by 2 methods. One is ISOIL, the other one is using extrap soil DNA kit Plus Ver.2.

ISOIL is a kit for extracting DNA from soil samples. By using an extracting solution with a special composition, DNA can be extracted not only from non-volcanic ash soil but also from volcanic ash soil, something which has been considered difficult to achieve up to now. With ISOIL, DNA is isolated with a heat extraction method in the presence of a surface-active agent. Since soil DNA is extracted without applying physical force, high molecular weight DNA can be obtained. Therefore, soil DNA extracted with ISOIL is suitable for applications involving gene resources, e.g., the construction of a metagenome library.

In the following step, we took advantage of electrophoresis method to sort DNA molecules by length. Pieces of DNA are suspended in a tray of gel and subjected to an electric field, which causes them to migrate toward one end of the tray. The DNA separates out into bands, with the distance from the electrode corresponding to length of the strand.

At the last step, we applied the polymerase chain reaction (PCR) which is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. In practice, PCR can fail for various reasons, in part due to its sensitivity to contamination causing amplification of spurious DNA products. Fig.6 shows that DNA bands in sample A1, A2, C1, C2, D1, D2, E1, E2, R1, R2 and H2 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs. We successfully extract 16S ribosomal RNA gene sequences which contain hypervariable regions that can provide species-specific signature sequences useful for bacterial identification (Fig.7). Betaproteobacteria ammoniamonooxigenase (amoA) gene was extracted successfully (Fig.8).

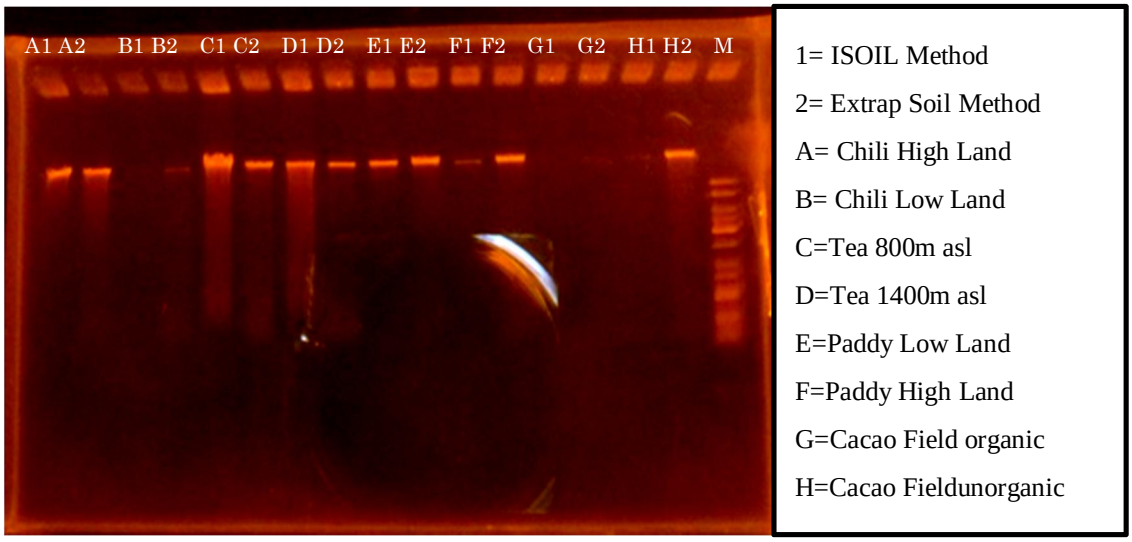

Fig.6 Ethidium bromide-stained PCR products after gel electrophoresis.

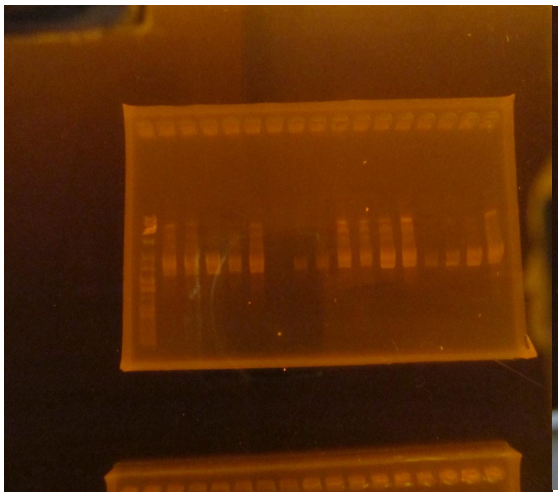

Fig.7 16S ribosomal RNA

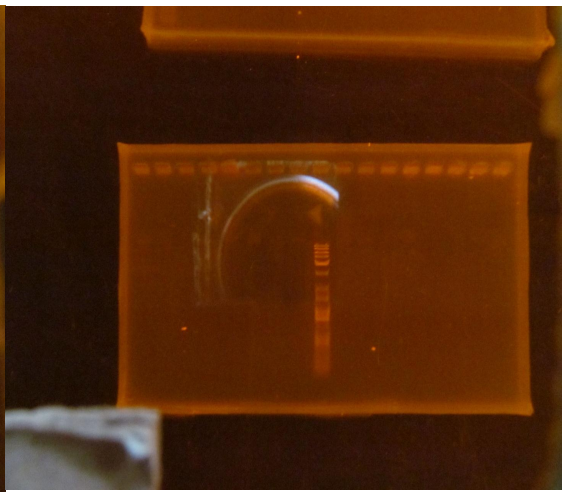

Fig. 8 Betaproteabacterial Ammoniamonooxigenase (amoA)

4. Conclusion

According to the results of the investigation, soil samples are generally Clay and Clay Loam textures and pH values of soil samples are strong acid and medium acid reactions. Organic matter amount and total N content of the soil samples are generally sufficient and high. Phosphorus contents of soil samples were generally sufficient. Calcium deficient was determined in soil samples, because great amount of soil samples were strong acid reaction. For this purpose, lime should be applied to these soils, but the pH value of soils should not be excessively high.

Tea soils are basically poor in fertility and productivity depends on the organic

matter content which builds the structure and sustains microbial activity. Hence, there is a need for regular and frequent application of nitrogen and potassium to even out the above effects. Thus splitting the application can increase the agronomic efficiency of nitrogen and potassium. The fertilizer program should carefully be chosen to increase tea production and cocoa plantation. The choice of fertilizer program should not only be based on soil analysis but also leaf samples analysis. Therefore, soil and leaf samples analysis results have been evaluated together to solve nutrition problems for many plants in the world. The physical and biological conditions for plants need to be controlled in addition to the soilôs chemical condition. It is possible that the plant cannot root and absorb nutrition in the soil even though each nutrient element is balanced in the soil. This is why it is also necessary to pay attention to improving the physical and biological conditions for plants as well as for chemical conditions for the soil.

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