



*International Journal of*  
**Environmental and Rural Development**

**Volume 12 Number 1**

**June 2021**

**International Society of Environmental and Rural Development**



# International Journal of Environmental and Rural Development

Official Journal of the International Society of Environmental and Rural Development

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## Assessment of Soil Properties using GIS Technologies in a Selected Area in Myanmar

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Received 24 December 2020    Accepted 12 May 2021    (\*Corresponding Author)

**Abstract** This study evaluates selected soil properties and maps these under different cropping patterns of Sipintharyar village, Zeyarthiri Township, Myanmar. A total of 130 soil samples were collected at a depth of 0-20 cm, with sample points selected using a Global Positioning System. Soil fertility maps were created using Kriging interpolation in ArcGIS software 10.5. Soil textures in the study area were loam, loamy sand, clay loam and sandy loam. The soils were strongly acidic to moderately alkaline and contained a very low status of soil organic matter (84%), available potassium (89%) while total nitrogen was at a medium (56.92%) level. The coefficient of variation (CV) showed that soil pH was the least variable (9.91%) parameter examined, with mean values ranging from 4.95 to 8.47, while available potassium was highly variable (86.95%) with content values ranging from 1 to 578 ppm. Other selected properties such as bulk density, total nitrogen, CN ratio, electrical conductivity and soil organic matter were found, respectively, to have the following variabilities; 11.68%, 33.84%, 34.86%, 72.23% and 52.07%. Levels of soil organic matter were highly significant, and positively correlated with total nitrogen and available potassium. These variations in soil properties are probably related to the different cropping patterns and fertility management practices employed in the study area.

**Keywords** soil properties, global positioning system, Arc GIS

## INTRODUCTION

The agriculture sector in Myanmar accounted for 30% of gross domestic product, 60% of employment, 29% of value addition, and 23% of exports in 2016 (Agriculture Guide, Myanmar, 2018). Therefore, an evaluation of the fertility status of soils of an area is an important aspect in promoting sustainable agricultural production (Singh and Mishra, 2012). In Myanmar, the major soil fertility issues are understood only at the higher level with limited information at local levels. Soil fertility and productivity have a direct relationship in ensuring food security for the increasing world population. The optimum plant growth and crop yield might be influenced by not only the total amount of nutrients present in the soil at a particular time, but also on the availability of these, a process controlled by physicochemical properties (Bell and Dell, 2008). In addition, nutrient properties vary over time and space. Wakene and Heluf (2003) stated that the periodic evaluation of important soil properties and

their responses to changes in land management is required to apply proper soil fertility management techniques, and to improve and conserve the fertility and productivity of soils. Additionally, the spatial variability of soil fertility and its classification can be mapped by applying GIS, to clearly show the specific locations where attention is required with respect to management of plant nutrients (Jatav et al., 2013). Currently, there is little information on the spatial variability of these soil fertility parameters and very few efforts in generating soil fertility maps for the agricultural soils in Myanmar. Furthermore, without detailed soil related information at a specific local level, sustainable crop production cannot be achieved. From this, understanding the spatial variability of soil fertility in specific agricultural fields is essential in optimizing the application of agricultural inputs and ensuring maximum crop yields in Myanmar.

## OBJECTIVES

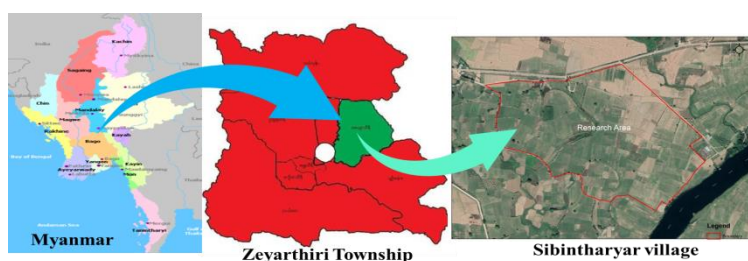
The main objective of this research is to evaluate the spatial variability of selected soil properties and to map soil fertility status under different cropping patterns in the study area.

## METHODOLOGY

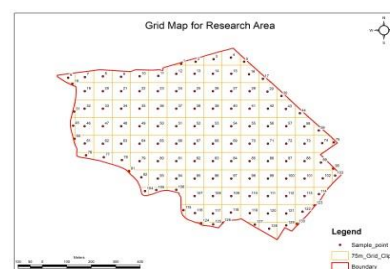
### Study Area, Soil Sampling and Data Collection

Total study area is 0.6 km<sup>2</sup> and it is located at Sipintharyar village (19°44'43" N - 19°45'22" N and 96°17'42" E - 96°18'02" E), Kyidaung village Tract, Zeyarthiri Township, in central Myanmar (Fig. 1). It receives a mean annual rainfall of about 1265 mm and has an average temperature of 26.8°C.

The grid map preparation was created using ArcGIS (Ver.10.5) software for the collection of soil samples, in March 2020. The soil samples were collected after harvesting of crops and during the cultivation of horticultural crops, with samples from 300 m × 300 m grid points for determining soil texture, and 75 m × 75 m for the other soil parameters, and at 0-20 cm depth by using auger and hand-hoe from the selected points (Fig. 2). Ten soil samples were taken from each grid to form a composite a representative sample, with a 130 soil samples in total. After labelling and packing, samples were tested at the laboratory of the department of Soil and Water Sciences, Yezin Agricultural University, Myanmar. After air drying, the samples were gently crushed, sieved (2 mm) and properly stored for analysis. Furthermore, interviews were conducted with farmers using structured questionnaires to identify variations in management practices. Sixty farmers were selected as sample respondents and interviewed. The data collected related to method of land preparation, fertilizer management and the cropping patterns practiced by the respondents in the study area.



**Fig. 1 Location of study area**



**Fig. 2 Soil sampling grid points**

### Laboratory Analysis

The composited soil samples were analyzed to determine soil texture, adopting a pipetted method (Ryan et al., 2001), with bulk density determined by gravimetric method. Soil pH and electrical conductivity were measured in an extract, with a soil: water (1:5) suspension, using a digital pH-meter and EC meter (Hesse, 1971). Total nitrogen was determined by the Modified Kjeldahl Digestion method (Ohyma et al., 1991). Heanes (Rayment and Lyons, 2011) wet digestion method was used to determine soil organic matter and available potassium was determined by Atomic Absorption Spectrophotometer.

### Statistical Analysis and Soil Fertility Mapping

The soil results were analyzed by using statistix (8<sup>th</sup> version). The coefficient of variation (CV) was also determined to measure nutrient variability according to Ogunkunle (1993), where, soil properties having a CV between 0 and 15% are considered least variable, those with 15 and 35%, moderately variable, and larger than 35%, highly variable. These results were used to create soil fertility maps. The soils were classified into different categories of fertility i.e., very low, low, medium, high, and very high on the basis of the measured soil parameters. Nutrient index was also calculated by Ramamoorthy and Bajaj (1969).

## RESULTS AND DISCUSSION

### Spatial Distribution in Soil Properties

The results of the descriptive statistics of soil samples are presented in Table 1. There is a large variation in soil properties in the study area. Electrical conductivity shows the highest variability with 72.23% CV, followed by soil organic matter, with a CV value of 52.07%. However, least variability across sample areas was found for bulk density and soil pH, with CV values of 11.68% and 9.91% respectively. Moderate variability occurred for the CN ratio and total nitrogen, which have CV values of 34.86% and 33.84%, respectively.

**Table 1 Descriptive statistics of soil physicochemical parameters**

Variables	Unit*	Minimum	Maximum	Mean	SD	CV%
Sand	%	24.44	78.80	56.38	18.04	31.99
Silt	%	7.72	33.36	17.04	8.23	48.29
Clay	%	13.06	42.20	26.58	11.04	41.53
Bulk Density	g cm <sup>-3</sup>	0.80	1.51	1.21	0.14	11.68
pH	-	4.95	8.47	6.94	0.69	9.91
Electrical Conductivity	dS m <sup>-1</sup>	0.03	0.72	0.17	0.12	72.23
Organic Matter	%	0.31	3.55	1.41	0.73	52.07
Total Nitrogen	%	0.11	0.39	0.23	0.08	33.84
Available Potassium	ppm	1.00	578.00	87.08	75.72	86.95
Carbon : Nitrogen (CN ratio)	-	1.19	7.85	3.43	1.20	34.86

SD: Standard Deviation, CV: Coefficient of Variation;

\* Units represent for the columns of minimum, maximum and mean in the table

### Soil Texture and Bulk Density (BD)

Sandy loam was the dominant soil textural class throughout the study area (Fig. 3), indicating a similarity in soil forming processes and parent materials. According to USDA soil texture classification system, six points in a 12 point of soils describes a sandy loam (58.33%) textural class; whereas two



points describe clay loam (16.67%) and loam (16.67%) and one point represents loamy sand (8.33%), respectively. The spread of BD values ranged from 0.8 to 1.51 g cm<sup>-3</sup>, with a mean value of 1.21 g cm<sup>-3</sup>. From the survey, all farmers use machine, animal and manpower from the land preparation to the harvesting process, for crop production and grow the different cropping patterns in every year.

### Soil pH and Electrical Conductivity (EC)

The pH values of the soil samples ranged from strongly acidic to moderately alkaline, (4.95 to 8.47) with a mean value of 6.94. The spatial distribution map of soil pH is presented in Fig. 5 and the pH can be seen as mostly neutral (47.69%), strongly alkaline (1.54%), moderately alkaline (26.92%), slightly acidic (13.85%), moderately acidic (9.23%) with only 0.77 % strongly acidic. Gazey and Davies (2009) indicated that the pH values between 5.5 and 8.0 are considered as ideal for plant growth.

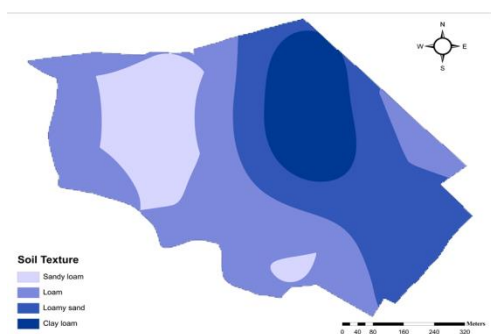


Fig. 3 Spatial variability of soil texture

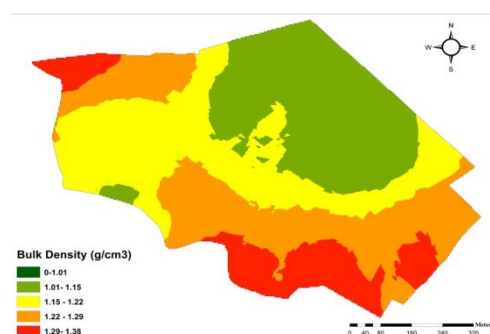


Fig. 4 Spatial variability of soil bulk density

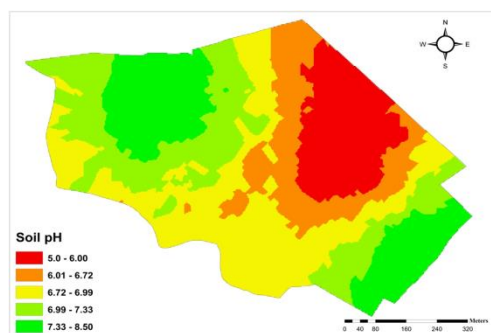


Fig. 5 Spatial variability of Soil pH

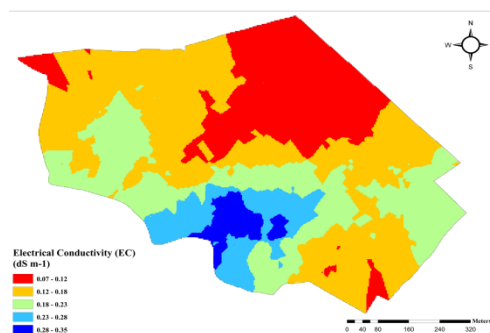


Fig. 6 Spatial variability of EC

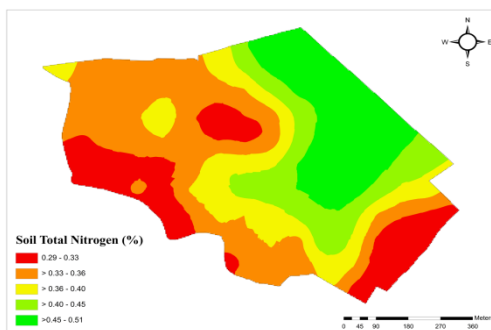


Fig. 7 Spatial variability of total N

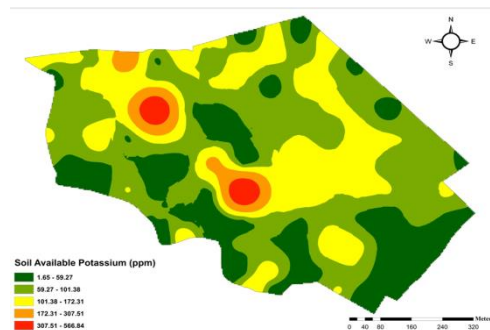
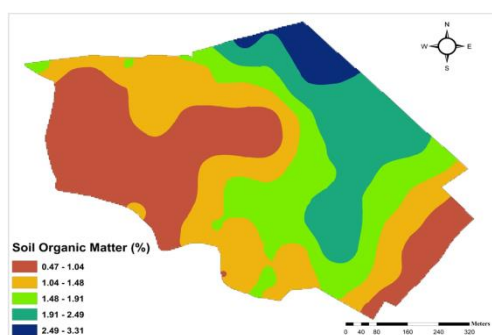


Fig. 8 Spatial variability of available K

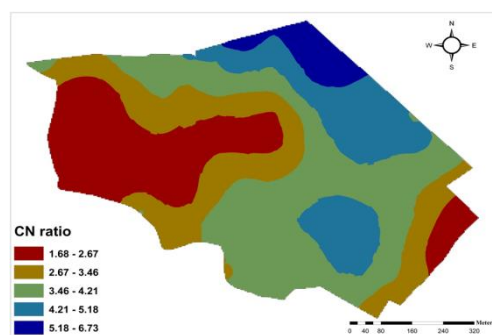
Therefore, the observed pH values are suitable for different crops and the availability of most of plant nutrients should not be subject to restrictions within the observed pH range. The values of EC range from 0.03 to 0.72 dS m<sup>-1</sup> with an average of 0.011 dS m<sup>-1</sup> (Fig. 6). According to the Soil Guide (Moore, 2001), these EC values are situated between a low EC value (0.03 – 0.47 dS m<sup>-1</sup>; 96.92%) and a medium level (0.52 – 0.72 dS m<sup>-1</sup>; 3.77%), which would have no effect on plant growth.

### Total Nitrogen (TN) and Available Potassium (AK)

Variation in the TN content of the soil samples ranged from 0.11% to 0.39%, with a mean value of 0.23%. The study confirms that about 20.77% of the sampled area has low levels of TN, while 56.92% has medium levels and 22.31% of the sampled area has high levels (Fig. 7). The areas of medium to high (79.23%) levels of TN would probably be as a result of the addition of nitrogen fertilizer and the intercropping with legumes. The AK levels appears to be low, with 89% of the sampled area being in the lower range, whereas 9% and 2% of the samples showed medium and high levels (Fig. 8), respectively. The low levels of AK may be due to infrequent application of potassium fertilizers in their cultivation, and this only through the use of compound fertilizers.



**Fig. 9 Spatial variability of SOM**



**Fig. 10 Spatial variability of CN ratio**

### Soil Organic Matter (SOM) and Carbon : Nitrogen (CN Ratio)

The spatial distribution of SOM is displayed in Fig. 9. The SOM content of soil ranges from 0.31% to 3.55% with a mean value of 1.41%. Most (84%) of the study area has very low levels of SOM. Based on the survey data, this may be as a result of the complete removal of crop residues, the failure to add organic manures and the burning of crop residues after harvesting. This finding is in line with Alemayehu, K. and Sheleme, B. (2013) who report that SOM recorded in cultivated fields as being lower than for other land uses because of the effect of continuous cultivation and SOM oxidation. There is a very low spatial variation of CN ratio throughout the study area, with ratios ranging from 1.19 to 7.85, and with a mean value of 3.43 (Fig. 10).

### Nutrient Index Value (NIV) and the Relationship among Soil Parameters

NIV of the SOM, CN ratio and AK content for the studied area clearly reveals these parameters are at low levels, while soil TN was at a medium level. The ranking of nutrients according to NIV is total N > available K > Soil organic matter > CN ratio. According to the results, TN content was highly significant and positively correlated with SOM and AK at a 1% confidence interval level, and CN ratio at a 5% level, and SOM also shows a highly significant positive correlation with AK and the CN ratio (Table 2).

**Table 2 Correlation among the different soil parameters**

	<i>BD</i>	<i>pH</i>	<i>EC</i>	<i>TN</i>	<i>SOM</i>	<i>C:N</i>
<i>pH</i>	0.255 **					
<i>EC</i>	0.225**	-0.029 <sup>ns</sup>				
<i>TN</i>	-0.437**	-0.490**	0.018 <sup>ns</sup>			
<i>SOM</i>	-0.333**	-0.399**	-0.081 <sup>ns</sup>	0.791**		
<i>C:N</i>	-0.034 <sup>ns</sup>	-0.095 <sup>ns</sup>	-0.093 <sup>ns</sup>	0.212*	0.730**	
<i>K</i>	-0.119 <sup>ns</sup>	0.003 <sup>ns</sup>	0.053 <sup>ns</sup>	0.431**	0.302**	0.059 <sup>ns</sup>

\*Correlation is significant at the 0.05 level; \*\*Correlation is significant at the 0.01 level

(*EC*: electrical conductivity, *BD*: bulk density, *SOM*: soil organic matter, *TN*: total nitrogen, *C:N*: carbon nitrogen ratio, *K*: Available potassium)

## Crop Patterns and Soil Fertility Management Practices

The most common cropping patterns in the study area are rice-black gram, rice-fallow, maize-tomato, maize- tomato and lablab bean intercropping, okra-tomato and okra-Japanese mustard-onion. Moreover, some farmers grow mango, guava, banana and ambarella. Almost all farmers, except rice growers, practice mixed cropping, crop rotation and intercropping systems for fertility management. According to the survey data, 78% of farmers have no knowledge about the soil fertility testing or analysis, whereas the remaining 22% know about this but cannot afford the cost of analysis. The most common types of inorganic fertilizers used by the respondents were urea, foliar and NPK compound fertilizers.

## CONCLUSION

The ranges of soil pH and EC measured should not be detrimental to crop cultivation. However, the distribution of soil TN percentage are 50% of the study area at medium, 25% low and 25% at high levels, whereas most of the levels for SOM, CN ratio and AK are at low levels. The study also shows that NIV of TN and SOM are only at medium levels, while that of CN ratio and AK are at low levels. Based on the survey results, farmers in the study area, in their crop management practice, use nitrogen (urea) fertilizer regularly, but without applying potassium fertilizer, apart from manure and NPK compound. The spatial variability in soil properties appear to be largely due to the differences in crop management practices, diverse cropping patterns and the variety of chemical fertilizers available. Therefore, these soil fertility maps may greatly assist farmers in identifying nutrient levels for the specific areas they crop and so help them to improve their crop fertility management programs, leading to an increase in productivity and higher incomes.

## ACKNOWLEDGEMENTS

I would like to gratefully acknowledge the JICA-TCP, Japan International Cooperation Agency, through its Capacity Development program at Yezin Agricultural University, for providing the research grant. My thanks also go to the supervisory committee, Staff at DOA, and to the farmers in the study area who have provided technical and manual support through their participation in the study.

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# Preparation of Effective Microorganisms Based Compost Using Some Selected Wastes for Improvement of Plants Growth

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Received 11 December 2020 Accepted 16 May 2021 (\*Corresponding Author)

**Abstract** Solid waste disposal is the most pressing problem facing mankind throughout the world. The solid waste management plays a significant role to create a sustainable environment. Some vegetable wastes such as rice husk, cotton husk, coconut husk, pigeon-pea husk and chicken manure were selected for chemical analysis. The effective microorganism (EM) solution was prepared from kitchen vegetable wastes except onion and garlic peels to ferment for two months. The microorganisms that contain in prepared EM solution were studied by using microscopic morphology. The pH of prepared EM solution was measured by using pH meter. The compost was prepared from the vegetable waste materials and prepared EM solution by using aerobic method. The yield percent of compost was determined by calculation method. The yield percent of prepare compost was found to 55%. The mineral contents of compost and soil sample were measured by using EDXRF spectroscopy. The physicochemical properties of vegetable wastes, prepare compost and soil sample were determined. Planting the seedlings of some selected useful vegetables in various ratios of the prepare compost and soil sample. The growth rates of plant rates were found to be effective by the planting experiment.

**Keywords** physicochemical properties, EM, compost, EDXRF, planting

## INTRODUCTION

Compost is organic matter that has been decomposed in a process called composting. This process recycles various organic materials otherwise regarded as waste products and produces a soil conditioner. Compost is rich in nutrients. Compost is decomposed organic material, such as leaves, grass clippings, and kitchen waste. There are two composting types in which the difference is by the nature of the decomposition process; namely as aerobic and anaerobic method. In aerobic composting that oxygen is present, mainly to break the organic matter in the waste into a stable end product such as carbon dioxide, ammonia, water and heat. Anaerobic composting developed an intermediate compound such as methane, organic acid and hydrogen sulphide (Sita et al., 2016).

Composting is a technique which can be used to reduce the amount of organic waste through recycling and the production of soil fertilizers and conditioners. Compost is primarily used as a soil conditioner and not as much as a fertilizer because it contains a high organic content (90-95%) but generally low concentrations of nitrogen, phosphorus, potassium as well as macro and micro nutrients compared to commercial fertilizers (Knight, 1997). Effective Microorganisms (EM) are mixed cultures

of beneficial naturally-occurring organisms that can be applied as inoculants to increase the microbial diversity of soil ecosystem. They consist mainly of the photosynthesizing bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi. There is evidence that EM inoculation to the soil can improve the quality of soil, plant growth and yield (Kengo and Hui-lian, 2000).

The main objectives were to examine the properties of compost from the vegetable waste and investigate the effect on plant growth. The vegetable wastes are very effective in soil nutrient. The principal goal of nature planting is to produce abundant and healthy crops without the use of chemical fertilizers and pesticides and without causing adverse effects on the natural environment.

## **METHODOLOGY**

### **Sample collection:**

The degradable vegetable wastes such as rice husk, cotton husk, coconut husk and pigeon pea husk were collected from Samar village, Kyaukse Township, Mandalay Region. Chicken manure was collected from Taungthamam village, Mandalay Region, Myanmar. Chicken manure was dried under the sunlight. Soil sample was collected from Mandalay University Campus for planting. All selected samples were ground with blender and used for throughout the experiment.

### **Determination of physicochemical properties of selected wastes:**

Some physicochemical properties of selected vegetable wastes and chicken manure were determined (AOAC, 1990).

### **Preparation of effective microorganism solution:**

The kitchen waste materials used for preparation of effective microorganism solution were collected from the home kitchen. The mixture of vegetable wastes from home kitchen (10 kg) was made the small pieces and kept in 10 liters air tight container to ferment for two months. Effective microorganism (EM) solution was prepared by using anaerobic digester method.

### **Isolation and microscopic morphology of prepare EM solution by using sub-micrometer:**

Isolation of microorganism from prepared EM solutions was done at Department of Biotechnology, Mandalay Technology University. The microscopic morphology of microorganism in prepare EM solutions was also studied.

### **Determination of pH of EM solution:**

The pH of EM solution was determined by pH meter (AOAC, 1990).

### **Preparation of compost by using aerobic digester method:**

Compost was prepared by mixing of rice husk (0.25 kg), cotton husk (0.25 kg), coconut husk (0.25 kg), pigeon-pea husk (0.25 kg), chicken manure (0.25 kg) and EM solution 10 liters in container by using aerobic digester method.

### **Determination of yield percent of prepared compost:**

The prepared compost was dried and the yield percent was determined based upon the total weight of selected materials used.

### **Determination of mineral contents of compost and planting soil:**

The mineral contents of prepared compost and planting soil were measured at department of Chemistry, University of Monywa, by applying EDXRF (Energy Dispersive-X-Ray Fluorescence Spectroscopy).

### **Determination of pH of prepare compost and planting soil:**

The pH of sample was determined by pH meter (AOAC, 1990).

### **Determination of moisture content of compost and planting soil:**

The moisture content was measured by oven dry method (AOAC, 1990).

**Determination of ash content of prepare compost and planting soil:**

The ash content was measured by oven drying method (AOAC, 1990).

**Determination of organic matter:**

The sample is ash at 500-600°C by placing a suitable weight (0.5-1.0g) of the sample in a silica crucible and heating it in a muffle furnace for 4-6 h. Loss of weight on ignition can be used as a direct measure of the Organic Matter (MO) (AOAC, 1990).

**Estimation of available nitrogen by using alkaline permanganate method:**

The amount of nitrogen released by alkaline permanganate solution was estimated by distillation procedure. The distillate was collected in known amount of standard acid and the excess acid was titrated against standard alkali solution by using methyl red as an indicator. The nitrogen so estimated is designated as available nitrogen (Subbiah and Asija, 1956).

**Determination of available phosphorus by using Olsen's method:**

2.5 g of sample was taken in a conical flask and 1.0 g of carbon black was added. 50 mL of 0.5 M  $\text{NaHCO}_3$  solution was added to the flask and shaken for half an hour. It was filtered through the filter paper. 5 mL of filtrate was pipette out into a 25 mL volumetric flask. 5 mL of molybdate reagent was added and washed down the steam of the flask and mixed. 1 mL of dilute stannous chloride solution was added and made up to 25 mL with distilled water. The contents were mixed thoroughly. After ten minutes the color intensity was read in the spectrophotometer by using read filter (or 700 nm). The value (mg of phosphorus) was read from the standard curve (Olsen et al., 1954).

**Determination of potassium by using atomic absorption spectroscopic method:**

The potassium content of prepare compost and planting soil were determined by Atomic Absorption Spectrophotometer at Department of Agriculture (Fishman and Downs 1966).

**Planting:**

The roselle, nannan, pumpkin, lady's finger, cow pea and mustard were seedlings in pots. After 20<sup>th</sup> days, all the seedlings of the same size were selected and planted in planting experiment. The three plant-based filter were used in the different ratio of compost and soil sample. In the first plant-based filter, no fertilizer was used. In the second plant-based filter, 1:10 of prepare compost and soil sample were mixed. In the third plant-based filter, 1: 5 prepare compost and soil sample were mixed. All plant-based filter was regularly watered. The duration from the time of selected plants to the end of the study period was 60 days. The growth rate of the plants was measured from selected plants from each plant-based filter and average values were recorded. The plant growth was measured at every ten days during this research work.

**RESULTS AND DISCUSSION****The Results of Physicochemical Properties of Vegetable Wastes**

The results of physicochemical properties of vegetable wastes are shown in Table 1. According to Table 1, the pH values of samples were lie between 6 and 8. It values showed the slightly alkaline condition. The organic matter content of cotton husk was found to be higher than the other samples. Thus, all selected samples contain suitable inorganic matter such as N, P and K contents found to be suitable amount respectively (Dharmakeerthi et al., 2007).

**Isolation of Microorganism and Bacterial Identification from Prepare EM Solution**

The results of isolation of microorganism and bacterial identification from prepare EM Solution were describe in Table 2.

**Table 1 Physicochemical properties of vegetable wastes**

Sample description	Unit	Chicken manure	Rice husk	Coconut hush	Cotton husk	Pigeon pea husk
pH	-	7.12	7.43	7.62	6.62	7.21
Moisture	%	21.60	12.93	8.45	6.24	10.18
Organic Matter	%	41.02	79.36	88.12	89.97	20.50
Available N	%	2.66	0.41	0.31	3.36	2.15
Available P	%	0.52	0.02	0.01	0.48	0.28
Available K	%	1.12	0.93	0.16	0.51	1.25

**Table 2 Cultural and microscopic morphology of isolated bacteria**

Sample name	Colony morphology			Microscopic morphology			
	Size (mm)	Color	Elevation	Shape	Size (um)	Gram' reaction	Shape
EM1	1	Yellow (opque)	raised	round	1-2 × 2-4	+	Small Rod
EM2	3- 4	White (opque)	flat	Irregular	1-2 × 2-4	+	Rod (spore)
EM3	3- 4	White (opque)	raised	Irregular	2-3 × 3-4	+	Rod (spore)
EM4	4-6	White (opque)	raised	round	2-3 × 3-4	+	Rod (spore)

According to cultural and microscopic morphology, four bacterial strains were observed in liquid of EM solutions with different conditions. Four bacterial strains (EM 1, EM 2, EM 3, EM 4) from prepare EM samples were isolated on Nutrient media. According to the cultural and microscopic morphology, EM 1 predicted that positive gram stain bacterial from the family of *Pseudomonadaceae*, and EM 2, 3 and 4 could be assumed that positive gram stain bacterial from the family of *Bacillaceae* (Khan et al., 2018).

### The pH of Prepare Effective Microorganism Solution

The pH value of prepared effective microorganism solution was found to be 4.29. The value is towards slightly acidic and to neutralized selected vegetable wastes (Khan *et al.*, 2018).

### The Yield Percent of Prepared Compost

The yield percent of prepare compost was found to be 55%.

**Table 3 Relative abundance (%) of elemental composition of prepared compost and planting soil**

Element	Symbol	Prepare compost (%)	Planting soil (%)
Silicon	Si	40.413	56.573
Calcium	Ca	26.922	4.410
Iron	Fe	5.143	11.110
Potassium	K	22.596	5.546
Sulfur	S	2.997	2.225
Titanium	Ti	0.933	1.127

### Mineral Content of Prepared Compost and Planting Soil



The relative abundance (%) of elemental composition of prepared compost and soil were shown in Table 3. The elemental analysis indicated that the amount of silicon was highest value in prepare compost and planting soil. The calcium content in prepare compost was higher than planting soil. The sulphur content of prepare compost and planting soil was nearly equal. The other mineral contents were found to be small amount in prepare compost and planting soil (Pravina et al., 2013).

### Results of Physicochemical Properties of Prepared Compost and Planting Soil

The results of physicochemical properties of prepared compost and planting soil were showed in Table 4. The pH value of prepare compost and planting soil was nearly equal and the ash content of prepare compost was higher than planting soil. The moisture content of planting soil was decreased compare to the prepare compost (Clark et al., 1998).

**Table 4 pH, moisture and ash value of prepared compost and planting soil**

Sample	pH	Moisture (%)	Ash (%)
Planting soil	8.81	2.24	10
Prepared compost	8.76	12.19	45

The available nitrogen value of prepared compost and planting soil were described in Table 5. According to the results from the determination of available nitrogen content of prepare compost was higher than planting soil. The phosphorus and potassium contents in prepare compost were higher than planting soil (Clark et al., 1998).

**Table 5 Available nitrogen, phosphorus and potassium value of prepared compost and planting soil**

Chemical properties	Prepared compost (% by dry mass)	Planting soil (% by dry mass)
Available Nitrogen	2.08	0.12
Available Phosphorus	1.32	0.46
Available Potassium	1.02	0.44

### Planting

Duration from the time of roselle, nannan, pumpkin, lady's finger, cow pea and mustard were to the end of the study period was 60 days. The plants growth was measured at every 10 days during this research work and average values were record.

**Table 6 The growth rate of Roselle**

Day	Roselle		
	Blank	1:10	1:5
10	4.11	4.40	4.81
20	6.14	6.43	6.84
30	8.17	8.46	8.87
40	10.21	10.50	10.91
50	12.23	12.52	12.93
60	14.25	14.54	14.95

**Table 7 The growth rate of Nannan**

Day	Nannan		
	Blank	1:10	1:5
10	2.11	2.4	2.81
20	3.12	3.41	3.82
30	4.13	4.42	4.83
40	5.18	5.47	4.88
50	6.20	6.49	6.90
60	7.22	7.51	7.92

**Table 8 The growth rate of Pumpkin**

Day	Pumpkin		
	Blank	1:10	1:5
10	8.10	8.39	8.80
20	10.12	10.41	10.82
30	12.13	12.42	10.83
40	14.14	14.43	14.84
50	16.18	16.47	16.88
60	18.21	18.50	18.91

**Table 9 The growth rate of Lady's finger**

Day	Lady's finger		
	Blank	1:10	1:5
10	8.10	8.39	8.80
20	10.11	10.40	10.81
30	12.13	12.42	12.83
40	14.15	14.44	14.85
50	16.18	16.47	16.88
60	18.20	18.49	18.90

**Table 10 The growth rate of Mustard**

Day	Mustard		
	Blank	1:10	1:5
10	4.15	4.44	4.85
20	6.16	6.45	6.86
30	8.18	8.47	8.88
40	10.20	10.49	10.90
50	12.21	12.50	12.91
60	14.22	14.51	14.92

**Table 11 The growth rate of Cow pea**

Day	Cow pea		
	Blank	1:10	1:5
10	8.11	8.40	8.81
20	10.13	10.42	8.83
30	12.14	12.43	12.84
40	14.16	14.45	14.86
50	16.17	16.46	16.87
60	18.20	18.49	18.90

In this planting experiment contains three conditions. The first condition is blank, the second condition is 1:10 ratio and the third condition is 1:5 ratio of prepare compost and planting soil. According to these results, the third condition was found in the best growth rates of planting because its uses the most amount in prepare compost.

## CONCLUSION

This experiment was done in an effort to solid wastes management by investigating homemade composting with effective microorganism solution. From this analysis, vegetable waste materials were used in evaluation provided a better environment for EM to grow produce quality compost. The degradable vegetable wastes, cow dung and EM play a significant role in stabilizing the mixture and accelerate the composting process. The pH value of EM was found to be 4.29 and it is slightly acidic condition. According to microorganism morphology, four types of bacterial strains were observed in EM solution. All are gram positive bacteria. The observed bacteria are degraded the vegetable waste materials and creates the suitable environment conditions for decomposition of organic materials used. The elemental analysis indicated that silicon was found to be the highest amount in prepared compost and planting soil. Since silicon generates the resistance in many plants to disease and pests, it may contribute to reduce the rate of application of pesticides and fungicides. The physicochemical properties of vegetable wastes and prepare compost were lie within the limiting range and it is suitable for planting. The selected useful vegetables were treated with different ratio of prepared compost and planting soil. The highest amount of prepare compost used in planting is most growth rates in all selected plants. Thus, the prepared compost was low cost, easily available and supporting the improvement of growth rates of plants. According to the results of experimental work, compost from kitchen waste product could be employed as an alternative, eco-friendly and low cost for supporting of plants growing.

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## Spatial Variability and Mapping of Soil Properties Using GIS-Based Geostatistic in Myanmar

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Received 29 December 2020    Accepted 16 May 2021    (\*Corresponding Author)

**Abstract** Precise information on the spatial variability of soil is a crucial component for productive intensive agriculture, sustainable development, and the management of natural resources. The primary purpose of the study was to investigate the spatial variability of soil properties of the study site at Yezin Agricultural University Field, Myanmar using geostatistics. A total of 94 composite soil samples were collected from a depth of 0 to 20 cm, in a systematic grid (50 x 50 m<sup>2</sup>) at the site in May 2019. Soil pH, electrical conductivity (EC), soil organic matter (SOM), total soil nitrogen (TSN), available phosphorus (Ava-P), and available potassium (Ava-K) were measured using standard analytical methods. Data were analyzed geostatistically based on semivariogram. The exponential model best fitted the semivariogram for pH, EC, Ava-P, and Ava-K; SOM was adapted from the Gaussian model while TSN was adapted from the spherical model. The nugget/sill ratio showed a strong spatial dependence exists for EC, Ava-P, and Ava-K and a moderate spatial dependence for pH, SOM, and TSN. Most of the soil was found to be strongly acidic. It was also found that EC, SOM, and Ava-P are very low in most of the study area. Most of the study area was found to have low TSN levels, while Ava-K content was low over the entire area. With such an analysis, it is possible to plan better nutrient management practices for agricultural production and environmental protection. Therefore, geostatistical analysis with ordinary kriging is a useful tool for studying the spatial variability of soil properties.

**Keywords** spatial variability, soil properties, geostatistic, semivariogram, kriging

## INTRODUCTION

An understanding of the distribution of soil properties is essential for ecological modelling, environmental predictions, precise agriculture, and management of natural resources (Wang et al., 2009). However, soil properties vary spatially from a small to a larger regional area, and are affected by intrinsic (parent materials and climate) and extrinsic factors (soil management practices, fertility status, crop rotation) (Cambardella and Karlen, 1999). Therefore, demands for more accurate

information on spatial variability of soils are significant for intensive agriculture, sustainable development, and natural resource management (Karlen et al., 2011).

Many studies have used a classical statistical method to quantify spatial characteristics in soil properties (Salehi et al., 2013). However, physico-chemical characteristics of soil often exhibit spatial dependency, which cannot be recorded with classical statistical methods (Lin et al., 2005). To overcome this problem, many researchers apply geostatistical interpolation methods to estimate the spatial variability of soil properties (Cambardella et al., 1994; Webster and Oliver, 2007).

Geostatistics is a set of statistical tools that can be used to investigate and predict the spatial structure of georeference variables and generate soil property maps (Patil et al., 2011). Based on the geostatistical analysis, several studies have been carried out to characterize the spatial variability of various soil properties (Weindorf and Zhu, 2010). Among the various geostatistical methods, ordinary kriging is widely used to map spatial variations in soil fertility because it offers a higher level of predictive accuracy (Song et al., 2013). Therefore, it is important to extend the availability of soil resource information maps to allow the planning of appropriate soil management practices, including fertilization for agricultural production and environmental protection.

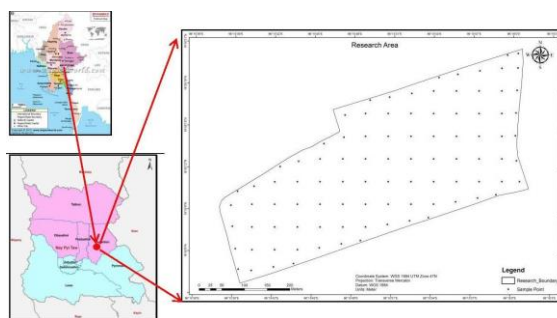
## OBJECTIVES

The objective of this research is to investigate the spatial variability and mapping of spatial distribution of selected soil properties status in the study area using GIS-based geostatistical analysis.

## METHODOLOGY

### Study Area, Soil Sampling and Laboratory Analysis

The study area was Yezin Agricultural University Field (19°49'47"19°50'21"N and 96°15'32"-96°16'15"E), Zeyarthiri Township, Nay Pyi Taw Union Territory, in central Myanmar. It has an elevation ranging from 121.547 m to 125.205 m above sea level (Fig 1). The area of the research site is 18.19 ha. The study area has an average temperature of 26.8°C and a mean annual rainfall of about 1420 mm. Summer and monsoon rice are the main crops in the study area and these include both rain-fed and irrigated rice cultivations.



**Fig. 1 Georeferenced sampling sites of the research area**

A total of 94 composite soil samples were collected at a depth of 0 to 20 cm based on a systematic grid (50 x 50 m<sup>2</sup>) determined with the help of a hand-held GPS device, in May 2019. Soil samples were air-dried and ground so as to pass through a 2-mm sieve. Soil pH and electrical conductivity (EC) were

measured in a 1:5 soil/water extract, soil organic matter (SOM) was determined by Heanes wet oxidation method, total soil nitrogen (TSN) was analyzed by the Semi-micro Kjeldahl steam distillation method, available phosphorus (Ava-P) was measured by the Olsen-P method, and available potassium (Ava-K) was determined by extraction with 1M ammonium chloride (Rayment and Lyons, 2011).

### Geostatistical Analysis

The geostatistical analysis, consisting of semivariogram calculation, cross-validation, and mapping, was performed with the Geostatistical Analyst Extension Tool of ArcGIS 10.7. The spatial variation of soil properties was analyzed using geographical semivariogram to quantify the spatial variation of a regionalized variables which derives important parameters used for ordinary kriging (OK) spatial interpolation (Krige, 1951). The semivariogram is used as a fundamental tool to study the spatial distribution structure of soil properties. The semivariogram analyzes were performed before the application of OK interpolation, as the semivariogram model determines the interpolation function (Goovaerts, 1997), defined as:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2 \quad (1)$$

where,  $\gamma(h)$  is the experimental semivariogram value in a distance interval  $h$  (in meters or km),  $N(h)$  is the number of sample pairs that are located by a particular distance ( $h$ ) from each other.  $z(x_i)$  and  $z(x_i + h)$  are the values of a regionalized variable at location  $x_i$  and  $x_i + h$ , respectively (Wang and Shao 2013).

Theoretical semivariogram models fit the empirical semivariogram obtained from the data to generate geostatistical parameters, including nugget variance ( $C_0$ ), structured variance ( $C$ ), sill variance ( $C_0 + C$ ), and distance parameters ( $A$ ). The nugget/ sill ratio,  $C_0 / (C_0 + C)$  is calculated to characterize the spatial dependency of the values. A nugget/ sill ratio is classified as strongly spatially dependent if the ratio is less than or equal to 0.25, moderately spatially dependent if the ratio is between greater than 0.25 and less than or equal to 0.75 while it is classified as a weak spatial dependent if it is greater than 0.75 (Cambardella et al., 1994).

Several semivariogram models were evaluated to select the best fit with the data. The model, spherical, Gaussian, or exponential that offers the best fit varies depending on the soil parameters (Ramzan and Wani, 2018). A cross-validation technique was used to evaluate and compare the performance of the OK interpolation method. The lowest RMSE (Root Mean Square Error) value indicates the best fit for the variogram model (Panday et al., 2018). The predictive maps of soil properties are then created using a semivariogram model through OK.

### Assessment of Accuracy of Interpolation Map

The effectiveness of interpolation was evaluated based on Goodness-of-Prediction Estimate ( $G$ ) Eq. (2). A “ $G$ ” value of 100% indicates a perfect prediction, positive values (i.e., 0 to 100%) show that the predictions are more reliable than the use of the sample mean, and negative values indicate that the predictions are less reliable than using the sample means (Laekemariam et al., 2018).

$$G = \left[ 1 - \frac{\sum_{i=1}^N [z(x_i) - \hat{z}(x_i)]^2}{\sum_{i=1}^N [z(x_i) - \bar{y}]^2} \right] \times 100 \quad (2)$$

Where,  $z(x_i)$  is the observed value at location  $i$ ,  $\hat{z}(x_i)$  is the predicted value at location  $i$ ,  $N$  is the sample size, and  $\bar{y}$  is the sample mean.

## RESULTS AND DISCUSSION

### Geostatistical Analysis

The semivariogram parameters obtained from the best-fit model are in Table 1. An exponential model produced the best fit to semivariogram for pH, EC, Ava-P, and Ava-K. This model is one of the standard models used in the study of soil properties (Cambardella et al., 1994; Reza et al., 2016). The spherical model was the best suited to the semivariogram of TSN, while a Gaussian model was the best fit for SOM.

The range for all soil properties varies from 96.76 m to 276.68 m, and therefore the length of the spatial autocorrelation is much longer than the sampling interval of 50 m. According to Goovaerts (1997), the current sample design is appropriate for this study, and it is expected that the interpolated map will display good spatial structure.

In the present study, the nugget/ sill ratio showed that EC, Ava-P, and Ava-K were strongly dependent spatially whereas, pH, SOM, and TSN were moderately dependent spatially. The strong spatial dependency suggests that intrinsic factors, such as climate, parent material, topography, soil properties, and other natural factors, play important roles in spatial variability. The weak spatial dependency indicates that the spatial variability is mainly caused by extrinsic factors, such as fertilization, local farming practice, cropping systems, and other human activities. The moderate spatial dependency shows that spatial variability is caused by a mix of extrinsic and intrinsic factors (Bhunia et al., 2018; Cambardella et al., 1994).

The G-values are greater than zero for all soil parameters. This value indicated that spatial prediction using semivariogram parameters is better than assuming that the mean of observed values is the best value for an unsampled location. This result also shows that semivariogram parameters obtained from fitting experimental semivariogram values describe the spatial variation reasonably (Reza et al., 2010).

**Table 1 Geostatistical parameters of the fitted semivariogram models for soil properties**

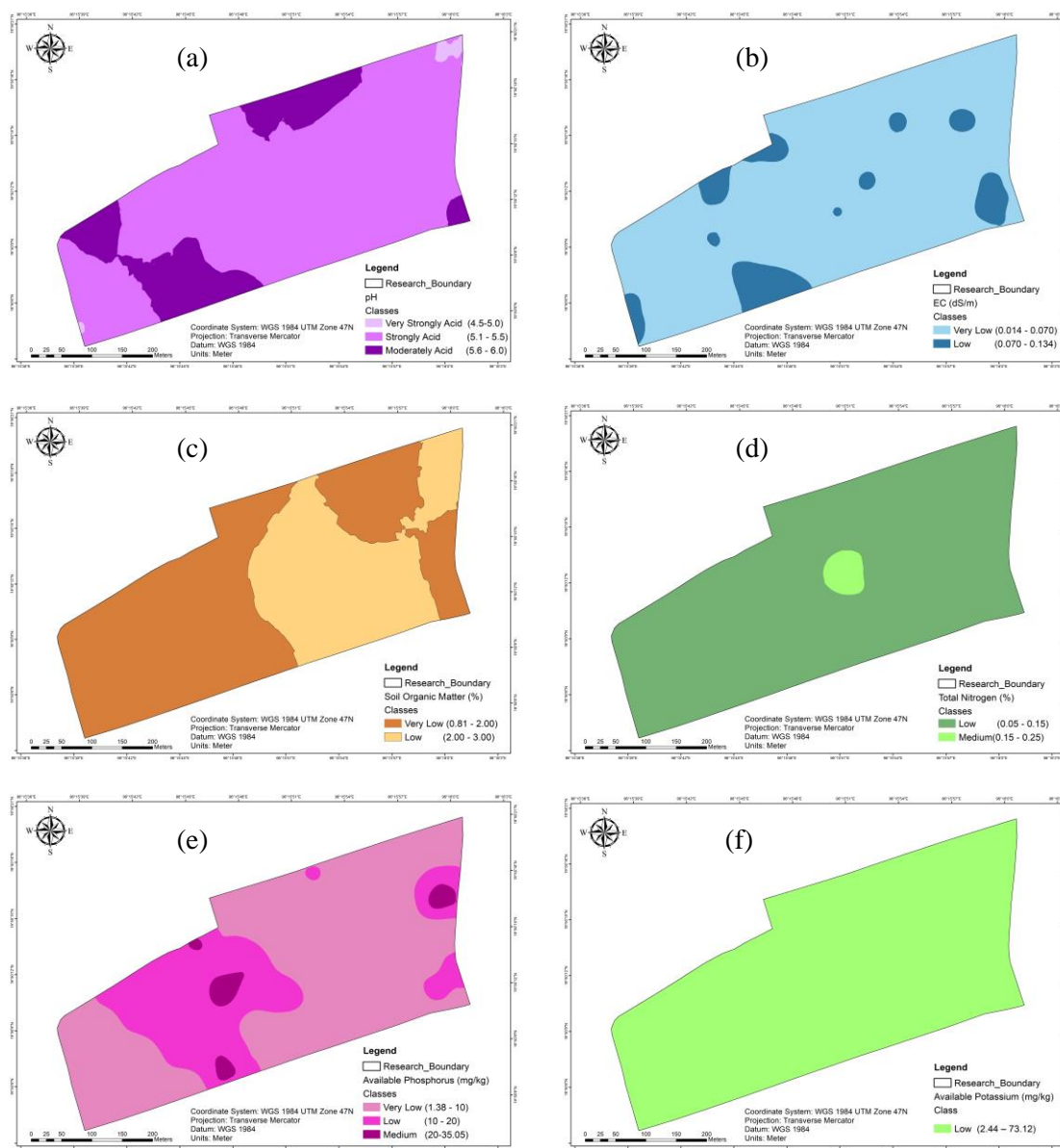
Parameters	Model	Nugget	Sill	Range (m)	Nugget / Sill	DSD	RMSE	G (%)
Soil pH	Exponential	0.1037	0.1822	253.34	0.57	Moderate	0.41	6.11
EC	Exponential	0.0000	0.4822	96.76	0	Strong	0.03	13.25
SOM	Gaussian	0.0900	0.2869	276.68	0.31	Moderate	0.34	47.43
TSN	Spherical	0.0005	0.0008	227.30	0.38	Moderate	0.03	25.08
Ava-P	Exponential	0.0856	0.6445	178.09	0.13	Strong	6.71	25.66
Ava-K	Exponential	0.0000	214.0108	108.58	0	Strong	12.26	39.90

*DSD: Degree of Spatial Dependence, RMSE: Root Mean Square Error, G: Goodness of Prediction*

### Spatial Distribution of Soil Properties

The parameter of the fitted semivariogram models is used for OK to produce a spatial distribution map of soil properties in the study area. The spatial distribution of soil properties such as pH, EC, SOM, TSN, Ava-P, and Ava-K are shown in (Fig. 2a-e). The distribution of the predicted soil pH map (Fig. 2a) shows that 0.77%, 80.92%, and 18.31% of the study area were very strongly acid, strongly acid, and moderately acid, respectively. Most of the soils were strongly acid which may be caused by a mixture of the nature of the soil mineralogy, the use of acidic fertilizers, low input of organic materials,

and removal of base nutrients (Rawal et al., 2018). The predicted map of EC (Fig. 2b) shows that 89.22% and 10.78% of the soil in the study area, can be described as very low and low, respectively. According to the soil guide (Moore, 2001); low EC levels only have a minimal impact on plant growth.



**Fig. 2 Spatial distribution maps for (a) pH, (b) EC, (c) SOM, (d) TSN, (e) Ava-P and (f) Ava-K**

The distribution of SOM (Fig. 2c) ranged from very low (62.73%) to low (37.27%), but low levels were most prevalent. The lower organic matter content in these soils can be attributed to the poor management practices such as intensive cropping, the complete removal of crop residues, and lack of addition of organic fertilizer sources (Gebreselassie, 2002). The majority of the soils were low (97.75%) in total nitrogen content, whereas 2.25% of the study area is rated at a medium level (Fig. 2d). The low nitrogen levels in most areas could be as a result of continuous cereal-based cropping, lower external organic-N inputs (like plant residues, animal manures), N (nitrate ions) leaching problem, and addition of a low amounts of SOM (Patil et al., 2011). The Ava-P map (Fig. 2e) shows that, in terms of



area coverage, 68.83%, 28.42%, and 2.75% of the study area has very low, low, and medium levels respectively. This result indicates that, in general, the majority (97.25%) of the study area is deficient in phosphorus. The reason for low soil phosphorus levels may be due to the intensive cropping system, low pH (acidic) soils, the imbalanced use of fertilizer, and nutrient mining (Sertsu and Ali, 1983). The whole of the study area can be classified with a low rating for Ava-K content (Fig. 2f). The lowest Ava-K in the study area might be due to the lowest SOM and the continuous removal of potassium by cereal crops, as the field has been intensively cultivated for a long period.

## **CONCLUSION**

Most of the soil was strongly acidic. The selected soil properties had a distribution showing low content levels in most of the study area. The distribution of variability of the soil properties across the landscape might be sufficient to construct fertility maps. The generated spatial distribution and fertility maps can serve as a powerful tool for farmers, decision-makers, and planners to understand the existing soil conditions and make sensible decisions to better manage the soil for sustainability and productivity. These results show that geostatistical analysis with kriging is an effective tool for studying the spatial variability of soil properties and that it will be useful technique for future soil sampling campaigns in Myanmar.

## **ACKNOWLEDGEMENTS**

It is a pleasure to express special gratitude to the supervisory committee and Australia Centre for International Agricultural Research (ACIAR) which supports research grant and technical support for this study under the project title of “Management of nutrients for improved profitability and sustainability of crop production in central Burma (SMCN– 2014 -044)”.

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## Assessment of Insect Damage and Growth Performance of Dipterocarps Planted at Rainforestation Demonstration Farm at VSU, Baybay City, Leyte

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Received 30 December 2020   Accepted 16 May 2021   (\*Corresponding Author)

**Abstract** Assessment of associated insects and leaf damage caused by insects is essential concerning decisions to contribute to developing suitable rehabilitation techniques. Few studies have been done to identify the insect species associated with dipterocarp species and determine the damage caused by insects and their growth performance. Six species of dipterocarps, namely: *Dipterocarpus alatus* (hairy leaf apitong), *Hopea philippinensis* (gisok-gisok), *Shorea malibato* (malibato), *Shorea assamica* (manggasinoro), *Shorea polita* (malaanonang), and *Shorea almon*, were studied. This study was conducted to determine the insect-associated fauna using the visual and handpicking method of insect collection, leaf damage assessment using the Bioleaf app, and the morphological traits (i.e., basal diameter and plant height) on the growth performance of dipterocarps. There were eight orders of insects associated with the dipterocarps: Coleoptera, Diptera, Hemiptera, Heteroptera, Hymenoptera, Lepidoptera, Odonata, and Orthoptera. There was a significant difference ( $p \leq 0.05$ ) in the leaf damage among the six dipterocarps species after 25 months from planting. *Shorea assamica* had the highest leaf damage ( $8.68\% \pm 0.09$ ), and *Shorea almon* had the least leaf damage ( $2.57\% \pm 0.09$ ). In terms of basal diameter, the species with the highest significant increment ( $p \leq 0.05$ ) was *Shorea polita* ( $2.49 \pm 0.67$  mm), while *Shorea almon* had the least growth increment ( $0.98 \pm 0.67$  mm) 25 months after planting. *Dipterocarpus alatus* grows faster for the plant height than other species with a significant increment ( $p \leq 0.05$ ) of  $32.90 \pm 0.19$  cm, while *Shorea assamica* had the least increment of ( $4.95 \pm 0.19$ ) cm. The study indicated eight orders of insects associated with the dipterocarps showing significant damage on the *S. assamica*. Despite the insect association, the plants grow significantly with the rapid increase observed on *D. alatus*.

**Keywords** dipterocarp, defoliation, insect fauna, leaf damage, bioleaf

## INTRODUCTION

Herbivorous species, mostly insects, are a significant global biodiversity component, comprising approximately 25% of all described species. Insect herbivores are among the many biotic factors known to help maintain forest diversity through selective predation on vulnerable tree species' seedlings altering forest community composition (Norghauer and Newbery, 2013). Insect herbivores, directly and indirectly, influence plant community composition by altering the recruitment, mortality, or individual growth rates of plant species, as supported by Maron and Crone, 2006.

Dipterocarps are known worldwide because of their economic and ecological functions. It has a good timber quality exported to other countries in finished products such as plywood and sawn timber (Corlett and Primack, 2005). According to Langenberger (2005), some species of dipterocarps are indicators for site suitability in local reforestation programs. Moreover, insect herbivores increase tree seedling recruits (Dyer et al., 2010). Annual rates of leaf damage are higher in tropical forests than in temperate broad-leaved forests (Coley and Barone, 1996). In the natural Dipterocarp forest, insects are the primary source of damage as leaf feeders, borers, suckers, and gall formation (Appanah, 1998).

Despite the knowledge about the damages caused by the insects' attack, few studies have been done in the Philippines to identify the insect species associated with *Dipterocarpus alatus* (hairy leaf apitong), *Hopea philippinensis* (gisok-gisok), *Shorea malibato* (malibato), *Shorea assamica* (manggasinoro), *Shorea polita* (malaanonang), *Shorea almon* and its growth performance. These species were chosen because of their conservation status according to the Updated National List of Threatened Philippine Plants and Their Categories by the Department of Environment and Natural Resources Administrative Order No. 2017-11. Assessing the damage caused by the insect is essential concerning assisting experts to make better decisions to contribute to efforts of developing suitable rehabilitation techniques and to recommend favorable dipterocarp species that can stand insect herbivory. Furthermore, the study used a novel approach in assessing leaf damage i.e., using the Bioleaf app for the first time on dipterocarps which in the literature has been commonly used in assessing leaf damage on soybean (Machado, 2016)

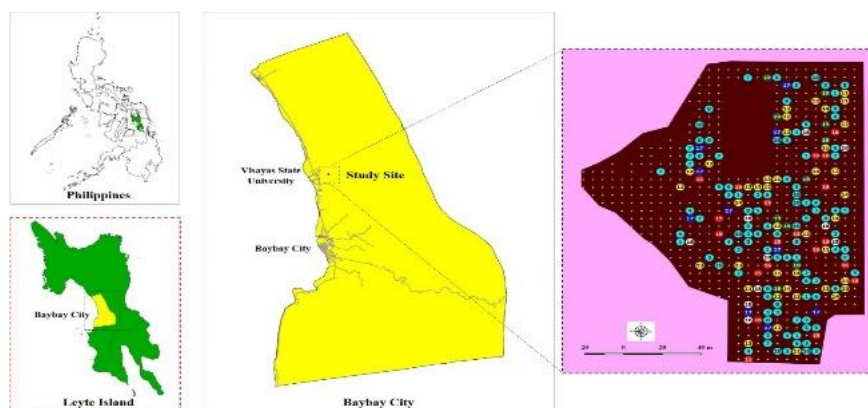
## OBJECTIVES

1. To identify the insect species associated with the dipterocarps planted in the dipterocarp germplasm and;
2. To assess the leaf damage of insects and growth performance of dipterocarps planted in the dipterocarp germplasm of VSU, Baybay City, Leyte, Philippines.

## METHODOLOGY

### Location of the Study Site

The study site was located at the Dipterocarp germplasm of the Terrestrial Ecosystems Division (TED), Institute of Tropical Ecology and Environmental Management (ITEEM) field laboratory established near the Reforestation Research Training Center (RRTC), Visayas State University-Main Campus, Baybay City, Leyte, Philippines (Fig. 1). It has a total area of approximately 1.80 hectares planted to 30 species of dipterocarps. The seedlings were randomly planted with a planting distance of 5 meters x 5 meters, constituting 21 seedlings per species. Six species of dipterocarp trees are randomly selected among the species of dipterocarps planted in the germplasm.



**Fig. 1** The study site is located in Visca, Baybay City, Leyte, Philippines.

### Assessment of Insect Fauna Associated with Dipterocarps

To identify the insect species inflicting damage on the trees and affected their growth performance, visual observation and handpicking was used as an insect collection method (Fig. 2). This included documenting all insects encountered and their damage among the sample plants. Insects observed were photographed, documented, and were collected by handpicking. They were brought to the laboratory to validate and identify the type of damage to the tree species. Insects that were collected were identified according to Order level. Sampling was done every morning (6:00 to 8:00 a.m.) and afternoon (3:00 to 5:00 p.m.). Samples were separately placed in jars with a killing agent and brought to the laboratory for processing, identification, and recording. Insects collected from each sample tree were separately kept for counting and sorting (Fig. 3). The different arthropod species were classified according to the following categories (Wall work, 1976 as cited by Ceniza, 1995). Insects can be considered “accidental” if the species occurs in 1-24 % of samples; “accessory” if the species occurs in 25-49% of samples; “constant” if the species occurs in 50-74% of samples; and “absolute” if the species occurs in 75-100% of samples.



**Fig. 2** Visual and handpicking

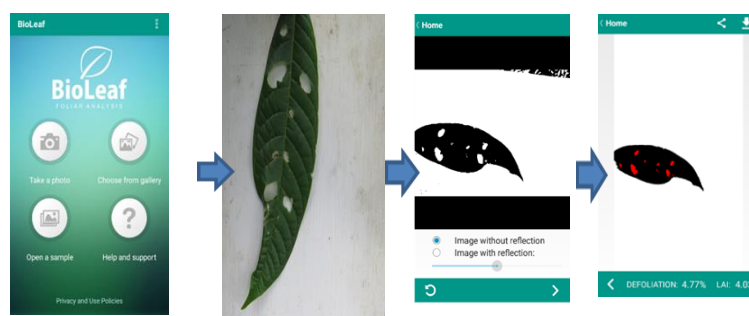


**Fig. 3** Sorting of insects in labeled plastic wares and vials

### Assessment of Leaf Damage Using the Bioleaf App

The study followed the methodology on insect herbivory assessment by Herve et al. 2017, which is suitable for this study. Measures were made on a fixed number of leaves per tree, choosing two facing opposite branches at the top and two facing opposite branches at the middle, and two facing opposite branches bottom of the tree crown. Ten leaves were randomly chosen at the top of a branch, ten at the

middle, and ten leaves at the bottom per tree per species. If there were not enough leaves on the branch, one could choose another branch at the same height. If there are not enough branches, one can use the main axis. Assessed leaves were different from one assessment to the next because some may fall, some may have appeared, and damage may have accumulated. Bioleaf foliar analysis was used to estimate the damage (Machado et al., 2016), a professional mobile application to measure foliar damage caused by insect herbivory, developed by a Brazilian team of researchers released in 2016. It then estimated the defoliation percentage related to the total area using images captured from the camera or loaded from the photo gallery. Pictures of the leaf images taken from the field were loaded into the bioleaf app, automatically reading the injured leaf regions caused by insect herbivory and estimating the total area's defoliation percentage (Fig. 4).



**Fig. 4 Process flow for the bioleaf foliar analysis**

### **Growth Performance of Dipterocarp Species**

Ten sample plants per species of dipterocarp were measured every three months. The basal diameter (mm) was measured at the base of the stem using a Vernier caliper. The sample plants' base was marked with white ink to ensure a permanent measurement point from the present to the following data collection (Fig. 5). Meanwhile, the plant height (cm) was measured using a meter stick. It was taken from the stem's base up to the stem's tip (Fig. 6).



**Fig. 5 Measuring the basal diameter**



**Fig. 6 Measuring the total plant height**

### **Data Encoding and Statistical Analysis**

All data gathered were collated, encoded, and summarized using an electronic spreadsheet editor, Microsoft Excel 2013. The data were analyzed using the Statistical Package for Social Science (SPSS version 20). The mean of basal diameter and plant height variability were analyzed using the one-way analysis of variance (ANOVA). Moreover, in a case where the significant variations at  $p \leq 0.05$  were identified, Tukey and Least Squares Differences (LSD) were carried out to compare means.

## RESULTS AND DISCUSSION

### Insect Species Associated with Dipterocarp Species

Table 1 shows the list of insect orders associated with the dipterocarps. It included eight insects, namely, Coleoptera, Diptera, Hemiptera, Heteroptera, Hymenoptera, Lepidoptera, Odonata Orthoptera. These groups were composed of chewers and suckers, the major groups causing damage to the dipterocarp species.

**Table 1 List of insect order associated with the dipterocarp species**

Dipterocarp species	Insect orders with constancy class
<i>Dipterocarpus alatus</i>	Orthoptera-(Ab), Coleoptera- (A), Hemiptera- (A), Lepidoptera (Ab), Odonata (A)
<i>Hopea philippinensis</i>	Orthoptera-(C), Coleoptera- (Ac), Hemiptera- (Ac), Lepidoptera (Ab)
<i>Shorea almon</i>	Orthoptera-(A), Coleoptera- (A), Hemiptera- (A), Lepidoptera (Ab)
<i>Shorea assamica</i>	Orthoptera-(Ab), Coleoptera- (Ab), Hemiptera- (Ab), Lepidoptera (Ab), Heteroptera (Ab), Diptera (C), Hymenoptera (Ab), Odonata (Ac)
<i>Shorea malibato</i>	Orthoptera-(C), Coleoptera- (Ac), Hemiptera- (Ac), Lepidoptera (Ab)
<i>Shorea polita</i>	Orthoptera-(C), Coleoptera- (A), Hemiptera- (A), Lepidoptera (Ab)

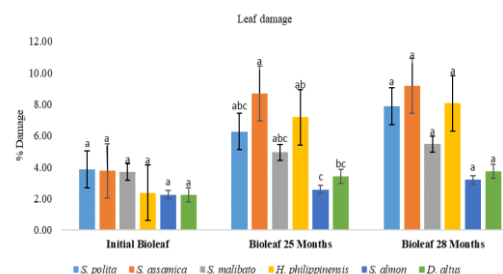
Note: Accidental 1-24% Ac- Accessory 25-49% C- Constant 50-74% Ab- Absolute 75-100%

### Leaf Damage

The leaf damage samples of six dipterocarp species were shown in Fig. 7. The statistical analysis results showed significant ( $p \leq 0.05$ ) differences in the leaf percentage damage among the six dipterocarps species during the data collection 25 months after planting. Fig. 8 shows the results on the leaf percentage damage of dipterocarp species in the three sampling periods. *Shorea assamica* had the highest leaf damage ( $8.68\% \pm 0.087$ ), and *Shorea almon* had the least leaf percentage damage ( $2.57\% \pm 0.087$ ).



**Fig. 7 Leaf damage samples of six dipterocarp species**



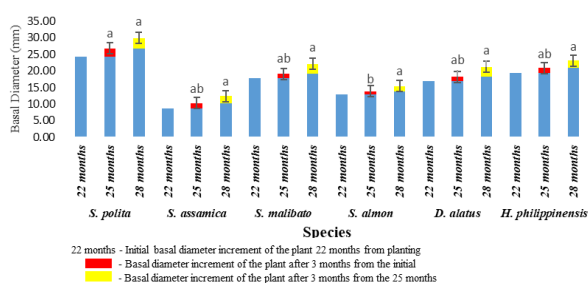
**Fig. 8 Percentage damage of leaves of six dipterocarp species**

Note: Values in the figure with different letters (a-b) designation across treatments during data collection periods are statistically significant at  $p \leq 0.05$ .  $N = 10$  in total for all treatments per species per period.



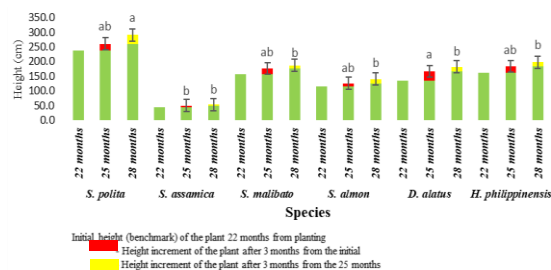
## Basal Diameter and Plant Height

The different dipterocarp species' growth performance showed increments and variations after a 3-month sampling period, 25 months after planting. Increments are used as a measure of performance in our forest stands for a particular period (Assmann, 1970). This was calculated as the difference between the initial growth of the height and basal diameter of the plant at 22 months and its growth after 3 months and 6 months. The most significant growth increment was the *Shorea polita* ( $2.49 \text{ mm} \pm 0.67$ ) regarding basal diameter. At the same time, *Shorea almon* had the least growth increment ( $0.98 \pm 0.67$ ). However, it can be seen from the results that regardless of the dipterocarp species, there was an increase in the growth of the basal diameter between the periods of the first three months and six months (Fig. 9). At least one species in every Dipterocarp genus differed in terms of basal diameter among other species. There was a significant increase in dipterocarp species for the plant height after six months of data collection, 28 months after planting (Fig. 10). There is evidence that at least one species in each Dipterocarp genus differs in plant height among other species. Notably, *Dipterocarpus alatus* ( $32.90 \text{ cm} \pm 0.19$ ) had a considerable increment in plant height, while *Shorea assamica* ( $4.95 \text{ cm} \pm 0.19$ ) had the least increment, 28 months after planting.



**Fig. 9 Basal diameter increment of six dipterocarp species**

Note: Values in the figure with different letters (a-b) designation across treatments during data collection periods are statistically significant at  $p \leq 0.05$ .  $N = 10$  in total for all treatments per species per period.



**Fig. 10 Height increment of six dipterocarp species**

Note: Values in the figure with different letters (a-b) designation across treatments during data collection periods are statistically significant at  $p \leq 0.05$ .  $N = 10$  in total for all treatments per species per period.

## CONCLUSION

There are eight orders of insects associated with dipterocarps: Coleoptera, Diptera, Hemiptera, Heteroptera, Hymenoptera, Lepidoptera, Odonata, and Orthoptera, showing significant damage on the *S. assamica*. There was a significant difference ( $p \leq 0.05$ ) in the leaf damage among the six dipterocarps species during the data collection 25 months after planting. *Shorea assamica* had the most significant leaf damage, and *Shorea almon* had the least leaf damage. The different dipterocarp species' growth performance showed significant increments ( $p \leq 0.05$ ) and variations after a 3-month sampling period, 25 months after planting. In terms of basal diameter, the species with the most significant growth increment was *Shorea polita*. At the same time, *Shorea almon* had the least growth increment. *Dipterocarpus alatus* grow significantly ( $p \leq 0.05$ ), which had the largest increment for the plant height despite the insect association, while *Shorea assamica* had the least increment. The assessment of associated insects is more relevant in assessing the damage of insects. Thus, it is suggested that a further detailed study and analysis on the family and species level of other groups should be conducted to enhance the recent findings.



## **ACKNOWLEDGEMENT**

This study was supported by the Environmental Leadership and Training Initiative (ELTI) and Visayas State University (VSU).

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# Spatial Variability of Soil Salinity and its Influence on Rice Yield in Salt-Affected Areas using Remote Sensing Techniques

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Received 1 March 2021    Accepted 16 May 2021    (\*Corresponding Author)

**Abstract** Soil salinity has become one of the major constraints to sustain crop production in Myanmar, especially in the dry zone areas. Salinity stress and its spatial distribution has been a useful for crop monitoring. Unevenness in early crop growth stage can support to identify yield-limiting factors such as soil salinity, nutrient availability, and soil moisture. Remote sensing techniques have been used to collect reflectance numbers from crop canopies and to analyze the vegetative index (VI). VI has been related with percentage of ground cover, chlorophyll content of plant, and nitrogen use efficiency. This study aims to monitor the influence of salinity stress on the growth, yield and chlorophyll content of rice by using remote sensing techniques. This study was conducted in the salt-affected soils at Myittha Township, the central dry zone of Myanmar during the rainy season of 2019. Electrical conductivity (EC) and chlorophyll content in rice plant were collected at early growth and tillering stages of rice. Sentinel-2 satellite imagery was used in the analysis for those two growth stages. Total grain yield of rice was also measured. The acquired images analysis was implemented with ArcGIS 10.7 software to calculate vegetative indices. The results showed that significant relationships were found between plant chlorophyll content and the normalized difference vegetation index (NDVI) values under different salinity levels. A relationship was also observed between NDVI and rice grain yield. Identifying the spatial distribution of salinity stress using the spectral vegetation indices would be effective for increasing rice yield in the salt-affected areas.

**Keywords** salinity stress, Sentinel-2 satellite imagery, vegetation index

## INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop for more than half of the population of the world. It is cultivated over 167 million hectares with the production of 780 million tones (FAO STAT, 2017). It is grown under various environmental situations in both upland and lowland rainfed ecosystem. Crop production in Myanmar is affected by many factors such as soil salinization, drought, and low soil fertility (Oo et al., 2017). Among them, soil salinization is an increasingly severe problem in rainfed rice production.

In Myanmar, salt-affected soils are found in coastal and inland regions. Coastal salinity is affected by seawater intrusion/ infiltration during flood resulting salt accumulation in the top soil in the summer season. It is commonly occurred in Ayeyarwady, Yangon, Yakhain and Taninthari regions. Inland salinity is commonly happened in dry zone areas of the central Myanmar such as Mandalay, Magway and Sagaing regions. Salinization in central dry zone is becoming a prominent abiotic problem declining rice production which little or lack of attention was paid in the past (Swe and Ando, 2017). They reported that sodic/saline soils have been settled in certain areas, because of the excessive

application irrigation for several years continuously. The excessive applications of irrigation water elevated the ground water level sufficiently to increase salts concentration through evaporation. It is related principally to the presence of sodium carbonate and sodium bicarbonate in these specific areas. Inland salinity or irrigation salinity is due to over-watering, seepage from irrigation channel, impaired natural drainage and high water table. In the low land soil from those salt affected areas, high rate of evaporation and evapotranspiration of rice crop increase the capillary transport of water and solutes from the groundwater to the root zone. When there is a condition of no or negligible leaching of these salts, the soils will be affected with salts within a few years.

The nutritional monitoring of plants using remote sensing is a vital component of precision agriculture (Liaghat and Balasundram, 2010). Remote sensing has received increased interest as a non-destructive tool for determining the nutrient status of growing crops due to the time and expense involved with traditional soil and plant analysis. Remote sensor can estimate chlorophyll content and nitrogen status by their reflectance in the visible region of the electromagnetic spectrum (Thomas and Gausman, 1977). By using remote sensing images, normalized difference vegetation index (NDVI) is essential parameter for the vegetation growth of crops as well as assessing plant nitrogen content.

The concept of precision agriculture is based on the fact that crop productivity varies spatially and temporally within a field, depending on soil, environment and operational activities. One direction in precision agriculture research is to identify the correct management plot based on the variability in yield limiting factors within a field, and to implement optimal management practices for each plot. The effects of salinity stress on crops are complex and it is difficult to interpret the results if investigates are not designed carefully and if suitable measurements are not made (Negrao et al., 2017). Therefore, classifying the soil salinity variability and its influence to crop yield are essential for farmers to reclaim the salt-affected soils.

## OBJECTIVE

This study was to determine the influence of salinity stress on the growth, yield and chlorophyll content of rice by using remote sensing techniques.

## MATERIALS AND METHODS

### Study Area

In order to determine the influences of salinity stress on the growth and yield of rice, the experiment was conducted in the salt-affected area at Hteinkangyi village in Myittha Township, Mandalay Division where is situated in the dry zone area of Myanmar during the rainy season of 2019. Geographical coordinates are between 21°14'16.36" N latitudes and 96°8'23.88" E longitudes. Its elevation is 106-111 m above sea level. The total area of the study site is 40 hectares. The study site has been affected by salinity for 20 years. The study location map is shown in Fig. 1.

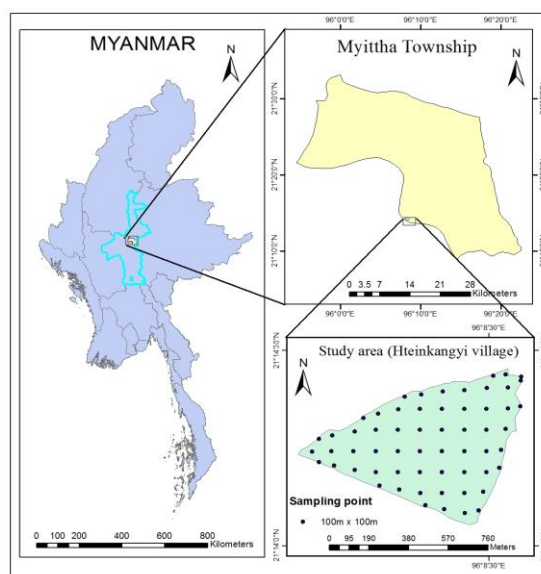
### Field Data Collection

The field data was collected during the rainfed rice growing season of 2019. Apparent electrical conductivity was collected by using a portable electrical conductivity meter (Field Scout (FS) direct soil EC meter) before the rice cultivation. Electrical conductivity ( $EC_e$ ) in saturated paste extracts was calculated by using the formula ( $2.7FS+0.8$ ).

Chlorophyll content was measured randomly from three mature leaves in each sampling point using a Minolta SPAD-502 leaf chlorophyll meter at the early growth and tillering stages of rice. Rice grain yield was recorded at harvesting. Pawsan and manawthuka varieties were cultivated in the study area.

## Vegetation Index (VI) Calculation

The multispectral images from Sentinel-2A Satellite were used in this study. Sentinel-2A has four bands, namely red (R), green (G), and blue (B) and near-infrared (NIR) at 10 meter spatial resolution. The Sentinel-2 image was acquired at the time of early growth and tillering stages of rice. The normalized difference vegetation index (NDVI) was calculated for those two growth stages of rice. This index is widely used for measuring crop health - a healthy plant will absorb visible light (especially blue and red), while the fortified leaf structure will reflect a high amount of NIR. NDVI calculation is simply  $(\text{NIR} - \text{red}) / (\text{NIR} + \text{red})$ , returning values between -1 and 1, ranging from non-vegetated (water or barren) to healthy plants (Rouse et al., 1974; Tucker, 1979).



**Fig. 1 Map of study area (Hteinkangyi Village)**

## Data Analysis

The collected data were examined by descriptive statistics to attain the minimum and maximum values, mean, standard deviation (SD), coefficient of variation (CV) of each parameter. All statistical analyses were implemented using Statistix 8.0 software and Excel program (2010). The spatial distribution maps of soil electrical conductivity, SPAD values and rice grain yield were accomplished by ArcGIS 10.7 software.

## RESULTS AND DISCUSSION

### Spatial Variability of Soil Salinity and Rice Grain Yield

The spatial variability of soil salinity is presented in Fig. 2. Soil salinity was found at three levels as  $2.0\text{--}3.9 \text{ dSm}^{-1}$  (low salinity),  $4.0\text{--}6.0 \text{ dSm}^{-1}$  (moderately salinity) and  $6.1\text{--}7.6 \text{ dSm}^{-1}$  (high salinity). This distribution map can help the farmers to manage or remediate their salt-affected soils accordingly to soil EC level.

Rice grain yield can be varied as affected by soil salinity. The result showed that rice grain yield ranged from  $3.00$  to  $4.83 \text{ ton ha}^{-1}$  with the mean value of  $4.11 \text{ ton ha}^{-1}$  ( $\text{SD}=0.39$ ) and CV of 9.46%. Spatial variability of rice grain yield in the study area was obvious (Fig. 2). This might be due to

differences in soil salinity become limiting. Previous study (Oo et al., 2019) reported that salinity stress on the growth of tested rice varieties was found seriously in  $7.5 \text{ dSm}^{-1}$  when compare with  $3.7$  and  $5.7 \text{ dSm}^{-1}$  salinity values in the Hteinkangyi Village.

### Chlorophyll Content of Rice Plant

Interpolated maps of chlorophyll meter (SPAD) values at the early growth stage and tillering stage of rice in the salt-affected soils are presented in Fig. 3. The results of this study showed that chlorophyll content was observed relatively large variation within a farmland at the early growth stage (Fig. 3a). It ranged from 23.50 to 38.90 SPAD reading. At the tillering stage, it ranged from 28 to 43 (Fig. 3b). The distribution maps of SPAD values showed that soil salinity affected the chlorophyll content in plant. The plant under low salinity level had the highest chlorophyll content indicating that it may not be affected by salinity. In contrast, the rice plant under the high salinity level produced the low chlorophyll content which was due to limitations of chlorophyll synthesis (Santos, 2004) and it resulted in increased reflectance of active light for plant photosynthetic (Clay et al., 2006) and decreased reflectance in the near-infrared light (Cui et al., 2009; Yoder and Pettigrew-Crosby, 1995).

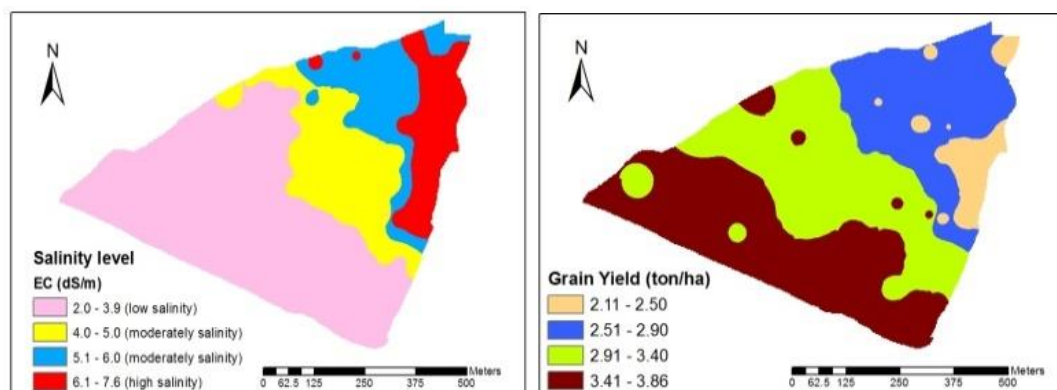


Fig. 2 Interpolated maps of salinity level and rice grain yield in the study area

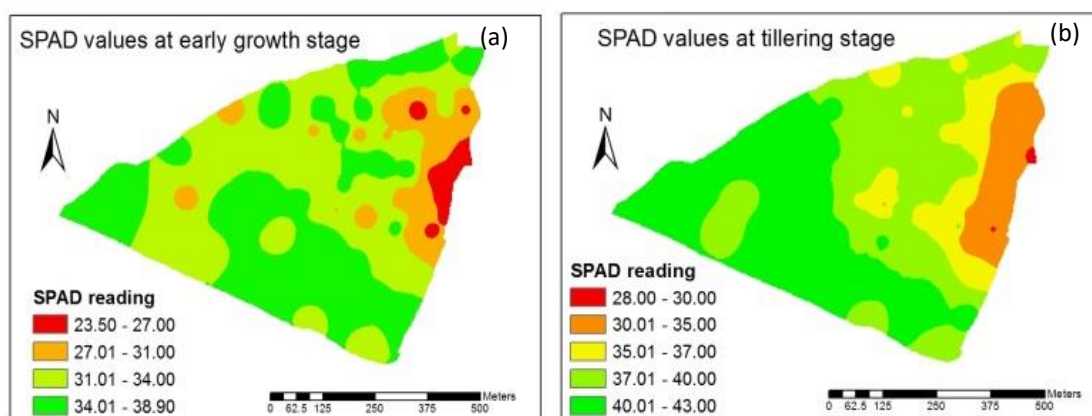
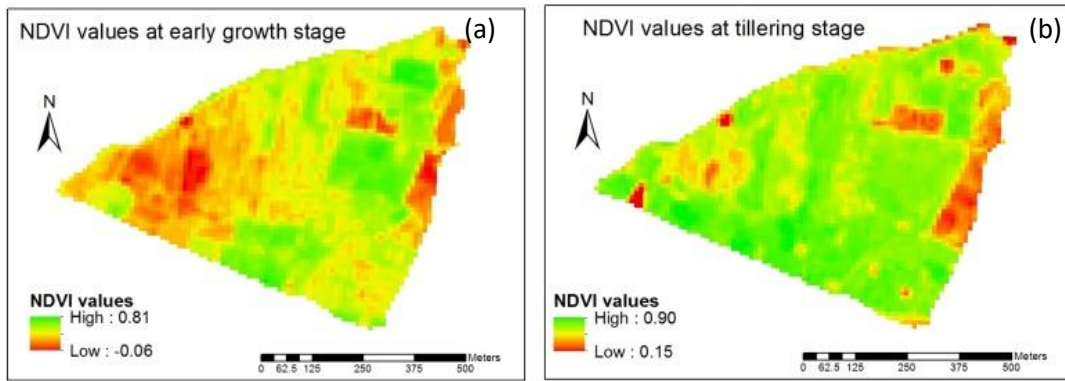


Fig. 3 Interpolated maps of chlorophyll meter (SPAD) readings in the salt-affected soils of Hteinkangyi Village (a) early growth stage and (b) tillering stage



**Fig. 4 NDVI maps for Sentinel-2 spatial resolutions at (a) early growth stage and (b) tillering stage of rice in the salt-affected soils of Hteinkangyi Village**

### Vegetation Index (VI)

The VI maps were produced at the early growth stage and tillering stage of rice using Sentinel-2 satellite imagery. The maps are shown in Fig 4. The NDVI map demonstrated the vast variation at early growth stage (Fig. 4a). The decreasing NDVI values at early growth stage may be attributed to decrease in the number of rice leaves. The NDVI values largely rise up at tillering stage (Fig. 4b). The high NDVI values (0.90) were observed in the tillering of rice when compared with the early growth stage. Scattered vegetation or death of rice plant was found under high salinity soils, indicating that the effect of salinity stress on rice is the shrinkage of leaf size, which leads to damage of the leaf, and lastly the death of the plant (Volkmar et al., 1998).

### Relationships among $EC_e$ , NDVI, SPAD Values and Rice Yield

In this study, there was negatively significant relationship  $EC_e$  and NDVI values for both growth stages (Table 1), indicating the higher the  $EC_e$  the lower chlorophyll meter in rice plant.  $EC_e$  values was negatively significantly correlated with rice yield ( $p < 0.000$ ), indicating the higher the  $EC_e$  the lower rice yield. The SPAD value was positively associated with the NDVI values for both growth stages. Rice yield was positively associated with the NDVI values and SPAD values for both growth stages. These results showed that NDVI and SPAD value was a strong predictor of rice grain yield. The results of this study can be useable information for the farmers in the study area.

**Table 1 Relationships among  $EC_e$ , NDVI, SPAD values and rice yield at two different growth stages in Hteinkangyi Village during the rice growing season of 2019**

	Variable	Regression equation	$R^2$	$p$ -value	Acquired growth stage
$EC_e$	NDVI values	$Y = -0.0547x + 0.7347$	0.342	0.000	Early growth
	NDVI values	$Y = -0.043x + 0.9014$	0.452	0.000	Tillering
	Yield	$Y = -0.2308x + 4.1096$	0.679	0.000	Harvesting
SPAD	NDVI values	$Y = 0.0251x - 0.152$	0.237	0.000	Early growth
	NDVI values	$Y = 0.0294x - 0.2964$	0.255	0.000	Tillering
Yield	NDVI values	$Y = 1.5546x + 2.3764$	0.267	0.000	Early growth
	NDVI values	$Y = 2.5674x + 1.28$	0.245	0.000	Tillering
	SPAD values	$Y = 0.0458x + 1.4948$	0.140	0.003	Early growth
	SPAD values	$Y = 0.097x - 0.6633$	0.453	0.000	Tillering

## CONCLUSION

This research evaluated the salinity and its influence on rice in the salt-affected areas in Hteinkangyi village, Myittha township, Myanmar employing with remote sensing technique and geographic information system (GIS). The maps created by ArcMap 10.7 presented virtual concept of salinity management system which could be useful for the farmers in the study area. The vast variability of soil salinity was found. Soil salinity was affected on the vegetation development and yield of rice under the high salinity area.

Sensor values and rice grain yields had positively significant relationships at all growth stages. The distribution maps are very applicable to farmers for reclaiming the salinity level to their farmlands. The results showed that vegetative index could be useful in detecting the salinity stress in salt-affected area. Based on this study, it could be suggested that the Sentinel-2 satellite imagery would be beneficial for monitoring crop development and managing the salt-affected soils.

## ACKNOWLEDGEMENTS

The authors would like to thank the JICA (Japan International Cooperation Agency), Project for Capacity Development of Yezin Agricultural University for financial support.

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## **Evaluation of the Acceptability of Cashew Apple Jam in Cambodia**

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Received 24 December 2020   Accepted 16 May 2021   (\*Corresponding Author)



**Abstract** This study aimed to evaluate the acceptability of cashew apple jams based on a sensory evaluation test in Cambodia. Sensory evaluation tests for 2 types of cashew apple jams were conducted in Phnom Penh, Cambodia, and in Setagaya, Japan, in September 2019 for 70 persons. The jam raw materials were cashew apple, sugar, low-methoxyl pectin, and citric acid. These materials were mixed, dissolved, condensed, and prepared to 50 °Brix sugar content while heating to approximately 80°C. The jam heated to 80°C was filled in a glass bottle. We called this sample a hot-pack jam. The hot-pack jam was reheated at 90°C for 20 min in a sterilization process. After 20 min, the jam was kept at room temperature for cooling with sterilization. We called the sample with the sterilization procedure a reheated jam. The color, sweetness, acidity, taste, flavor, jelly state, smoothness, and overall score of each sample were evaluated using a five-point hedonic scale (1: hate, 2: dislike, 3: neutral, 4: like, and 5: like very much) in the sensory test. The hot-pack and reheated jams were tested by 40 panelists of Cambodian students and faculty members at the Royal University of Agriculture, Cambodia (RUA), and Tokyo University of Agriculture, Japan (TUA). Thirty citizens evaluated only reheated jam at a supermarket in Phnom Penh. Almost all panelists answered that the reheated jam was sweeter than the hot-pack jam. Because the scores of both jams given by panelists ranged from 3 to 4, the cashew apple jam was considered to be acceptable and had a possibility to become a new processed food in Cambodia.

**Keywords** cashew apple, jam, sensory evaluation, acceptability

## INTRODUCTION

New processed food made from local sites is expected to increase income for farmers and support their independence. The cashew (*Anacardium occidentale* L.) is originally from Brazil and is also cultivated in tropical areas (Honorato and Rodrigues, 2010; Oliveira et al., 2020). The cashew nut is a part of the cashew fruit, which contains an edible pedicel called the cashew apple (Janick and Paull, 2008).

In Cambodia, the cashew plant is an industrial crop. The total yield of cashew nuts was 136,094 tons in 2018, and the largest cultivation areas in Cambodia were the provinces of Kampong Thom (58,624 ha), Ratanakiri (30,459 ha), Kratie (27,761 ha), and Kampong Cham (24,364 ha) (MAFF/GDA, 2018). However, only cashew nuts are commercialized; and the rest of the cashew fruits (cashew apples) become wastes. Providing a way to utilize cashew apple will support the economy of the agricultural industry. The cashew apple is rich in vitamin C (five times more than that in oranges) and minerals and is good for health (Akinwale, 2000). The cashew apple exhibits antibacterial properties, treating gastritis and ulcers in the stomach and preventing scurvy (Melo et al., 2003). Cashew apples can be eaten fresh, cooked in curries, used for fermented vinegar, consumed as alcoholic and nonalcoholic drinks, and used to make preserved food, chutneys, and jam (Ogunsina et al., 2008). Codex Alimentarius International Food Standards (CXS 296-2009, 2017) reported that jam is the product brought to a suitable consistency, made from the whole fruit, pieces of fruit, unconcentrated and/or concentrated fruit pulp or fruit puree. Jam is made of one or more fruits and is mixed with foodstuffs with sweetening properties with or without water. There are many reports about methods of preparing jam from various fruits and their sensorial attributes. Ajenifujah-Solebo et al. (2011) examined the possibility of producing jam from black plums and evaluated its physicochemical properties, nutritional properties, and consumer acceptability. Ho et al. (2020) studied the proximate composition, physicochemical characteristics, and sensory evaluation of reduced-calorie belimbi fruit (*Averrhoa belimbi*) jam with maltitol.

The appropriate procedure for processing fruit and making jam is different from the characteristics of using fruit. Jam in this study is processed from cashew apple pulp, sugar, low-methoxyl (LM) pectin, and citric acid. Some jam products are sold in Cambodia, but they do not appear to be common,

especially for local Khmer. Sensory evaluation is necessary to grasp whether a new cashew apple jam is preferred in Cambodia.

In this study, we investigated the acceptability of cashew apple jams based on a sensory evaluation in Cambodia. The sensory analysis of two types of cashew apple jams was conducted in Phnom Penh, Cambodia, in September 2019 with 70 persons. The jam is a gelled ready-to-eat product and has many uses, for example, bread, milk-product, sweets, and so on. The jam is easy to make without the need for any special devices and skills. The jam also can keep at room temperature. Therefore, we conducted the sensory evaluation of the cashew apple jam.

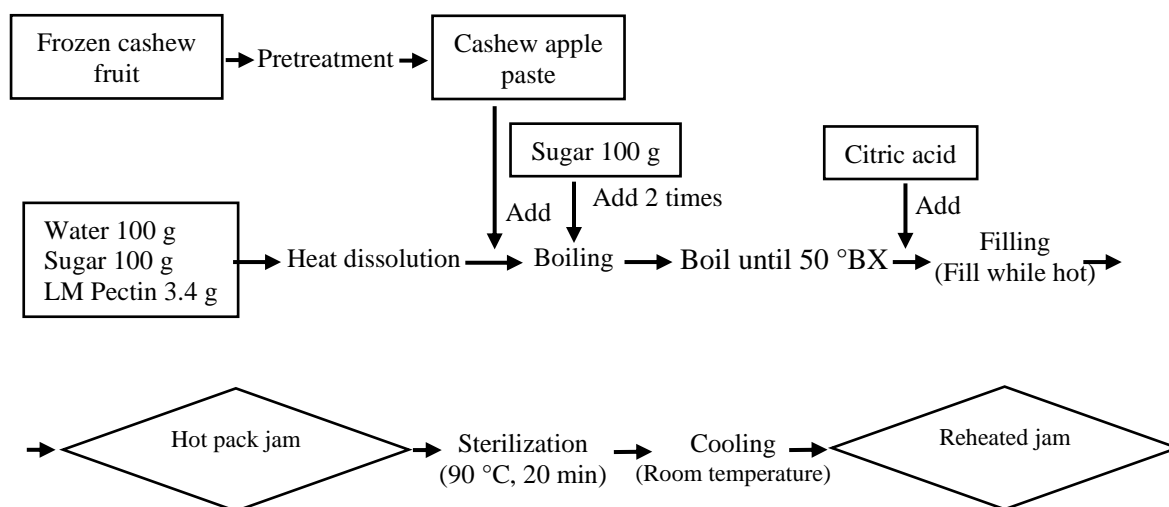
## OBJECTIVE

The objectives of this study were 1) to survey Cambodian citizens regarding the sensory evaluation of cashew apple jams and 2) to evaluate the acceptability for the sensory characteristics for cashew apple jams.

## METHODOLOGY

### Materials and Product Process of Jam

Cashew (*Anacardium occidentale* L.) fruit (cashew apple and nut) was purchased in Kampong Cham Province, Cambodia, and was stored in a refrigerator at approximately -18 °C until processing into jams. The cashew apple jam was made from 400 g of cashew apple paste, 300 g of sugar, 3.4 g of LM pectin, and 2.4 g of citric acid. Fig. 1 shows the production process of 2 types of cashew apple jams.



**Fig. 1 Product process of 2 types of cashew apple jams: a hot-pack jam and a reheated jam**

In the pretreatment of cashew apple paste, the frozen cashew fruit was thawed at room temperature. Then, the seed (cashew nut) and peer were removed from the cashew apple. The peeled fruit pulp (cashew apple) was crushed with a food processor for 2-3 min because there was substantial fiber in the pulp, and it did not fall apart even when boiled. During heat dissolution, LM pectin was mixed in 100 g of sugar in advance and dissolved in 100 g of boiling water. As pectin is difficult to dissolve in water, it must be mixed with sugar beforehand. After heat dissolution, 400 g of cashew

apple paste was added to the sugar and LM pectin solution. Keeping boiling this mixture, 100 g of sugar was added to this mixture twice. The boiling process took a total of 6-10 min. This mixture was boiled and concentrated up to 50 °Brix. After stopping heating, citric acid dissolved in a small amount of water was added to this concentrate. While hot, the jam was filled into a glass bottle up to 3-5 mm from the bottle lid. After closing the lid of the bottle, the bottle was turned upside down. We call this sample “hot-pack jam” in this manuscript. The hot-pack jam was sterilized for 20 min in hot water at a temperature of 90°C. We call the jam with the additional sterilization process “reheated jam”. After sterilization, the reheated jam was cooled to room temperature.

Fig. 2 shows photographs of the hot-pack and reheated jams. Each bottle contained 90 g of jam. Considering the sanitary conditions in Cambodia, the sterilization process was added to the hot-pack jam. As a result, as shown in Fig. 2, the browning phenomenon occurred in the reheated jam. The component composition of these jams was the following: moisture, 47.1%; protein, 0.40%; fat, 0.1%; ash, 0.2%; carbohydrate, 52.3% (sugar, 50.6%; dietary fiber, 1.7%).



**Fig. 2 The hot-pack and reheated jams**

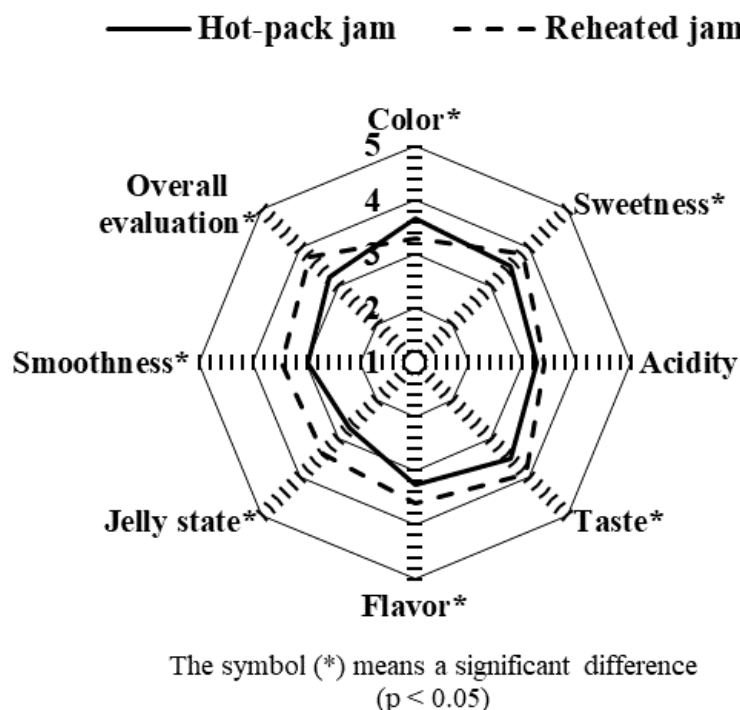
### Sensory Evaluation

Sensory evaluation plays an important role in assessing the acceptance of new food products and preferences for different consumers (Banaś et al., 2018). The color, sweetness, acidity, taste, flavor, jelly state, smoothness, and overall score of each sample were evaluated using a five-point hedonic scale (1: hate, 2: dislike, 3: neutral, 4: like, and 5: like very much) at the sensory test (preference type). The hot-pack and reheated jams were tested by 40 panelists consisting of Cambodian students and faculty members at the Royal University of Agriculture, Cambodia (RUA), and Tokyo University of Agriculture, Japan (TUA). These panelists are represented as the RUA group in this research. Thirty citizens evaluated only reheated jam at a supermarket in Phnom Penh, Cambodia. The time and space for sensory evaluation of jam were limited in this case. The reheated jam received favorable evaluation results in the RUA group. The reheated jam is safer than the hot-pack jam. Therefore, only reheated jam was evaluated at the supermarket. The panelist ages of the RUA group and citizens were 19-55 years (average: 22) and 18-64 years (average: 27), respectively.

### RESULTS AND DISCUSSION

The sensory evaluation results of both cashew apple jams from the RUA group are represented in Fig. 3. The average scores of sensory evaluation items for the hot-pack jam and the reheated jam ranged from 2.7-3.0 and 3.3-3.9, respectively. The reheated jam had higher scores than the hot-pack jam except for the color. The average values of all evaluation items for the hot-pack and reheated jams were tested for differences between population means (paired t-test), and there was a significant difference (5% significance level) between all items, except for acidity. Although the average value of

the color for the reheated jam was 3.3, this value was lower than that for the hot-pack jam (3.7). The occurrence of the browning phenomenon in the reheated jam affected this result. The average scores of the reheated jam were higher than those of the hot-pack jam except for the color. Because the score of the reheated jam for color was over 3: neutral, we considered that the color of the hot-pack jam was more favorable than that of the reheated jam but not unacceptable in the RUA group. The average sweetness, taste, and overall evaluation scores for the reheated jam were nearly 4.0, and the reheated jam received a high evaluation from the RUA group, which means that the reheated jam was more favorable. This result indicates that the addition of heat to the cashew apple jam after filling in the bottles influenced the final product characteristics and sensorial acceptance.

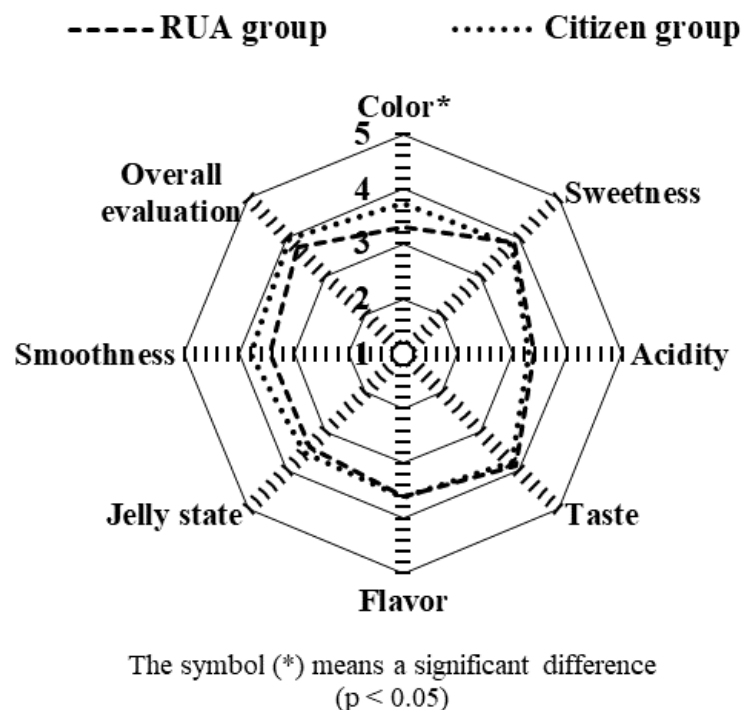


**Fig. 3 Sensory evaluation results of the hot-pack and reheated jams in the RUA group**

Fig. 4 shows the sensory evaluation results of the reheated jam from citizens, with a comparison to the results for the RUA group. The average score of the sensory evaluation items by the citizens ranged from 3.3 to 4.0, which was higher than that for the RUA group. Compared to the RUA group, the citizens highly evaluated the color of the reheated jam, and there was a significant difference (5% significance level) between the evaluation results of the color for both groups. A t-test for differences between population means for each evaluation item of the reheated jam (significance level of 5%) showed no significant differences between citizens and the RUA group, except for color. The average score of the overall evaluation for citizens was 4.0, and citizens also gave the reheated jam high evaluation scores.

Almost all panelists answered that the reheated jam was sweeter than the hot-pack jam. One of the possible reasons for this result is that the sweetness of the reheated jam increased because the sourness decreased due to volatilization caused by heating (sterilization). The cost of cashew apple jam made in Japan will be approximately 80 Japanese yen, including the glass container and the lid, based on the assumption that cashew apples can be obtained for free, the loss of materials and/or products during processing is negligible, and 600 units of jam (100 g per container) per 1 batch can be made. Because RUA group and citizens gave relatively high evaluation of the hot-pack jam and reheated jam, these

cashew apple jams were acceptable and had the possibility to become new processed foods in Cambodia. The sweeter jam was preferred in Cambodia. For improved food safety, we recommend adding sterilization to the jam-making process. The potential of cashew apples in jam making, product development, reduction of postharvest losses, creation of job opportunities, and so on would contribute to Cambodia's development.



**Fig. 4 Comparison of the sensory evaluation results of the reheated jam between the RUA group and 30 citizens**

## CONCLUSION

A sensory evaluation test of two types of cashew apple jams was conducted in Phnom Penh, Cambodia, in September 2019 with 70 persons. During the production of the hot-pack jam, the jam's raw materials were mixed, dissolved, condensed, and prepared to 50 °Brix sugar content while heating up to approximately 80 °C, and this jam heated to 80°C was filled in a glass bottle. Another jam, i.e., reheated jam, included a sterilization procedure to the hot-pack jam preparation process. The color, sweetness, acidity, taste, flavor, jelly state, smoothness, and overall score of each sample were evaluated using a five-point hedonic scale (1: hate, 2: dislike, 3: neutral, 4: like, and 5: like very much) for the sensory test (preference type). Both jams were evaluated relatively highly by the 70 panelists. The cashew apple jams were acceptable and had the possibility becoming new processed foods in Cambodia. Such a development will reduce postharvest losses, which have been a challenge to Cambodia's food technologists, as well as help create job opportunities and contribute to Cambodia's development.

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# Food Service Industry Development and its Effect to Distribution of Fresh Agri-products in Vietnamese Suburban Area

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Received 31 December 2020    Accepted 16 May 2021    (\*Corresponding Author)

**Abstract** This research aims to uncover how the emerging catering industry contingent to deindustrialisation changes the value chain of fresh agri-product in the Vietnamese suburban area. The results revealed that by entering both international and local businesses, the catering industry is competitive. Firstly, while the catering business in the North are small and operate on a local level those operating in the South are consolidated enterprises as they have different market conditions. Secondly, Fresh food ingredients provide direct contract with producers or wholesalers. In the south, catering companies tend to contract food wholesalers to purchase in bulk and distribute them to their branch preparation kitchens located in surrounding provinces. Third, the value chain created by catering company could make transactions and the volume of products handled concentrated, this could extend the transport distance of produce. However, the vertical coordination for the entire chain is inadequate. Furthermore, contract transactions are limited to only part of the chain, and the requirement for quality control is weak.

**Keywords** food service industry, fresh vegetable and fruits distribution, industrialization, suburban area, Vietnam

## INTRODUCTION

The food system in developing countries has transformed rapidly in the past several decades (Reardon et al., 2019). The two main themes are in trends regarding food systems in developing countries. These themes include, exports and imports with trade liberalization and globalization, and transformation in the food systems (Reardon, 2015). Consolidation and globalization of supermarket chains and its affect on small holders are also examined in many countries (Berdegué et al, 2005; Minten et al., 2009; Ruben et al., 2007; Wang et al., 2009). The supermarket plays an important role in food production with their ‘own brand’ products and the setting of safety, quality and manufactured food. The value chain is aimed at quality control of food governed by global retail chains effect on all the actors in the chain, from the input suppliers to the farmer and the food retailer to the consumer (Burch et al., 2013). Furthermore, the transaction system is changed from spot markets to contract transaction, food products are standardised and the area of food supplied is geographically expanding. The modern value chain offered by supermarkets are gaining the attention of scholars, as well as detailed research and analysis have been conducted (Maruyama and Trung, 2007; Mergenthaler et al., 2009; Moustier et al., 2010; Ruben et al., 2007).

In contrast, research regarding the middle stream (processing, wholesaling and storage) in developing countries are limited. Reardon (2015) reviled middle-stream growth and transformation, both in a “modern revolution” with large and often foreign companies, but also a “quiet revolution” with a small and medial enterprise and a substantial investment made by them. However, the food service industry, especially in rural and suburban areas, has recently begun rising due to urbanization and industrialization, which has not been considered in previous studies.

With the rapid growth in the industrial zone in rural and suburban Vietnam, the food service industry providing meals for labourers working in factories is emerging. With the development of the industrial and rural labour markets, non-farm labour is rising continuously. In 2005, the number of labourers in the country working in the primary sector was 23,563,000 (55.1%), with the number of labourers in manufacturing numbering 5,031,000 (11.9%). However, the number of people working in the primary sector dropped to 18,831,000 (34.4%), while the number of labourers in manufacturing increased to 11,287,600 (20.6%) in 2019. Employers in the industrial sector (factories) contracted by catering companies provide one to two meals per working day for employees as welfare support. While supermarkets are suspended in relatively small channel for Vietnamese lifestyle (Sigrid C.O. Wertheim-heck et al., 2015), as the emergence of the catering industry's contingency to industrialization and food demand growth in suburban area, the value chain of food is gradually changing. Nevertheless, the framework of catering companies providing meals to the factories, how they deal with food suppliers and how food value chains are restructured remains a matter of uncertainty.

## **OBJECTIVE**

This research aims to uncover how the emerging catering industry is industrialized and changes in the value chain of fresh agri-product (fruits and vegetables) in the Vietnamese suburban area.

## **METHODOLOGY**

A semi-structured interview was conducted with 15 catering companies (3 international and 12 local companies), which supply meals to factories in the industrial sector in northern and southern Vietnam, particularly in Hanoi City, Hai Duong Province, Ho Chi Minh (HCM) City and the Tien Giang Province. In addition to Hanoi and HCM, the centre of industrialization for North and South Vietnam, Hai Duong and Tien Giang Province, respectively, are used as a fresh food producer for the former cities, which are investigated to clarify changes in agri-food distribution.

This research answers the following questions:

- 1) What are the general characteristics of these catering companies and business conditions?
- 2) How do they procure and control the quality of fresh agri-products as ingredients?
- 3) How does the development or modernization of the food service industry affect agricultural distribution in the surrounding areas of the industrial sector?

Sample companies are selected due to introduction by department of health in each province and city, and the interviews were conducted in 2014 for North Vietnam and in 2019 for South Vietnam.

## **RESULTS AND DISCUSSION**

### **General Characteristics of Catering Companies Supply Meals to Factories at Industrial Zone**

Table 1 outlines each of the catering companies, with 15 companies divided into 3 groups by the scale of business. Companies that provided over 20,000 meals/day were categorised as large scale, those that provided 10,000 to 20,000 meals/day as middle, and 10,000 meals/day as small scale. The size of catering companies varied, the largest cater (L1) provides 60,000 meals/day and has 600 labourers, while the smallest (S5) distributes only 200 meals/day.



Most of informants were started their business in the 2000s, especially in 2011-2015, when foreign direct investment increased<sup>1</sup>. The largest company (L1) are jointly invested with a Japanese catering company and the Vietnamese local company. Their Headquarter is in HCM, then spreading their business into the north. Several former food wholesalers (L3, L4) started their catering business. Local restaurants have also entered the catering industry, but their business scale is relatively small (S1, S2 and S4). Middle scale companies are comprised mostly of those that left other catering companies to develop their own business. According to the informants, companies in the catering industry are continuously merging and splitting.

Two types of meal preparations were observed. These include those that set up the facilities<sup>2</sup> to prepare meals on spot, and those that prepare meals at a central kitchen location and then deliver them to each customer in the industrial sector. While the latter is recommended for hygienic reasons, most of companies could adapt to either style, depending on their customers' needs. Large catering companies with facilities (L1, L3, L4 and M3) and small catering companies which distribute a small number of meals/day (S2, S4, S5) tend to prepare these in their own kitchen and then deliver them to each factory.

Of the 15 catering companies, 4 of them invested in a central kitchen that met ISO and HACCP standards, yet quality certification is not popular for small scale companies. Furthermore, unstable annual contracts made middle and small scale catering companies hesitate to invest in new facilities.

**Table 1 Outline of sample catering companies**

	Large scale				Middle scale						Small scale				
	L1	L2	L3	L4	M1	M2	M3	M4	M5	M6	S1	S2	S3	S4	S5
Location	HN	TG	HCM	HCM	HD	HCM	HCM	HCM	TG	TG	TG	HD	TG	HD	TG
Year established	2013	2011	1998	2011	2008	2017	2003	2015	2004	N.A.	2007	2014	2009	2014	2011
Num. of branches	2	16	5	4	5	7	2	8	4	5	2	1	20	1	1
background	JP+V N	N.A.	WS	WS	N.A.	split out	FP	split out	N.A.	N.A.	FS	FS	N.A.	FS	Sub con.
Num. of employees	600	N.A.	350	55	400	130	2500	70	N.A.	N.A.	N.A.	21	N.A.	22	N.A.
Num. of meals(1,000M/day)	60	13	20	20	15	13	11	9	6-11	5	1.9	1.5	1	0.6	0.2
note	ISO2200	HACCP,ISO2000		ISO22000		ISO9001,BRC, HACCP									

\* NH=Hanoi, TG=Tien Giang, HCM=Ho Chi Minh, HD=Hai Duong

\*\*N.A.=no answer, WS=Whole sealer, FP=Food Processor, FS=Food Service (Restaurant)

\*\*\*Shaded companies are in north.

Source: field survey in 2014-2019

As Table 2 shows, the contract between catering companies and customers (factories) are typically annually arranged<sup>3</sup>. Contract sometimes broken, and often not renewed. It made their business unstable and flexible so large companies often outsource meal preparation to local small catering companies. In addition to existence of countless competitors, unstable business condition caused caters to continually merge and split.

Contract unit prices vary widely from 12,000-40,000 Vietnamese Dong (VND) per meal (Table 2). As explained next, the food safety requirement for industrial workers meals are not high. This minimum safety standard makes catering companies provide reasonably priced meals. According to the interview with department of Health, the average price for primary school lunch in HCM was 35,000

<sup>1</sup> Value of foreign direct investment to Vietnam was \$428.5 million (US) in 1991, which increased to \$2.4 billion (US) in 2000 and \$17.5 billion (US) in 2017.

<sup>2</sup> Depending on the contract, facilities are invested in by either the customers or catering companies. According to the informant, it costs 1.5-2.0 billion VND for the facility with capacity for 1,000 meals/day.

<sup>3</sup> It could also be every 3 months, 6 months, 12 months, 18 months or more. However, one year is the most common period for the contract.

(VND) per meal, while the average price the informant provided for the factory was 15,800 (VND) per meal. In most cases, the meals that catering companies provide for factories are segregated from those prepared for school meals because the quality required for both differ greatly.

**Table 2 Contents of contracts**

	Large				Middle						Small				
	L1	L2	L3	L4	M1	M2	M3	M4	M5	M6	S1	S2	S3	S4	S5
Num. customers	N.A.	N.A.	50	35	N.A.	7	N.A.	8	4	5	1	3	1	1	1
Main customer.	Fac.	Fac.	Fac., office, school	Fac., school	School, fac.	Fac.	office	Fac.	Fac.	Fac.	Fac.	Fac.	Fac.	Fac.	School, Fac.
Unit price (1,000VN D)	18 - 20	N.A.	15 - 40	20 - 35	12 - 15	15 - 30	20 - 30	14 - 20	20	16.5	15	14 - 17	15	13 - 20	15

source: field research in 2014-2019

### Supply Chain of Ingredients – Vertical Coordination-

Two main routes: contract with suppliers and spot purchasing, were observed for ingredient food (especially fresh food) procurement.

**Table 3 Purchasing fresh produce**

	Large				Middle						Small				
	L1	L2	L3	L4	M1	M2	M3	M4	M5	M6	S1	S2	S3	S4	S5
Supplier	Pro	WS	WS	WS/Pro	Pro	Coop	WS	WS	Coop	WS	WS	Coop	WS	WS	Pro
Contractor*	kitchen	HQ	HQ	HQ	kitchen	HQ	HQ	HQ	kitchen	HQ	N.C.	N.C.	HQ	N.C.	N.C.
required standard	own Stan.	—	—	V-GAP	own Stan.	own Stan.	—	—	V-GAP	—	—	—	—	—	—

\*N.C.=no contract

source: field research in 2014-2019

#### 1) Direct contract with producers/producers' coop (L1, M1, M2, M5, S2 and S5):

Catering companies purchase food directly from producers or producers' cooperatives which obtained the Food Safety and Hygiene Certification (FSHC) located near the preparation spot. The transaction volume was relatively small and transportation capabilities were limited. Each kitchen had the initiative to find suppliers, purchase process and maintain quality control. This purchasing system tends to be employed primarily by catering companies in the north rather than the south. There are three important reasons that direct purchasing is more common in north than in the south. Firstly, the meals provided each day are limited since the industrial zone in the north is not as developed and concentrated as in the south. Secondly, the consolidation of transactions are difficult because the food wholesale business is also not well developed to provide standardised ingredients for preparation locations. Lastly, even though catering companies can find food wholesalers with well-developed networks, it is difficult to purchase from a distant area because the cold chain is not maintained.

#### 2) Purchase from suppliers (L2, L3, M3, M4, M6, S1, S3 and S4):

Creating contracts with food suppliers are more prominent in the south. Several large catering companies (L2, L3, L4, M3, M4 and M6) deal with food suppliers that are based on the Binh Dinh, Hoc Mon and Tu Duc wholesale markets<sup>4</sup>. They aggregate orders for all their branch kitchens located in southeast and Mekong delta regions, then indicate suppliers to deliver to each kitchen. However, in some cases, each kitchen could be a contractor.

<sup>4</sup> The Binh Dinh market is the largest wholesale market and mainly provides fish and fruits from the Mekong Delta. The Hoc Mon market is in the west side of the city, mainly supplying meat. Finally, the Tu Duc market located in the east part of HCM accepts vegetables from the central highland to distribute to the entire city.

Using quality controlled or certified produce is not popular for caterers. The minimum level of safety standard officially set by the government, only requires a FSHC provided by the department of Health for each province. Furthermore, it supports caterers provide reasonably priced meals and are discouraged from forming tight relations with suppliers. In most of cases, the contract unit price is not affordable to prepare certified products. While the response to their customers mainly comes from overseas, several catering companies (L4 and M5) supplying meals with certified ingredients have special practices for production (Good Agricultural Practice (GAP)), and tracing their products origin. The companies, L4 and M5 needed to find producers who could produce V-GAP form a contract with them to ensure quality control. Furthermore, L1, M2 and M2 have their own standard for purchasing food. Caterers' behaviour for purchasing is determined by customers (factories) attribute for workers welfare. However, most of the enterprises in the Vietnamese industrial sector do not pay close attention to it.

### **Impact of the Value Chain on Agricultural Produces**

The development of such large scale catering in the south could be the changing value chain of agricultural products in the region. Food for urban areas has been supplied from surrounding production areas, and it has been clarified that the supply chain of fresh food in Vietnam was scattered and relied on small scale distribution actors even in the 2000s (Cho, 2001). Delays of infrastructural development, development of commercial capitals and procedure cooperatives, and the gradual growth of urban food demand due to the slow influx of migrants to cities have hindered the changes of distribution systems. For instance, the industrial park surveyed by the Tien Giang Province is an area where agricultural production is carried out and it used to be relatively large vegetables supplier not only in Tien Giang, but also for cities like HCM and Bien Hoa<sup>5</sup>. They were suppliers of food to urban area and to the local market. Food demand in Tien Giang was supplied by local produce. Thus, when a local restaurant provides meals for workers in the Tien Giang province, locally produced food from the local market could be prepared. However, developing large scale catering that has a headquarters in HCM employ different methods for efficient procurement of their ingredients. They purchase in bulk at the wholesale market in HCM then deliver the ingredients to each preparation kitchen. In the former, the food system is completed within the region, while in the latter, agricultural products produced in the region are transferred to the HCM market where they are purchased and distributed by a large collector, and then transferred again to the Tien Giang Province.

On the other hand, since the demand for quality food is not strong, development of the catering industry did not promote vertical integration of the whole value chain. For this reason, contracts between catering and wholesalers, which obtained FSHC, is popular.

### **CONCLUSION**

This research empirically investigated how the emerging catering industry impacted value chain of fresh food in the Vietnamese suburban area. The results suggest that first, both international and local enterprises have entered the catering industry. In northern area, since industrial sectors are geographically sparse, catering companies are also scattered, and meals are mostly prepared by local small businesses so that the impact to conventional procurement value chain is limited. While in the south, since large industrial sectors invested more, catering companies developed and merged to provide large number of meals to more extensive places. Second, the fresh food ingredients are provided by producers, or wholesalers. In the south, more catering companies tend to contract food wholesalers to purchase bulk amounts then distribute them to their branch preparation kitchens located

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<sup>5</sup> Central city of Binh Duong province, where a lot of industrial parks are concentrated.

in surrounding provinces. Finally, the value chain created by caters could make transaction and volume of fresh product handled more concentrated and extend the transport distance of produce. However, because of low requirement for quality, the value chain is not integrated and coordinated strongly.

## **ACKNOWLEDGEMENTS**

This work was supported by JSPS KAKENHI, Grant-in-Aid for Young Scientists (B), JP17K15337.

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# The Influence of Packaging Materials and Storage Duration on Seed Quality of Sesame (*Sesamum indicum* L.)

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Received 1 January 2021    Accepted 17 May 2021    (\*Corresponding Author)

**Abstract** The process of seed deterioration can lead to some physiological changes, such as a progressive decrease in germinability, an increase mean time for germination, an increase in the number of abnormal seedlings and a subsequent lower tolerance to adverse storage conditions. This study was conducted to determine the seed quality losses associated with the use of different types of packaging materials used for storage under farm condition. The study was conducted in collaboration with sesame farmers in Pwintphyu Township, from August 2016 to March 2017 (pre-monsoon seed storage) and in Pakokku Township, from January 2017 to August 2017 (post-monsoon seed storage). The sesame seeds were stored in the farmers' houses using two types of packaging materials; woven polypropylene bags and pioneer super bags. The data were recorded before storage and at two-month intervals during the eight month storage period. The results show that the germination percentage and germination index means of the pre-monsoon seeds stored in woven polypropylene bag are significantly higher than for those stored in super bags. However, the packaging material used has no significant influence on the germination percentage and germination index of post-monsoon sesame seeds. In regard to storage duration, there was significant variation in the germination percentage and germination indexes depending on storage method for both pre-monsoon and post-monsoon seeds. Although the seedling vigour measures of index I and II for pre-monsoon seeds stored in woven polypropylene bag are significantly higher than for those of seeds stored in super bags, there is no difference in seedling vigour indexes I and II for post-monsoon seeds with the use of different packaging methods. However, the seedling vigour indexes I and II of both pre- and post-monsoon seeds varied significantly with duration of storage. Germination percentage and germination indexes are different for the two packaging materials examined, for pre-monsoon sesame storage. The germination percentage and germination indexes of stored seeds in super bags are significantly lower than that in woven polypropylene bags, whereas, this effect was not observed in post-monsoon sesame. Black sesame cultivated in both pre-monsoon and post-monsoon seasons has a different dormancy period, and any effect of packaging materials on this dormancy release time is not evident, for both seasons. Therefore, hermetic (super bag) storage can be used in post-monsoon sesame without adverse effects on germination. However, without further research, hermetic storage appears to not be suitable for pre-monsoon sesame, due to its negative effect on germination.

**Keywords** sesame, germination percentage, germination index, seedling vigour index

## INTRODUCTION

A seed's storage environment greatly influences the period of seed survival. The type of packaging used during the storage of seeds helps in lessening the speed of deterioration by maintaining the initial moisture content of the seeds stored, and by diminishing the seeds' respiration rate (Brooker et al., 1992). Longevity of seed viability in storage is influenced by the initial quality of the seed stored as well as the storage conditions. However, irrespective of initial seed quality, unfavourable storage conditions, particularly air temperature and air relative humidity, contribute to acceleration of seed deterioration in storage (Walters et al., 2010; Hansen, 2011). Maintaining seed viability and vigour during storage also depends on the type of packaging employed. Seeds in packaging which enables the exchange of water vapor with the environment can absorb water under high relative humidity, easily decaying seeds (Filho, 1998).

## OBJECTIVES

The study was conducted to determine losses in seed quality associated with the use of different types of packaging materials used during storage and under farm conditions.

## METHODOLOGY

### Experimental Sites and Design

The study was conducted in collaboration with the sesame farmers in Pwintphyu Township, during the period August 2016 to March 2017 and in Pakokku Township from January 2017 to August 2017, with both of these townships situated in Magway Region, Myanmar. The black sesame seeds (Samou Nei) were stored in the farmers' houses using two types of packaging materials, woven polypropylene bags and pioneer super bags. The woven polypropylene bag is permeable bag and pioneer super bag is non-permeable poly-ethylene bag with thickness of 0.078 mm. The pioneer super bags (which consists of a two layer bag, with a bag inside a polypropylene outer), and as much air as possible was removed from super bag before it was taped closed. The bags were stored together with other bags using different storage methods. This storage treatment was undertaken in the houses of six farmers in each township. The data were recorded before storage and at two-month intervals during the eight month storage period. Germination tests (germination percentage, germination rate), vigour tests (seedling vigour index I, seedling vigour index II) were conducted to determine seed quality.

### Germination Percentage

The germination test was performed using the top paper method. Four replications of one hundred seeds, randomly distributed on wet filter paper in petri dishes 9 cm in diameter, were examined. Each dish was placed into an incubator set to a constant temperature of 25°C throughout the testing period. The germinated seeds (2 mm radicle elongation) were counted daily, up to the tenth day, to calculate the germination rate (ISTA, 2004).

### Germination Index

The germination index was computed using the following formula:

$$G.I = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \frac{N_3}{D_3} + \dots + \frac{N_n}{D_n}$$

where,

N1, N2, ..., Nn: Number of seedlings on day 1st, 2nd and nth day after sowing

D1, D2, ..., Dn: Number of days after sowing

### Seedling Vigour Index

This was calculated by measuring the germination percentage and seedling length of the same seed sample. The seedlings were grown by the rolled paper towel method. Fifty seeds each, in four replications, were germinated in moist paper towel, in such a way that the micropyles were oriented towards the bottom to avoid root twisting. The rolled paper towel mediums were kept in an incubator, with temperature maintained at 25 °C. After 10 days, the paper towel substrates were removed and five normal seedlings were randomly selected. Their lengths were measured and a mean seedling length calculated. To determine dry weight, the seedlings were removed from the substrate and dried in an oven at 100°C temperature for 24 hours, then cooled and weighed. The seed vigour indexes were calculated by multiplying the germination percentage by seedling dry weight (g) (Index I) or by seedling length (mm) (Index II). The seed lot showing the higher seed vigour index was considered to be more vigorous (Adbdul-Baki and Anderson, 1973).

Vigour index -I: Germination (%)  $\times$  total dry weight of seedling (g)

Vigour index -II: Germination (%)  $\times$  seedling length (mm)

### Statistical Analysis

All data were analyzed with analysis of variance (ANOVA) by using Statistix (version 8.0) and comparison of treatment means was done using the LSD test at 5 % level of significance.

## RESULTS AND DISCUSSION

### Effect of Different Packaging Materials and Storage Durations on the Quality of Pre-monsoon Sesame

#### Germination percentage:

The means of germination percentage of seeds stored in woven polypropylene bags was significantly higher than that of those stored in super bags. These results indicate that woven polypropylene bags are more suitable to store pre-monsoon sesame seeds to maintain good quality seeds. In hermetically storage, high moisture contents in stored grain can loss in germination and viability. The dryness of stored grain in pre-monsoon may not be enough for safety storage in hermetical bags. The mean value of germination percentage of pre-monsoon sesame varied with storage duration. The minimum germination percentage for sesame seeds was found at the initial storage time and this percentage increased significantly after two months storage and again after four months storage. The increase in germination percentages were positively correlated with the duration of storage up to a period of six months then decreased slightly. It can be said that the physiological quality of sesame stored for six-month did not decrease during storage. Therefore, the results show that the best storage duration for pre-monsoon sesame seeds, in relation to germination rates is six-month (Table 1).

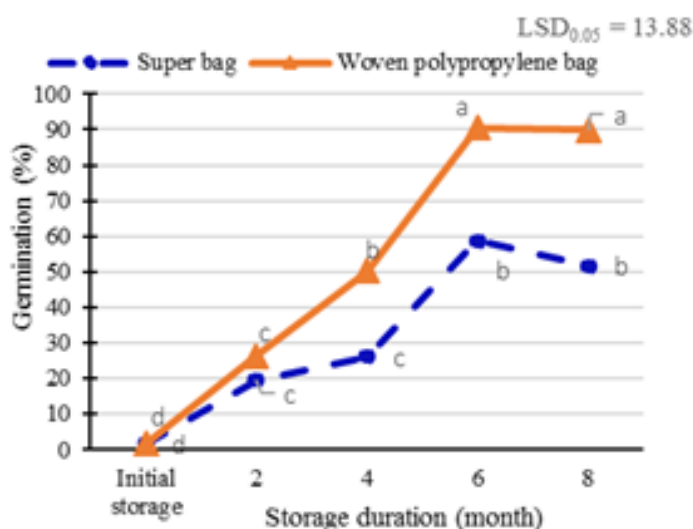
These results compare with those reported by Prashant et al. (2016), whose study of the variability of germination percentage with the duration of seed storage for *Seasamum indicum*, found that maximum germination percentages occur after short term storage (68-75%), followed by mid-term, and minimum germination percentages occurred in seeds stored for long terms. The mean germination percentage of pre-monsoon sesame seeds was significantly different, and was affected by packaging

materials and storage duration (Table 1). However, it can be seen that the variations in the germination percentage means during the storage periods depend primarily on the packaging material used for storage. The best germination of pre-monsoon sesame could be achieved from seed stored in woven polypropylene bags for six months (Fig. 1). This agrees with the study carried out by Lima et al. (2014) that concluded that the physiological quality of sesame stored for six months does not decrease during storage.

**Table 1 Mean effects of packaging materials and storage durations on the quality of pre-monsoon sesame**

Treatment	Germination (%)	Germination index	Seedling vigour index I	Seedling vigour index II
<b>Packaging materials (P)</b>				
Super bag	31.583 b	6.821 b	0.055 b	226.520 b
Woven polypropylene bag	51.708 a	12.197 a	0.086 a	390.570 a
<b>LSD<sub>0.05</sub></b>	6.207	1.610	0.012	50.666
<b>Storage duration (D)</b>				
Initial storage	1.583 d	0.720 e	0.003 d	14.010 d
2 months	22.979 c	6.264 d	0.043 c	181.910 c
4 months	38.208 b	9.885 c	0.064 b	228.050 c
6 months	74.604 a	16.625 a	0.119 a	637.790 a
8 months	70.854 a	14.052 b	0.124 a	480.970 b
<b>LSD<sub>0.05</sub></b>	9.814	2.546	0.019	80.109
<b>Pr &gt; F</b>				
P	<0.0001	<0.0001	<0.0001	<0.0001
D	<0.0001	<0.0001	<0.0001	<0.0001
P x D	0.0010	0.0001	0.0099	0.0002
<b>CV (%)</b>	28.66	32.57	32.08	31.58

Note: In each column, means with the same letter are not significantly different at 5 % level.



**Fig. 1 Combination effect of packaging materials and storage durations on germination percentage of pre-monsoon sesame**

#### Germination index:

The maximum germination index is seen from seed stored in woven polypropylene bags followed by seeds stored in super bags. The mean germination indexes varied significantly with the duration of storage. It is the effect of dormancy of stored seed, the germination index of stored seeds increased



with storage duration due to dormancy release and reached the maximum after six months storage when the dormancy fully release. Germination indexes significantly differed for the two packaging materials, except at the two-month storage interval, and this difference was greatest at six months, when seeds stored in woven polypropylene bag storage displayed the greatest germination index.

#### **Seedling vigour index I and II:**

The seedling vigour indexes I and II, for seeds stored in woven polypropylene bag were significantly higher than for those seeds stored in super bags. Seedling vigour depends on the germination percentage, seedling length and seedling dry weight. And maintaining the viability and seed vigor during storage also depends on the type of packaging employed (Filho, 1998). The maximum reading for seedling vigour index I is seen after eight months of storage, although this is not significantly different to the index I measurement at the six-month storage interval. The pre-monsoon sesame seeds stored for longer than four months show a higher seedling vigour index I when stored in the woven polypropylene bag. In relation to seedling vigour index II, the lowest measurements occur in the initial storage but these germination index rates increase with duration of storage. The results indicate that the most vigorous seedlings are those stored in woven polypropylene bags for six months.

### **Effect of Different Packaging Materials and Storage Durations on the Quality of Post-monsoon Sesame**

#### **Germination percentage:**

The mean values of germination percentages of post-monsoon sesame are statistically similar for the two packaging materials, super bags and woven polypropylene bags. The packaging materials do not show an influence on the germination percentages of post-monsoon sesame seeds. However, the differences in the mean germination percentages of post-monsoon sesame are significant when storage duration is considered (Table 2). The interaction effects between packaging materials and storage duration exhibit non-significant effects on mean germination percentages for post-monsoon sesame (Fig. 2). It can be seen that mean germination percentages, which vary with storage duration, are not dependent on the packaging material used for storage. It can also be seen that water proof, air tight containers are a suitable packaging type to maintain germination of post-monsoon sesame above 90% at the eight-month interval of storage.

#### **Germination index:**

Although the germination index for seeds is similar for the two packaging materials, these indexes varied significantly with storage duration. However, the interaction effects between packaging materials and storage durations were not significant in germination index measurements for post-monsoon sesame (Table 2). It can be said that the changes of germination index during storage are not influenced by the packaging material used for storage.

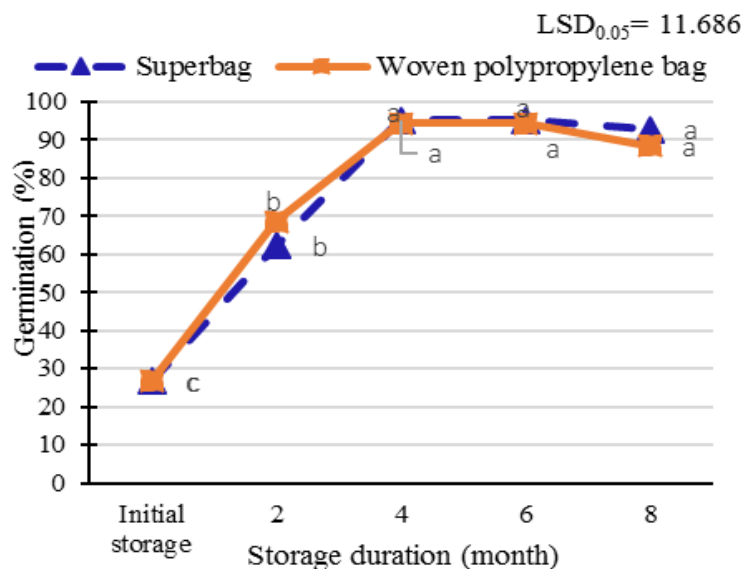
#### **Seedlings vigour index I and II:**

Seedling vigour indexes I and II for seeds stored in different packaging is not different, however, these index measurements varied significantly with storage duration. Seedling vigour indexes I and II at the initial storage interval, exhibit minimum values and significantly differ to seeds stored for longer durations. The combination effect of packaging materials and storage duration on seedling vigour indexes is not significant (Table 2). This shows that the variations in seedling vigour indexes observed over time are not governed by the packaging materials. Although germination percentage and the germination index are numerically higher for seeds stored in super bags, seedling vigour indexes I and II are higher for seed in woven polypropylene bags.

**Table 2 Mean effect of packaging materials and storage durations on the quality of post-monsoon sesame**

Treatment	Germination (%)	Germination index	Seedling vigour index I	Seedling vigour index II
<b>Packaging materials (P)</b>				
Super bag	74.317 a	18.637 a	0.142 a	618.290 a
Woven polypropylene bag	74.375 a	19.148 a	0.143 a	620.640 a
<b>LSD<sub>0.05</sub></b>	5.226	1.626	0.016	45.080
<b>Storage duration (D)</b>				
Initial storage	26.750 c	8.800 c	0.045 c	174.050 d
2 month	65.333 b	17.645 b	0.119 b	566.170 c
4 month	94.625 a	22.585 a	0.182 a	669.290 b
6 month	94.521 a	23.412 a	0.173 a	951.510 a
8 month	90.500 a	22.017 a	0.195 a	734.290 b
<b>LSD<sub>0.05</sub></b>	8.263	2.572	0.025	71.278
<b>Pr &gt; F</b>				
P	0.9822	0.5298	0.8417	0.9167
D	<0.0001	<0.0001	<0.0001	<0.0001
P × D	0.7791	0.1849	0.6766	0.8691
<b>CV (%)</b>	13.52	16.55	21.08	13.99

Note: In each column, means with the same letter are not significantly different at 5 % level.

**Fig. 2 Combination effect of packaging materials and storage durations on germination percentage of post-monsoon sesame**

## CONCLUSION

Black sesame seed cultivated in both pre-monsoon and post-monsoon seasons has different dormancy periods. The maximum germination rates occur after 6 months storage for pre-monsoon sesame but at 4 months storage for post-monsoon sesame. There was no observed differences in the effects of the materials used for storage on the dormancy release time for sesame seeds, for either pre- or post-monsoon seasons. Hermetic storage can be used for post-monsoon sesame without adverse effects on germination. However, hermetic storage is not suitable for pre-monsoon sesame, as it has a negative effect on germination. More research should be conducted to investigate the reasons for this.

## **ACKNOWLEDGEMENTS**

This study was supported financially and technically by the Japan International Cooperation Agency, Technical Cooperation Program (JICA-TCP).

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# Evaluation of Site Contamination from Storage of Fertilizer

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Received 14 January 2021    Accepted 18 May 2021    (\*Corresponding Author)

**Abstract** Australia has extensive agricultural production of crops and livestock production. Regular fertilizer application maintains production rates of grain crops. Large quantities of phosphorus-based fertilizers are produced from phosphate rock by sulfuric acid extraction at designated production works at coastal locations and transported by train to distribution points in selected agricultural areas. Bulk fertilizer is stored in large sheds adjacent to railway lines and large or small trucks then make deliveries to farms. When such sites are disused or abandoned, contaminated soil may remain from fertilizer constituents. Occasionally fertilizer storage sites may have experience accidental contamination from railway engine or truck hydrocarbons. The objective of this study is to examine the extent of fertilizer contamination at agricultural storage sites, identify environmental risks associated with the handling practices of storage sites, and distinguish from other contamination from transport vehicles. Soil samples were collected at two locations using a grid-base sampling program to identify contaminants. Following preparation soils were analysed for phosphorus, sulfate, sulfur, petroleum hydrocarbons (aliphatic and aromatic), heavy metals and pesticides using standard procedures. A ‘within a target area’ was used to assess contamination levels by comparing the 95% Upper Confidence Limit (UCL) of the average concentration of each analyte of concern against respective ‘contamination’ criteria for industrial sites; exceedance of the 95% UCL against site criteria identified contamination. Statistical analysis of the data for different analytes in soil at fertilizer storage sites showed the relationship between contaminant levels and dispersion over respective sites and extent of contamination from fertilizer handling activity and other vehicle-related site contamination. In conclusion accurate estimation of contamination of sites can be provided using the grid-base sampling approach and 95% Upper Confidence Limit (UCL) criteria to distinguished transport vehicle-related contamination from phosphate-based fertilizer on site.

**Keywords** fertilizer storage, transport vehicles, contamination, risk management

## INTRODUCTION

Australia has extensive agricultural production of crops and livestock. Regular fertilizer application has maintained production rates over many years (Fitzpatrick, 1968). Phosphorus-based fertilizers are produced by sulfuric acid extraction at Australian coastal production works and transported by train to distribution points. Bulk fertilizer is stored in large sheds adjacent to railway lines and large or small trucks then deliver to farms. The storage sheds usually have bare soil floors and over periods of decades fertilizer levels can build up. The disused or abandoned sites may leave soil contaminated with fertilizer phosphate, sulfate and sometimes sulfur (FMA, 1998). Occasionally fertilizer storage sites have experienced contamination from railway engine derailment or truck hydrocarbons (NSW EPA, 1994). The Australian National Environmental Protection Measure (NEPM) soil contamination guidelines are risk-based Health and Ecological criteria (Noller, 2020) and some guidelines may be applicable for assessing fertilizer contamination.

## OBJECTIVE

The objective of this study is to examine the extent of fertilizer contamination from phosphate, sulfate and sometimes sulfur at agricultural storage sites, identify environmental risks associated with the handling practices of storage sites, and distinguish from other contamination due to transport vehicles.

## METHODOLOGY

Soil samples were collected using a grid-base sampling program to identify analytes of concern (NSW EPA, 1995) at two comparative fertilizer storage sites; one location (A) experienced contamination due to a rail derailment following fertilizer storage ten years earlier and another location (B) was used for fertilizer only storage for several decades (Noller, unpublished data). Following preparation, soils were analysed by Queensland Health Scientific Services laboratory, Coopers Plains, Queensland for phosphorus, sulfate, sulfur, petroleum hydrocarbons (aliphatic and aromatic), heavy metals and pesticides using standard procedures (ANZECC, 1992; NEPM, 1999; NSW EPA, 1994; NSW EPA, 1995).

The criteria from the NSW EPA (1998) Guidelines for the NSW Site Auditing Scheme were used, together with the assessment criteria of the Australian National Environmental Forum health-based soil investigation Levels for Soil and Groundwater NEPM (1999) at industrial sites. A ‘within a target area’ statistical evaluation was used to assess contamination levels by comparing the 95% Upper Confidence Limit (UCL) of the average concentration of each analyte against respective ‘contamination’ criteria for industrial sites; exceedance of 95% UCL against site criteria identified contamination using the assessment criteria of the NSW EPA Sampling Design guidelines (NSW EPA, 1995). “Hotspots” could be identified when analytical results from individual sites were 250% of the relevant criteria value, and when the standard deviation of data was <50% of the criteria.

The 95% upper confidence limit (UCL) of the arithmetic average concentration is calculated using Eq. (1) (NSW EPA, 1995) as follows:

$$\text{UCL average} = \bar{x} + t_{\alpha, n-1} \times \text{sd} / \sqrt{n} \quad (1)$$

UCL average = Upper confidence limit of the arithmetic average concentration of the sampling area at the 1- $\alpha$  confidence level.

$\alpha$  = The probability that the ‘true’ average concentration of the sampling area might exceed the UCL average determined by Eq. (1).

$n$  = Number of sample measurements.

$n-1$  = Degrees of freedom

$\bar{x}$  = Arithmetic average of all sample measurements.

$t_{\alpha, n-1}$  = The test statistic at an  $\alpha$  level of significance and degrees of freedom of  $n-1$  obtained from table of values of Student’s  $t$ .

sd = Standard deviation of the sample measurements.

## RESULTS AND DISCUSSION

Table 1 gives results for soil sulfate, total phosphorus and elemental sulfur concentrations at the two fertilizer storage Locations (A and B). Both show extensive soil contamination, compared with contamination criteria, from sulfate, phosphorus and sulfur (B only) with higher concentrations observed at Location B due to extensive use over several years. Such contamination is quite different to low level contamination of agricultural soils (Noller, 2020), but significant over long-term accumulation in soil (Weissengruber et al., 2018). Comparison of the statistical evaluations using the 95% Upper Confidence Limit (UCL) of average concentration of each constituent (NSW EPA, 1995)

and exceedance of 95% UCL against the respective contamination criteria against site criteria (ANZECC, 1992; NEPM, 1999) enabled the following features of ‘hotspots’ to be identified:

- Location A Sulfate. The difference of samples below the contamination criteria from all samples showed that 7 sites are contaminated and indicated the extent of the ‘hotspot’ for sulfate.
- Location A Total phosphorus. The difference of samples below the contamination criteria from all samples showed that 13 sites are contaminated and indicated the extent of the ‘hotspot’ for total phosphorus and was more extensive than for sulfate.
- Location B Sulfate. The difference of samples below the contamination criteria from all samples showed that 7 sites are contaminated and indicated the extent of the ‘hotspot’ for sulfate.
- Location B Total phosphorus. The difference of samples below the contamination criteria from all samples showed that all 10 sites are contaminated and indicated the extent of the ‘hotspot’ for total phosphorus was also more extensive than for sulfate.
- Location B Sulfur. The difference of samples below the contamination criteria from all samples showed that 2 sites are contaminated and indicated the extent of the ‘hotspot’ for sulfur, was much less than for sulfate.

**Table 1 Soil sulfate, total phosphorus and elemental sulfur concentrations at fertilizer sites**

Test	Sulfate as S (mg/kg)	Total phosphorus as P (mg/kg)	Elemental sulfur as S (mg/kg)
<b>A. Once only storage site (600 m<sup>2</sup>) followed by train derailment contamination of soil</b>			
Number of samples (n-all)	20	20	-
Mean	4509	6132	-
Range	61-33400	130-25200	-
SD	8458	6935	-
Students $t_{\alpha=0.05}$	1.729	1.729	-
95% UCL mean	1239	9850	-
Contamination criteria	2000 <sup>a</sup>	2000 <sup>a</sup>	-
Number of samples (n<Contamination criteria)	13	7	-
Mean	527	926	-
Range	61-1600	130-1700	-
SD	532	631	-
Students $t_{\alpha=0.05}$	1.782	1.943	-
95% UCL mean	792	1389	-
Contamination criteria	2000 <sup>a</sup>	2000 <sup>b</sup>	-
<b>B. Storage site (5800 m<sup>2</sup> used continuously for 40 y with only fertilizer contamination)</b>			
Number of samples (n-all)	10	10	10
Mean	28542	10470	3615
Range	520-100100	2400-22700	100-32300
SD	33124	7277	10087
Students $t_{\alpha=0.05}$	1.833	1.833	1.833
95% UCL mean	47756	14691	9466
Contamination criteria	2000 <sup>a</sup>	2000 <sup>b</sup>	600 <sup>b</sup>
Number of samples (n<Contamination criteria)	3	0	8
Mean	11732	-	225
Range	520-1600	-	100-500
SD	575	-	183
Students $t_{\alpha=0.05}$	2.353	-	1.8603
95% UCL mean	1954	-	345
Contamination criteria	2000 <sup>a</sup>	-	600 <sup>b</sup>

Source: a. ANZECC (1992) and b. NEPM (1999)

**Table 2 Soil sulfate, total phosphorus, cadmium, lead and zinc concentrations at 9 sites, Location A**

Site A (surface 0cm)	Sulfate-S (mg/kg)	Total phosphorus-P (mg/kg)	Cadmium (mg/kg)	Lead (mg/kg)	Zinc (mg/kg)
Site 1	33,400	20,800	9.7	98	170
Site 2	5,600	10,200	2.2	43	160
Site 3	120	400	<1	25	32
Site 4	19,000	25,200	45	790	460
Site 5	360	6,300	4.3	62	220
Site 6	160	4,100	3	36	130
Site 7	930	8,600	7.7	78	220
Site 8	1,200	2,200	1.3	34	110
Site 9	250	1,700	1.3	400	240
Contamination criteria	2,000 <sup>a</sup>	2,000 <sup>b</sup>	100 <sup>b</sup>	1500 <sup>b</sup>	35,000 <sup>b</sup>

Source a. ANZECC (1992) and b. NEPM (1999)

**Table 3 Soil total petroleum hydrocarbon, polynuclear aromatic hydrocarbons, benzo(a)pyrene, and organo phosphorus pesticides concentrations at 9 sites, Location A**

Site (surface 0cm)	Total petroleum hydrocarbon (mg/kg)	PolyAromatic hydrocarbons (total) (µg/kg)	Benzo(a)pyrene (µg/kg)	Organo phosphorus pesticides (µg/kg)
Site 1	142	2,255	2,255	<10
Site 2	194	1,460	1,460	<10
Site 3	48	450	450	<10
Site 4	336	5,330	5,330	<10
Site 5	108	1,510	1,510	<10
Site 6	23	320	320	<10
Site 7	46	830	830	<10
Site 8	13	255	255	<10
Site 9	67	1,370	1,370	<10
Contamination criteria	28,000 <sup>b</sup>	100,000 <sup>a</sup>	100,000 <sup>a</sup>	10,000 <sup>a</sup>

Source a. NEPM (1999) and b. NSW EPA (1994)

Tables 2 and 3 give comparative data for soil sulfate, total phosphorus, petroleum hydrocarbons (aliphatic and aromatic), benzo(a)pyrene, heavy metals (cadmium, lead and zinc), and organo-phosphorus pesticide concentrations for 9 sites at Location A with rail derailment contamination being close to the railway line. Based on historical use of the agricultural storage site (Location A) for fertilizer, the type and concentration of contaminants found should be consistent with storage of phosphorus-based fertilizers. Therefore the presence of cadmium, lead, zinc, total petroleum hydrocarbon (TPH) and BTEX (all at detection limit; benzene <0.5 mg/kg, toluene <0.5 mg/kg, ethylbenzene <0.5 mg/kg and xylene <0.5 mg/kg), polyaromatic hydrocarbons (PAHs) comprising benzo(g,h,i)perylene, dibenz(a,h)anthracene, indeno(123c,d)pyrene, benzo(a)pyrene, benzo(b+k)fluoranthene, chrysene, benz(a)anthracene, pyrene, fluoranthene, phenanthrene, fluorene, acenaphthalene, acenaphthene and naphthalene, benzo(a)pyrene itself as a key carcinogen from fuel combustion, organo phosphorus pesticides, and are inferred collectively as not being consistent with the presence of phosphate-based fertilizers; cadmium is also present in phosphate rock used in fertilizer manufacture but is associated with zinc additives used in engine oils.

The data in Tables 2 and 3 is intended to show how fertilizer contamination can be displayed at the same sites at Location A together with rail derailment contamination. Sulfate and total phosphorus concentrations (Table 2) are consistent with the identification from Table 1 that some sites at Location

A are contaminated with fertilizer by comparison against the contamination criteria and comprise 'hotspots'. None of the cadmium, lead, zinc, total petroleum hydrocarbon (TPH) and BTEX, polyaromatic hydrocarbons (PAHs), benzo(a)pyrene or organo phosphorus pesticides (OCPs) concentrations given in Tables 2 and 3 exceed respective contamination criteria and cannot be described as 'hotspots' at Location A or as being environmentally significant. However, the association of engine fuel and oil constituents with a rail engine derailment is clear.

## **CONCLUSION**

Accurate estimation of contamination of sites has been demonstrated using the grid-base sampling approach and 95% Upper Confidence Limit (UCL) criteria to distinguish transport vehicle-related contamination from phosphorus-based fertilizer at two locations. Statistical analysis of the data for different constituents in soil at the fertilizer storage sites showed the relationship between contaminant levels and dispersion over respective sites (ANZECC, 1992; NEPM, 1999; NSW EPA, 1994; NSW EPA, 1995). Dispersion of constituents of phosphorus –based fertilizers can distinguish extent of contamination during the operational phases of fertilizer handling activity from other vehicle-related contamination. "Hotspots" were identified when analytical results from individual sites were 250% of the relevant criteria value, and when the standard deviation of the data set was <50% of the criteria.

## **ACKNOWLEDGEMENTS**

Keith Halford provided assistance with the sampling study.

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## Growth Performance of *Trema orientalis* L. (Blume) Ulm. Seedlings in Response to Mineral Nutrient Omission

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Received 31 December 2020 Accepted 18 May 2021 (\*Corresponding Author)

**Abstract** The Philippine forest land is among the top threatened biodiversity areas in the world and one of the major causes is deforestation. In rainforestation, a reforestation approach developed by Visayas State University (VSU) where pioneer tree species are planted first to copy the natural flow of forest succession. Studies on early successional tree species are limited, especially on determining the growth performance as affected by nutrient deficiency. The study was conducted under screen house condition at the Terrestrial Ecosystems Division (TED), Institute of Tropical Ecology and Environmental Management (ITEEM), VSU, Philippines. This study aimed at evaluating the effect of nutrient omission on morphological and physiological growth of *Trema orientalis* L. (Blume) Ulm at seedling stage. The experimental design was randomized complete block design, with 7 treatments and 3 replications with 35 seedlings in each replication which constituted a total of 735 seedlings. The root collar increment, plant height increment, number of leaves, leaf area, dry biomass and root shoot ratio were evaluated. After six months of fertilizer application, results showed that there was an increasing influence ( $p \leq 0.01$ ) to the root collar diameter increment, plant height increment, number of leaves and leaf area specially in  $-Ca$  treatment. The biomass production and root-shoot ratio was significantly affected ( $p \leq 0.01$ ) in control and  $-N$  treatments. Stunted growth and reduced leaf were exhibited in seedlings planted at  $-N$  and control treatments. Furthermore, the result showed that the omission of calcium has positive effects ( $p \leq 0.01$ ) while omitting nitrogen showed negative effects ( $p \leq 0.01$ ) on the morphology and physiology of *Trema orientalis*. Therefore, the omission of macronutrients at early seedling stage of *Trema orientalis* growth has positively ( $p \leq 0.01$ ) and negatively ( $p \leq 0.01$ ) affected the growth performance of the study plant.

**Keywords** biomass production, growth performance, nutrient omission, rainforestation

## INTRODUCTION

Forest has a vital role in the environment and climate change since it functions as a carbon sink. According to World Wildlife Fund (2018), without forest areas, excessive carbon would contribute to the carbon present in the atmosphere and enhances the greenhouse effect. The tropics and the

subtropics experienced the greatest loss in forest cover mainly due to extensive forestry land uses where forests are converted into crop production areas (Hansen et al., 2013). The Philippine forest land is among the top threatened biodiversity areas in the world (Apan et al., 2017). The country depends on the forest for timber and other resources which leads to irresponsible logging activities, swidden agriculture and mining (Perez et al., 2020). Deforestation also affects the country's resistance to natural disasters such as landslides and flash floods. A solution to this problem is by restoring forest covers through planting of native trees. The value of the forest has become more important considering the threats brought by climate change, as forests' mitigate the effects by retaining water, carbon sequestration and so on (FAO and UNEP, 2020). Reforestation promotes the importance of native species as it promotes biodiversity rehabilitation, conservation of remaining primary forests and natural resources, and the development of a closed canopy and high diversity forest farming system (Milan and Ceniza, 2009). Regeneration of forest largely depends on the survival and growth rates of native species that are planted or have arrived on their own (D'Antonio and Meyerson, 2002). Reforestation provides environmental and economic benefits as well as enrichment of biodiversity. It also improves chemical properties of soil and provides higher net income because of the availability of resources (Neidel et al., 2012).

In a disturbed and damaged ecosystem, the early successional tree species together with other pioneer plants are the first to grow because they can adjust to poor soil conditions and adverse environmental situations (Natividad, 2016). In reforestation, pioneer tree species are planted first to copy the natural flow of forest succession which creates diverse forest and variations (Neidel et al., 2012). Soils in the tropics are fragile and the overuse of soil leads to unproductiveness and affects the growth of plants (Pessaraklim, 1999). Just like other tropical countries, the Philippines is also affected by land degradation which is a major ecological problem (Asio and Milan, 2002). Land degradation is mainly due to anthropogenic activities that can lead to loss of soil nutrient, organic matter content, addition of heavy metals, compacted and polluted soils and increased of the pH level of the soil (Asio et al., 2009).

*Trema orientalis* is a medium to large sized tree, growing in the average 20 m and attaining diameters of 60 cm at breast height. A tropical tree belonging to family Ulmaceae that grows in poor soil condition and chromite overburdens. This tree is an effective cover to avoid leaching of heavy metals in the presence of mine waste dumps into neighboring environments.

Studies on early successional tree species are limited, especially on determining the growth performance as affected by nutrient deficiency. Thus, this study will focus on early successional species *Trema orientalis* and the effects of mineral nutrient omission, both physiologically and morphologically at seedling stage. The hypothesis for this study is "nutrient element omission will affect the growth performance of *Trema orientalis*."

## OBJECTIVES

1. To evaluate the morphological performance of *Trema orientalis* seedlings as affected by nutrient element omission under screen house conditions; and
2. To determine the influence of nutrient element omission on the physiological performance of *Trema orientalis* seedlings.

## METHODOLOGY

### Location of Study Site and Experimental Design

This study was conducted at the screen house in VSU, Baybay city, Leyte, Philippines. The study site falls under type IV climate wherein rainfall is more or less distributed throughout the year.

Randomized Complete Block Design (RCBD) was used as the experimental design with 7 treatments and 3 replications for each treatment. Each replication consists of 35 seedlings with a total of 735 seedlings. The treatments were the following: Treatment 1 (T1) – without the application of nutrient solution; Treatment 2 (T2) - nitrogen (-N) was omitted in the solution; Treatment 3 (T3) - phosphorus (-P) was omitted in the solution; Treatment 4 (T4) - potassium (-K) was omitted in the solution; Treatment 5 (T5) - calcium (-Ca) was omitted in the solution; Treatment 6 (T6) - magnesium (-Mg) was omitted in the solution; and Treatment 7 (T7) – complete (N, P, K, Ca & Mg) nutrient solution.

### Data Collection

The first destructive harvesting was done before treatment application. A total of 10 samples in which the roots, stem, and leaves were separated and then measured. After measuring and air drying, the samples were placed in a paper bag and were oven dried at 70 °C for 27 hours (Bande et al., 2013). Then the dried samples were measured using digital analytical balance to acquire the biomass of the roots, stem, and leaves. Six months after treatment application (February 2019) random selection was done and 15 samples were harvested per treatment. The morphology and physiology of the seedlings were initially documented and recorded. This includes the initial root collar diameter (RCD), shoot height, and number of leaves. A correction fluid was used to mark the exact point of the root collar. The root collar diameter was measured using the Vernier caliper (mm). This was done by placing the caliper at the marker and the height was measured using a ruler (cm). The number of leaves was determined through counting of functional leaves. After six months of fertilizer application, second collection of data was done using the same methodology and instruments in their respective parameters.

### Data Encoding and Statistical Analysis

Morphological (i.e. root collar diameter, height, and number of leaves) and physiological (i.e. root biomass, stem biomass, and leaf biomass) data were encoded and graphed using MS Excel 2016. Test was done for normality and homogeneity using Statistical Package for Social Sciences (SPSS) version 25. One-way ANOVA was used to determine significant effects of the treatments on root collar diameter increment, shoot height increment, number of leaves, leaf area, dry biomass (i.e., leaves, stem, roots, and total) and root-shoot ratio. Duncan Multiple Range Test (DMRT) and Least Square Differences (LSD) were used to compare treatment means and significant differences of independent variables with significant variations at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

### Morphological Performance

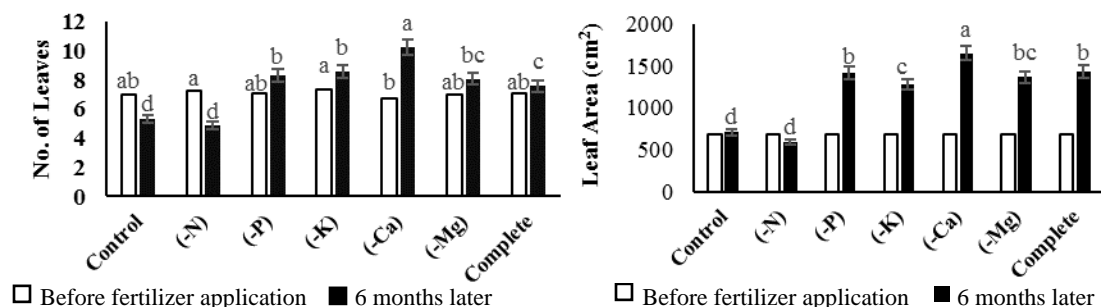
Table 1 presents that the root collar diameter increment and shoot height of *Trema orientalis* was significantly ( $p \leq 0.01$ ) affected by the nutrient element omission after six months of treatment application. It is further observed that -Ca treatments had the highest increment in root collar diameter ( $2.76\text{mm} \pm 0.09$ ) and shoot height ( $34.34\text{cm} \pm 0.70$ ) as compared to the -N and Control ( $0.92\text{mm} \pm 0.03$ ) treatments which showed the lowest growth increment of *Trema orientalis* after six months of fertilizer application (Table 4). Nitrogen is important for the formation of protoplasm which is essential for plant growth and development. Thus, omission of Nitrogen will result into stunted growth because cell division is reduced (Uchida, 2000).

**Table 1 Root collar diameter (mm) and shoot height (cm) increments of *Anabiong* planted under screen house condition six months after treatment application**

Treatment	Root collar increment (mm)	Shoot height increment (cm)
Control	$0.92 \pm 0.08^d$	$8.54 \pm 0.76^d$
-N	$1.15 \pm 0.08^c$	$7.38 \pm 0.76^d$
-P	$2.65 \pm 0.08^a$	$25.50 \pm 0.76^b$
-K	$2.49 \pm 0.08^b$	$26.03 \pm 0.76^b$
-Ca	$2.76 \pm 0.08^a$	$34.34 \pm 0.76^a$
-Mg	$2.41 \pm 0.08^b$	$26.61 \pm 0.76^b$
Complete	$2.36 \pm 0.08^b$	$22.25 \pm 0.76^c$

Note: Values with different superscript letters (a-d) within columns are statistically significant at  $p \leq 0.05$ .  
 Root Collar Increment  $n=720$ , Shoot Height Increment  $n=727$

Similarly, statistical analysis showed that the number of leaves and leaf area of *Trema orientalis* were significantly ( $p \leq 0.01$ ) affected by the nutrient element omission after six months of fertilizer application (Fig. 1). The -N and Control treatments significantly had the least leaves produced after this period and all treatments with Nitrogen produced significantly higher number of leaves. Results indicate that Nitrogen is necessary for the production of photosynthetic plant organ. On the other hand, omission of Calcium resulted to the significant increase ( $p \leq 0.01$ ) in leaf quantity as well as the leaf area of *Trema orientalis* after six months. In view of this observation, it is suggested that Ca maybe regarded not necessary for the production of leaves in *Trema orientalis* under certain growth stage. Results of the current study may also indicate inherent Ca content of the potting medium sufficient enough for the production of leaves.

**Fig. 1 Number of leaves and leaf area before and after 6 months of fertilizer application**

Note: Values with different letter (a-d) designation with the same color are significantly different at  $p \leq 0.05$ .  
 Number of leaves  $n=120$ , Leaf area  $n=15$

## Physiological Performance

The dry biomass as shown in Table 2 indicates that the -Ca treatment had the highest total biomass after 6 months of fertilizer application while the -N had the least biomass. -Ca treatment significantly had the highest root, stem and leaf biomass while -N and control treatments had the lowest increase in biomass after 6 months of fertilizer application. Furthermore, it was observed that among the plant organs roots had the most biomass allocation, regardless of what nutrient element was omitted.

## Root Shoot Ratio

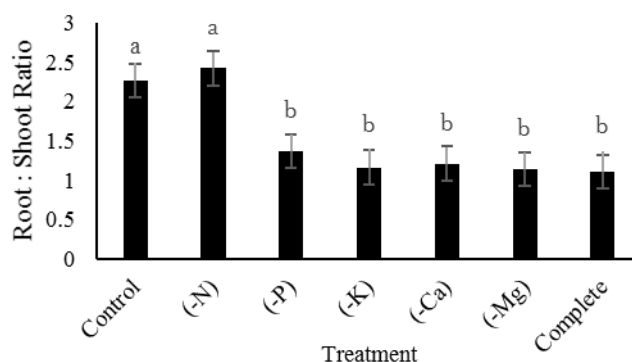
Overall observation shows that omission of N in the fertilizer solution resulted to the increase in the root-shoot ratio (Fig. 2). This observation confirms the findings of Davidson (1969) in ryegrass that decreasing the availability of N increased root: shoot ratio due to the increased sink strength of the

roots compared to the shoot sinks. Under the N depletion, soil environment roots tend to increase its length to aid the plant in obtaining more Nitrogen; hence, increasing its root biomass. The observation strongly indicates the importance of these nutrients in translocating photosynthates from shoot for root development.

**Table 2 Biomass (g) of the roots, stem and leaves of *Trema orientalis* after six months of treatment application**

Treatment	Roots (g)	Stem (g)	Leaves (g)	Total (g)
Control	$0.23 \pm 0.02^b$	$0.13 \pm 0.02^c$	$0.13 \pm 0.02^c$	$0.52 \pm 0.05^c$
-N	$0.26 \pm 0.02^b$	$0.11 \pm 0.01^c$	$0.11 \pm 0.01^c$	$0.45 \pm 0.04^c$
-P	$1.00 \pm 0.09^a$	$0.61 \pm 0.06^b$	$0.72 \pm 0.07^b$	$2.34 \pm 0.18^b$
-K	$1.18 \pm 0.10^a$	$1.00 \pm 0.20^a$	$1.10 \pm 0.14^{ab}$	$3.28 \pm 0.41^a$
-Ca	$1.16 \pm 0.09^a$	$0.84 \pm 0.06^{bc}$	$0.97 \pm 0.07^{ab}$	$2.98 \pm 0.17^{bc}$
-Mg	$1.06 \pm 0.08^a$	$0.77 \pm 0.09^{bc}$	$0.95 \pm 0.08^{ab}$	$2.77 \pm 0.24^{bc}$
Complete	$1.04 \pm 0.07^a$	$0.67 \pm 0.06^b$	$0.95 \pm 0.07^a$	$2.66 \pm 0.19^{bc}$

Note: Values with different superscript letters (a-c) within columns are statistically significant at  $p \leq 0.05$ ,  $n=105$



**Fig. 2 Root shoot ratio of *Trema orientalis* after six months of fertilizer application**

Note: Values with different letter (a-b) designation are significantly different at  $p \leq 0.05$  root:shoot ratio  $n=15$

## CONCLUSION

Omission of different macronutrient elements has a significant effect ( $p \leq 0.01$ ) on the growth increment of root collar diameter and shoot height, as well as on production of leaves and leaf area that subsequently affected the allocation of biomass in any of the plant parts and the root shoot ratio. This is because the macronutrients have specific functions as involved in the metabolic processes of the plants. Omission of calcium has positive effects on the morphology and physiology of *Trema orientalis*. Furthermore, the consistent low biomass production in -N treatment of the *Trema orientalis* strongly indicates that nitrogen primarily accounts for the growth rate of these plants. Therefore, the omission of macronutrient at the early seedling stage of *Trema orientalis* has positively ( $p \leq 0.01$ ) and negatively ( $p \leq 0.01$ ) affected the growth performance of the study plant.

## ACKNOWLEDGEMENTS

We extend our gratitude to the Nature-Life International through ViSCA Forest Reserved CO2 Conservation Project for their generous financial support of the study.

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## Potential Measurement as a Method for Monitoring the Soil Chemical Environment

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Received 8 January 2021   Accepted 19 May 2021   (\*Corresponding Author)

**Abstract** Soil chemical environment (SCE) affects soil degradation and productivity, thus understanding of the temporal changes of SCE is important for obtaining higher soil productivity. Widely used indices of SCE included ion concentration, pH, and redox potential (ORP), but these cannot monitor SCE continuously. Previously, a study proved that continued potential measurement can represent temporal changes in the water quality at the sea floor. However, no report related to monitoring the SCE with continued potential measurement has been found. Therefore, this study proposes a method for evaluating soil ORP using continued potential measurement, and examine the method's validity in representing changes in SCE due to soil reduction, bacteria activation, and soil oxidation. Laboratory experiments were conducted using soils from rice paddy fields. A brush-type carbon electrode was installed in the soil layer that then connected to a reference electrode for measurement of the soil ORP. Different soil conditions were created by mixing the paddy soil with cow manure compost or potassium sulfate. The soil ORP was automatically recorded every 15 min using a voltage meter. The ORP of paddy soil decreased temporally and stabilized at 50 days after the start of the experiment, suggesting that soil reduction occurred over those 50 days. When testing the potassium sulfate-paddy soil mixture, the soil ORP rapidly decreased during the first day after the experiment started. When testing cow manure compost-paddy soil mixture, a larger decrease in soil ORP was observed compared to that in the paddy soil alone. These findings suggest that soil reduction is promoted by adding potassium sulfate or cow manure compost. Among the soil samples tested, there was a range of soil ORPs and trends in the potential decreases. Based on the results of this study, it was found that continued potential measurement is effective in evaluating soil ORP, which represents temporal changes in SCE due to redox reactions after the addition of cow manure compost or potassium sulfate. In addition, the effects of bacteria activation are revealed during continued potential measurement of soil ORP.

**Keywords** potential measurement, carbon electrode, redox reaction, metabolism reaction, soil reduction, soil oxidation

## INTRODUCTION

Soil is a medium for plant growth and is one of the fundamental bases for agricultural productions, thus agricultural productivity is strongly influenced by the use of soil fertilizer (Hatfield, 2006). Over the last few decades, the relationship between cropping and managing systems and the soil chemical environment (SCE) has been discussed with regard to obtaining higher productivity by preventing soil degradation or erosion. The understanding of SCE is useful for ensuring healthy soil that can sustain crop production and environmentally friendly agriculture (Yan and Hou, 2018).

It has been suggested that the transition from conventional to organic or low-input farming has caused changes in the SCE and the processes related to soil fertility (Clark et al., 1998). As a decline in soil fertility results in a decrease in productivity (Buresh et al., 1997), management of soil fertility and soil health plays an important role in realizing sustainable agriculture (Prasad and Power, 1997). Since chemical reactions and nutrient circulation affect soil fertility, understanding changes in the SCE are essential in facilitating successful, high-productivity organic farming.

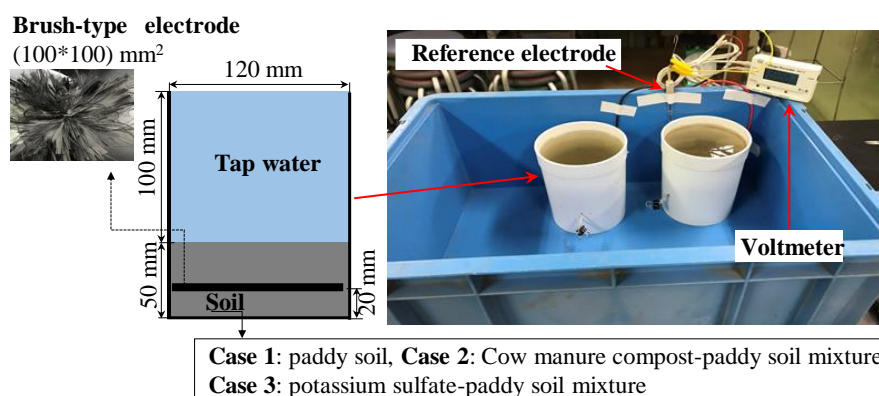
To date, parameters such as pH, organic matter contents, phosphorus availability, and cation exchange capacity have been widely used as indices for evaluating SCE. However, their measurements cannot be conducted continuously, thus changes in the SCE over time are underdetermined. Therefore, a method that can continuously monitor SCE is required. Potential measurement is a method that can monitor the redox environment of soil over time. This method may play an important role in organic farming. For obtaining a higher productivity of organic farming, the measuring SCE over time can provide useful information for controlling or improving redox and biological conditions of soils. This method was used by Nagama et al. (2018) when they suggested that water potential measurement can describe changes over time in the water quality at the sea floor. However, no report related to monitoring SCE with continued potential measurement has been found in the literature.

## OBJECTIVE

This study was aimed at proposing a method for measuring soil ORP and at examining the method's validity in representing changes in SCE due to soil reduction, bacteria activation, and soil oxidation. This was done by continued potential measurement to determine changes in the soil ORP of paddy soil. The paddy soil samples studies were pure, mixed with cow manure compost, or mixed with potassium sulfate.

## METHODOLOGY

The experimental device comprised a cylindrical bottle with an inner diameter of 120 mm and a height of 150 mm. The bottle was filled with paddy soil to a depth of 20 mm, and a brush-type carbon electrode was placed on the soil layer. Then, another soil layer at a depth of 30 mm was placed on the electrode. Finally, tap water was poured over the soil layers (Fig. 1). The paddy soil was collected during the agricultural off-season from a rice field in Ebina, Kanagawa, Japan. Approximately 150 mm of the surface soil was collected and transported to the laboratory.



**Fig. 1 Experimental device and measurement method**



The soil layer was prepared under different conditions to ensure differences in the SCE. For example, certain paddy soil was mixed with cow manure compost, and another mixture had potassium sulfate. The mixing ratio (mass basis) of paddy soil to cow manure compost or potassium sulfate was 9:1. The electrode material was carbon cloth (News Company, PL200-E), which was heated at 500°C for 1 h prior to use to improve its performance, as was suggested by Nagatsu et al. (2014). The heated carbon cloth with a width of 100 mm and a length of 100 mm was separated into fibers to form a brush-type electrode (Fig. 1).

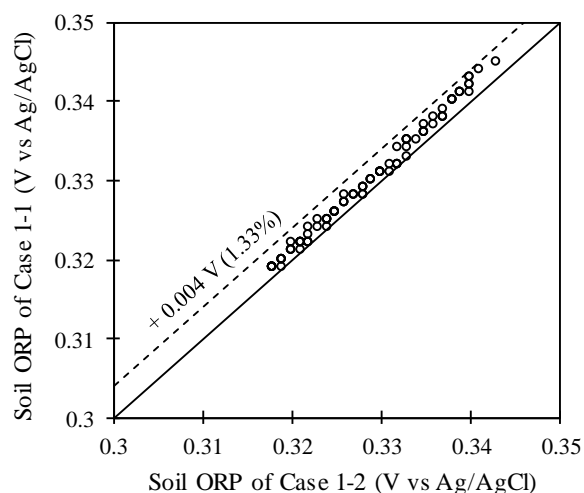
The bottles were then submerged in a container that was filled with tap water (Fig. 1). In the container, the electrode in the soil layer was connected to a reference electrode (Toyo Co., RE-7A) for measuring the electrode potential. This electrode potential was the soil ORP. The potential was recorded automatically every 15 min by a voltmeter (T&D Corp., VR-71). A plastic-coated copper wire was used for all the connections in the measurements.

## RESULTS AND DISCUSSION

### Accuracy of Potential Measurement

To examine the accuracy of potential measurement, two paddy soil samples were made under the same condition, and the soil ORP measurements of the two samples were compared (Fig. 2). The soil ORP measurements taken during the first day were used because they nearly reflected the electrode potential. As time increases, the SCE may differ from one sample to another, even though they were made under the same condition because soil reduction (organic matter decomposition) may differ.

As demonstrated in Fig. 2, the maximum variance in potential was only +0.004 V (1.33%), indicating this method's high accuracy. Therefore, it can be said that the proposed method, i.e., potential measurement, can accurately represent temporal changes in the SCE owing to redox reactions and bacteria activation in soils.



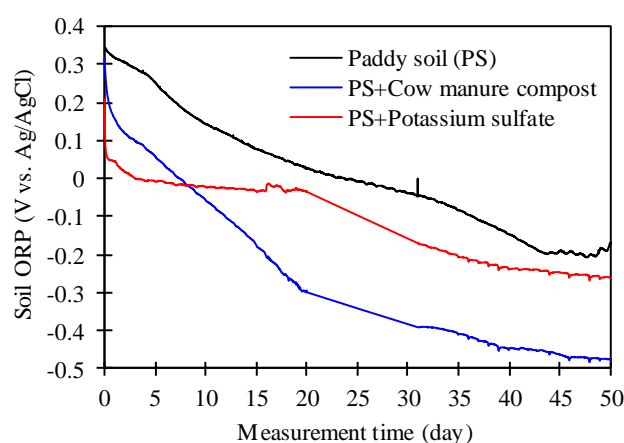
**Fig. 2 Comparison of the measured soil ORP from two samples under the same condition**

### Potential Measurement for Representing Soil Reduction

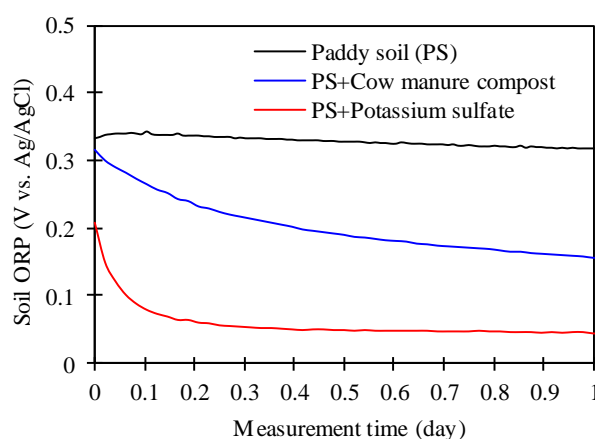
Fig. 3 illustrates the comparison of temporal changes in soil ORP for each sample. The paddy soil used was in an oxidized state as it was collected during the agricultural off-season. Thus, the initial potential

was 0.35 V which then temporally decreased and almost stabilized after 50 days. Approximately two months are required for complete soil reduction.

On day 50, the soil ORPs were  $-200$  mV for the paddy soil,  $-260$  mV for the potassium sulfate-paddy soil mixture, and  $-475$  mV for the cow manure compost-paddy soil mixture. Previously, it was reported that different redox reactions result in a difference in soil ORP (Liesack et al., 2000; Matocha, 2005). Specifically, soil ORP indicates redox reactions occurring in soils. The results of this study indicate that different redox reactions occur because of different soil compositions. Compared with paddy soil alone, a large decrease in soil ORP was observed when potassium sulfate was added to the paddy soil. This was due to the reduction reaction of sulfate in the soil. Since methanogens are present in cow manure, methane production occurred in the paddy soil the manure was mixed with, resulting in a large decrease in soil ORP. In summary, potential measurement is useful for understanding changes in SCE due to redox reactions in soils.



**Fig. 3 Comparison of temporal changes in soil ORP**



**Fig. 4 Comparison of decreasing trends of soil ORP during the first day of the experiment**

#### Potential Measurement for Representing Microbial Activation in Soils

Fig. 4 depicts a comparison of decreasing trends of soil ORP during the first day of the experiment. Interestingly, large differences in decreasing soil ORP were observed during this time. Compared with paddy soil, a large decrease in soil ORP was recorded when the soil was mixed with cow compost

manure, and an even larger decrease in soil ORP was recorded when it was mixed with potassium sulfate. These trends depend on the redox reactions occurring at the electrode and possible potential losses due to electron transfer at the electrode surface.

Following the Nernst equation (Eq. 1), the electrode potential ( $E$ ) depends on the standard potential of redox couple ( $E^0$ ) and the concentration of ions (logarithm term).

$$E = E^0 + (RT/nF) \ln (O_x/R_e) \quad (1)$$

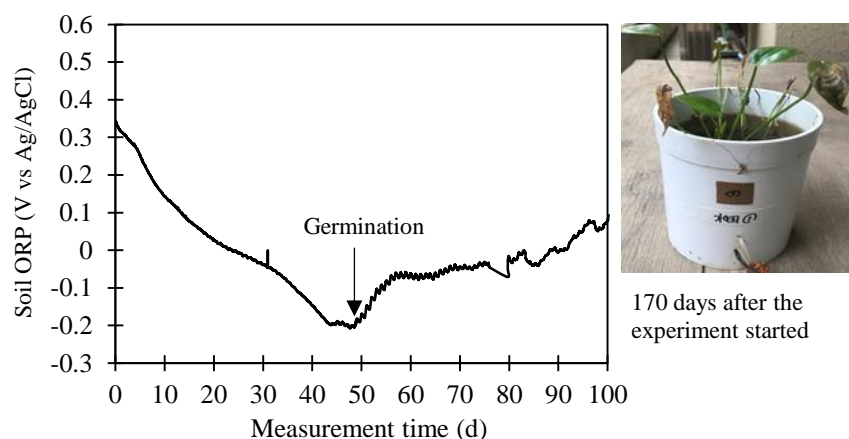
$R$  is the universal gas constant,  $T$  is the temperature in Kelvins,  $n$  is the number of electrons transferred in the reaction or half-reaction,  $F$  is the Faraday constant,  $O_x$  is the activity of the oxidized form, and  $R_e$  is the activity of the reduced form.

$E^0$  value are different between redox couples; for example,  $E^0 = 1.057$  V for the  $\text{Fe}(\text{OH})_3/\text{Fe}^{2+}$  redox couple and  $E^0 = 0.303$  V for  $\text{SO}_4^{2-}/\text{H}_2\text{S}$ . Therefore, large changes of  $E$  will be observed when the redox reaction varies from one to another redox couple. Compared with a biological reaction, a chemical reaction occurs rapidly, thus the observed rapid decrease of soil ORP in this study is due to the change of the redox couple from iron reduction in the paddy soil alone to sulfate reduction in the potassium sulfate-paddy soil mixture.

In addition,  $E$  depends on microbial activation at the electrode. According to Yamasaki et al. (2018), potential loss (activation loss) occurs during the flow of electrons into an electrode. In a potential measurement, activation loss should be minimized to obtain the equilibrium potential in a short time. Microbial activation at the electrode is one of the methods for minimizing activation loss. Wang et al. (2009) succeeded in improving electrode performance by lowering the potential losses by activating bacteria at the electrode. As seen in Fig. 4, the decrease of soil ORP when cow manure compost was used was larger than that when paddy soil alone was used. This may be explained in that microbial activation occurs in the cow manure compost. Therefore, changes in the redox couple and the benefits of microbial activation in soil can be understood through potential measurement.

### Potential Measurement for Representing Soil Oxidation

Measurement did continue after day 50 for the case of using paddy soil alone. Germination was confirmed on day 50. From the measurement taken (Fig. 5), soil ORP started to increase on day 50, indicating the generation of soil oxidation. On day 170, the soil potential reached 0.32 V which is close to the initial potential measurement taken. Additionally, the plant growth had occurred (Fig. 5).



**Fig. 5 Changes in soil ORP of paddy soil due to soil oxidation**

The increase in soil ORP was caused by soil oxidation owing to the oxygen supply from the overlying water layer. Plant growth may have also facilitated the oxygen supply of the soil layer. As the electrode was placed at a depth of 30 mm from the soil surface, the oxygen supply may have affected these long-term measurement results. In other words, the proposed method of potential measurement may also predict soil oxygen levels.

## CONCLUSIONS

In this study, a laboratory experiment was conducted to examine a method for monitoring SCE and to examine its validity in representing changes in SCE due to soil reduction, bacteria activation, and soil oxidation. It was found out that the proposed method, i.e., potential measurement, had high accuracy (with a variance of 1.33%) for representing SCE. A difference in equilibrium soil ORP was observed when mixing different fertilizers with paddy soil, indicating that redox reactions in soils can be predicted through potential measurement. In addition, different trends in decreasing soil ORP were observed, suggesting that chemical and biological reactions in soil can be understood from potential measurement. Finally, soil ORP started to increase on day 50 because of soil oxidation, indicating that potential measurement can predict soil oxygen supply that causes soil oxidation.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the students of the Rural Environmental Engineering Laboratory, Tokyo University of Agriculture, for their efforts in collecting data.

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## Study on the Water Quality of Indawgyi Lake Affected by Surroundings

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Received 30 December 2020 Accepted 19 May 2021 (\*Corresponding Author)

**Abstract** Water quality assessment was carried out at Indawgyi Lake, the biggest lake of Myanmar. The main aim of this research is to evaluate the current status of lake water quality affected by the surroundings and man-made activities for three seasons (cold, hot and rainy) in 2019. Some physicochemical properties such as temperature, pH, ORP, EC, NTU, TDS and DO of surface and deep water samples from each point and totally four different points were analyzed in situ by water quality monitor U-53-G(Horiba). The concentrations of Cr, Mn, Fe, Cu, As and Hg were also examined by atomic absorption spectrometer (AAS) and total hardness, total alkalinity, salinity and chloride were measured by their respective methods. Nitrate and phosphate were detected by LaMotte, SMART 3 colorimeter. The condition of COD and BOD of the lake water were also investigated by standard incubating methods. In addition, counting of coliform was performed to detect pathogenic microorganisms of lake water. The resulting values were compared with the standard limits of World Health Organization (WHO) and it was concluded that the lake was urgently needed to sustain the long-life existence and its water qualities due to the findings of some physicochemical, biological, toxic and pathogenic affects produced by the surroundings.

**Keywords** physicochemical properties, lake water, concentration, pollution, standard guideline value

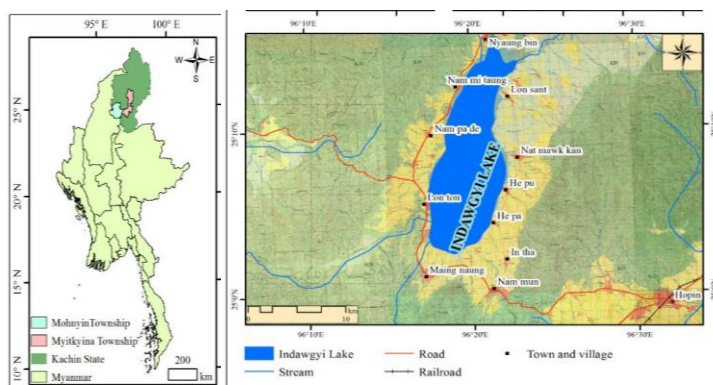
## INTRODUCTION

Water pollution becomes a serious threat and its affect to human and environmental is very wide. Freshwater ecosystems contain rivers, streams, ponds, lakes and wetlands. Fresh water is a main resource of habitat for organisms and its quality is closely related with surroundings. Now freshwater biodiversity has been challenged by many kinds of anthropogenic affects. Indawgyi Lake is not only the largest lake of Myanmar but also the third largest inland lake in Southeast Asia. It is located in the Mizoram-Manipur-Kachin Rain Forest Ecoregin in which it is the subtropical moist broad leaf forest biome. It is situated in the Mohnyin Township, Kachin State of northern Myanmar and 180 km away from the south-west of Myitkyina. Its measures are 13 km long from east to west, and 24 km wide from north to south. Lake water flows from the south to the north and only one water outlet, Indaw chaung at the northeast of the lake which finally combines into the Ayeyarwady River. Indawgyi area was officially recognized as a wildlife sanctuary on August 9<sup>th</sup> 2004 and it has also been regarded as a Ramsar site on February 2<sup>nd</sup> 2016. The entire lake basin has been declared as Biosphere Reserve on June 15<sup>th</sup> 2017 by UNESCO Man and the Biosphere Reserve Programme-MA (Indawgyi Wildlife Sanctuary, 2018). It has total area 815 km<sup>2</sup> including the lake and lakeside which is 259 km<sup>2</sup> with grasslands and the surrounding forested watershed. It has 546 feet (166 m) above the sea level. Over 400 species of birds,

109 species of water birds and 93 species of fishes and shrimps rely on the lake (Indawgyi Wildlife Sanctuary, 2018).

There are 38 villages around the lake and some villages are on the bank of the lake. Ethnic groups living in nearby villages are mostly the Red Shan and the Kachin and they earned for their livings from the agriculture, fishing and mining. They grow rice, orange, mustard, pineapple and dog-fruit. Now the lake has been facing many issues such as deforestation, disposal of mine tailings, poor management of waste, depletion of fisheries, climate change and reducing lake area.

Some water researches have done on it but there has not been still found a research that determines for both of surface and deep water qualities from each site until now. Therefore the aim of this research was to study the qualities of Indawgyi lake water affected by the surrounding not only from surface but also from bottom as the short term one year assessment in 2019. Seasonal sampling date were January 13 (as cold season), May 1 (as hot season) and July 25 (as rainy season) in 2019 (Daung Hawng, 2019).



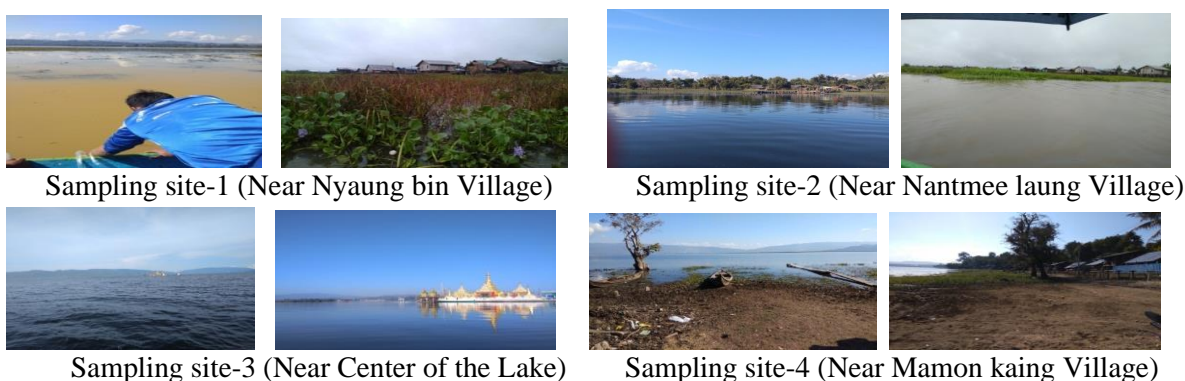
**Fig. 1 Map of the Indawgyi Lake, Mohnyin Township, Kachin State, Myanmar**

## OBJECTIVE

The objective of this research is to evaluate the current status of physicochemical and biological properties of Indawgyi lake water which is polluted by the surroundings for three seasons (cold, hot and rainy) in 2019 and to contribute the local people to get the awareness on the relation between water quality and the environment.

## METHODOLOGY

In this research, determination of physicochemical properties of Indawgyi lake water was carried out on 8 samples both of surface (sites 1, 2, 3 and 4) and bottom (sites 1(a), 2(a), 3(a) and 4(a)) of the lake water from 4 different sites. They were sampling site-1 (Near Nyaung bin Village), site-2 (Near Nantmee laung Village), site-3 (Near Center of the Lake), site-4 (Near Mamon kaing Village) respectively. Some villages are on the lakeside and near to the entrance of the permanent and seasonal inflowing streams. The depths of the water are different along the lake in everywhere randomly though the center is the deepest part, and so water-depth, location, collecting time and temperature were recorded for every sampling time. Some physicochemical properties of lake water such as pH, electrical conductivity (EC), nephelometric turbidity unit (NTU), dissolve oxygen (DO), total dissolve solid (TDS) and oxidation reduction potential (ORP) were measured by situ experiment by applying U-53-G (Horiba), (Trace20-HM 2000, Nanova). The concentrations of chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), arsenic (As) and mercury (Hg) were also examined by atomic absorption spectrometer (AAS) and total hardness, total alkalinity, salinity and chloride were measured by their respective methods. Nitrate and phosphate were detected by LaMotte, SMART 3 colorimeter. The condition of chemical oxygen demand (COD) and biological oxygen demand (BOD) of the lake water were also investigated by standard incubating methods. In addition, counting of coliform was performed to detect pathogenic microorganisms of the lake water.

**Fig. 2 Sampling sites****RESULTS AND DISCUSSION****Table 1 Physicochemical and biological parameters of cold season**

Parameters	Site 1	Site 1(a)	Site 2	Site 2(a)	Site 3	Site 3(a)	Site 4	Site 4(a)	Mni	Max	Aver	SD	n	WHO (2017)
pH	7.41	7.17	7.69	7.43	7.44	7.1	7.54	7.36	7.1	7.69	7.393	0.189	3	6.5-9.2
Ec (mmhos/cm)	0.13	0.13	0.2	0.2	0.14	0.14	0.14	0.14	0.13	0.2	0.155	0.030	3	1.5
Turbidity (NTU)	46.8	50.32	2.16	6.29	1.45	1.19	3.13	5.18	1.19	50.32	16.803	21.077	3	5
DO (mg/L)	8.01	7.17	6.45	7.44	6.53	6.46	7.96	4.29	4.29	8.01	6.661	74.518	3	-
TDS (mg/L)	86	85	88	92	91	91	93	96	85	96	90.3	3.694	3	600-1000
ORP (mV)	223	153	243	162	271	293	265	154	153	293	221	56.946	3	-
Total Alkalinity	120	100	200	140	140	140	120	120	100	200	138	29.761	3	-
COD (mg/L)	7.9	8	7.8	7.7	8	8.29	9.4	10.3	7.7	10.3	8.539	0.929	3	10
BOD( mg/L)	4.2	4	5.5	3.9	4.3	4.3	3.7	5.9	3.7	5.9	4.54	0.791	3	6
Salinity	0	0	0	0	0	0	0	0	0	0	0	0	3	-
Total hardness (mg/L)	60	60	68	64	64	64	66	66	60	68	64	2.828	3	500
Cl <sup>-</sup> (mg/L)	4.96	3.19	3.19	1.42	4.96	3.19	3.19	1.42	1.42	4.96	3.19	1.338	3	200
Mn (mg/L)	0.01	0.01	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.1	0.028	0.032	3	0.4
Fe (mg/L)	0.16	0.3	0.03	0.16	0.02	0.03	0.03	0.09	0.02	0.3	0.103	0.099	3	0.3
Cu (mg/L)	0.06	0.06	0.06	0.05	0.05	0.05	0.06	0.05	0.05	0.06	0.055	0.005	3	2
Hg (mg/L)	0.02	0.03	0.05	0.01	0.04	0.02	0.02	0.04	0.01	0.05	0.029	0.014	3	0.006
Cr (mg/L)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0	3	0.05
As ( µg/L)	0.095	0.04	0.024	0.003	0.016	0.011	0.141	0.065	0.003	0.141	0.044	0.048	3	0.01
NO <sub>3</sub> <sup>-</sup> (mg/L)	0.62	0.51	0.48	0.23	0.15	0.33	0.4	0.31	0.15	0.62	0.353	0.155	3	50
PO <sub>4</sub> <sup>3-</sup> (mg/L)	0.08	0.09	0.06	0.08	0.09	0.01	0.08	0.15	0.01	0.15	0.072	0.039	3	-
Depth (m)	0.05	1.45	0.15	2	0.55	5.25	0.3	3.54						
E-coli (CFU/100mL)	4	4	4	4	4	5	5	5						0
Temperature(°C)	20.76	19.89	23	22.65	22	21.65	22.9	21.66						
Time (hr)	12.29	12.27	2.22	2.24	3.14	3.17	4.48	4.52						

**Table 2 Physicochemical and biological parameters of hot season**

Parameters (units)	Site 1	Site 1(a)	Site 2	Site 2(a)	Site 3	Site 3(a)	Site 4	Site 4(a)	Mni	Max	Aver	SD	n	WHO (2017)
pH	9.04	8.49	8.31	8.06	7.9	7.66	9.04	8.18	7.66	9.04	8.338	0.502	3	6.5-9.2
Ec(mmhos/cm)	0.12	0.12	0.14	0.14	0.14	0.14	0.14	0.14	0.12	0.14	0.134	0.009	3	1.5
Turbidity (NTU)	6.02	7.29	1.86	2.53	2.06	1.81	5.76	8.22	1.81	8.22	4.558	2.660	3	5
DO (mg/L)	9.43	9.4	8.98	8.16	7.36	6.96	9.57	8.66	6.96	9.57	8.505	0.988	3	-
TDS (mg/L)	80	80	90	90	90	90	90	90	80	90	87	4.629	3	600-1000
ORP (mV)	141	198	224	247	217	240	139	196	139	247	198.8	41.223	3	-

Total Alkalinity	140	120	160	160	160	160	160	160	120	160	150	14.880	3	-
COD (mg/L)	8.7	9.2	8	7.5	9.5	11	14	18	7.5	18	11.14	3.575	3	10
BOD (mg/L)	3.8	4	2.5	2.7	5	6	6.5	7	2.5	7	4.7	1.709	3	6
Salinity	0	0	0	0	0	0	0	0	0	0	0	0	3	-
Total hardness (mg/L)	62	92	80	76	82	78	72	74	62	92	77	8.619	3	500
Cl <sup>-</sup> (mg/L)	4.96	4.96	3.19	7.09	10.64	4.96	8.87	7.09	3.19	10.64	6.559	2.435	3	200
Mn (mg/L)	0.15	0.12	0.01	0.09	0.13	0.1	0.14	0.14	0.01	0.15	0.104	0.045	3	0.4
Fe (mg/L)	0.05	0.05	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.05	0.026	0.018	3	0.3
Cu (mg/L)	0	0	0.001	0.001	0.03	0.003	0.17	0.17	0	0.17	0.0545	0.077	3	2
Hg (mg/L)	0	0	0	0	0	0	0.01	0.01	0	0.01	0.003	0.005	3	0.006
Cr (mg/L)	0.005	0.005	0.02	0.02	0.005	0.009	0.005	0.007	0.005	0.02	0.0101	0.007	3	0.05
As (µg/L)	0.013	0.014	0.027	0.044	0.062	0.074	0.075	0.086	0.013	0.086	0.0494	0.029	3	0.01
NO <sub>3</sub> <sup>-</sup> (mg/L)	0.42	0.6	0.08	0.2	0.24	0.12	0.12	0.07	0.07	0.6	0.252	0.188	3	50
PO <sub>4</sub> <sup>3-</sup> (mg/L)	0.03	0.07	0.05	0.11	0	0.03	0.14	0.08	0	0.14	0.065	0.046	3	-
E-coli (CFU/100mL)	4	4	5	5	5	5	5	5						0
Depth (m)	0.65	1	1.25	2	1.75	5.15	0.6	2.30						
Temperature(°C)	28.07	27.73	28.39	28.41	28.14	27.88	29.36	29.26						
Time (hr)	12.17	12.19	12.58	1	2.37	2.39	4.12	4.13						

**Table 3 Physicochemical and biological parameters of rainy season**

Parameters (units)	Site 1	Site 1(a)	Site 2	Site 2(a)	Site 3	Site 3(a)	Site 4	Site 4(a)	Mni	Max	Aver	SD	n	WHO (2017)
pH	6.9	7.46	7.46	6.09	8.15	7.85	7.94	6.5	6.09	8.15	7.259	0.732	3	6.5-9.2
Ec (mmhos/cm)	0.09	0.09	0.13	0.13	0.14	0.14	0.13	0.13	0.09	0.14	0.121	0.021	3	1.5
Turbidity (NTU)	96.9	12	13	27.4	7.91	9.83	12.3	11.8	7.91	96.9	29.595	30.086	3	5
DO (mg/L)	4.4	4.4	4.5	4.2	4.6	3.8	4.3	5.9	3.8	5.9	4.58	0.610	3	-
TDS (mg/L)	60	60	80	80	80	80	90	90	60	90	77	11.650	3	600-1000
ORP (mV)	390	405	216	434	442	373	281	286	216	442	348.5	82.285	3	-
Total Alkalinity	100	120	160	160	160	160	140	200	100	200	150	30.237	3	-
COD (mg/L)	9.8	10.6	9.2	9.5	13.5	15	17	20	9.2	20	13.38	3.998	3	10
BOD (mg/L)	7.8	8.3	8.5	9.3	10	12	15	17.5	7.8	17.5	11.37	3.523	3	6
Salinity	0	0	0	0	0	0	0	0	0	0	0	0	3	-
Total hardness (mg/L)	52	50	72	60	74	64	64	66	50	74	62.6	8.548	3	500
Cl <sup>-</sup> (mg/L)	0.2	0.2	0.09	0.09	0.15	0.12	0.1	0.1	0.09	0.2	0.134	0.0467	3	200
Mn (mg/L)	0.06	0.06	0.02	0.02	0.06	0.09	0.1	0.1	0.02	0.1	0.063	0.0320	3	0.4
Fe (mg/L)	0.3	0.3	1.12	1.12	0.12	0.12	0.08	0.08	0.08	1.12	0.444	0.450	3	0.3
Cu (mg/L)	0.08	0.15	0.05	0.05	0.12	0.11	0.09	0.09	0.05	0.15	0.094	0.034	3	2
Hg (mg/L)	0.001	0.001	0.003	0.003	0.002	0.002	0	0	0	0.003	0.0015	0.001	3	0.006
Cr (mg/L)	0.02	0.02	0.05	0.05	0.03	0.03	0.03	0.04	0.02	0.05	0.034	0.011	3	0.05
As (µg/L)	0.01	0.011	0.012	0.003	0.01	0.01	0.029	0.05	0.003	0.05	0.0188	0.0153	3	0.01
NO <sub>3</sub> <sup>-</sup> (mg/L)	0	0	0.1	0.11	0.03	0.03	0.14	0.15	0	0.15	0.071	0.062	3	50
PO <sub>4</sub> <sup>3-</sup> (mg/L)	0.03	0.03	0.03	0.03	0.05	0.05	0.13	0.13	0.03	0.5	0.191	0.207	3	-
E-coli (CFU/100mL)	5	5	5	5	5	5	5	5						0
Depth (m)	0.65	1.65	0.55	2.9	0.45	8.5	0.75	3.33						
Temperature(°C)	27.82	27.58	30.36	30.13	30.33	30.27	30.33	30.37						
Time (hr)	1.32	1.34	2.39	2.4	3.28	3.3	4.58	4.59						

Sampling time for three seasons was afternoon but significant temperature difference could be seen in (Tables 1, 2 and 3). The highest water temperatures were found in the rainy season with the range 27.58-30.37 °C while the hot season was moderate with variation 27.73-29.36 °C and the cold season showed as the least temperature with change 19.89-22.9 °C. Ongoing from surface to the depth



along a vertical column, most of water samples showed their decreased temperatures but the difference was small. Therefore the whole lake water seems to be well mixed which result from stirring by frequent water transportation. Precipitation could be only seen in the early morning of cold season. Although alkaline pH values were found in the hot season with the range 7.66-9.04, the rainy season showed some acidic pH within the range 6.09-8.15. The lake reached its lowest water level due to the reduction of seasonally inflowing streams into the lake except annually streams and therefore lake water alone remained with the alkaline pH. In the rainy season, the lake and its all wetland areas were flooded and therefore possible mine disposal and sewage directly touched with the lake water. It was leading to have acidic pH value. DO is an important index of physical phenomenon and it is influenced by many factors that affect the oxygen solubility, temperature, water movement and salinity etc. DO range in the cold season was 4.29-8.01 mg/L, 6.96-9.57 mg/L in hot season and 3.8-5.9 mg/L in the rainy season respectively. It was denoted that the DO value declined in the depth water than top water and the highest amount was determined in the hot season of site 4, 9.57 mg/L (Table 2) and the lowest value was detected in the rainy season of site 3 (a), 3.8 mg/L (Table 3).

In the cold season, COD values of sampling points with the range 7.7-10.3 mg/L showed the lesser amount than WHO standard limit, 10 mg/L except for site 4 (a), 10.3 mg/L. Three site 3(a), 4 and 4(a) out of eight sites exceeded the standard limit in the hot season with the variation 7.5-18 mg/L and five sampling points except site 1, 2 and 2 (a) were above WHO limit in the rainy season with the range 9.2-20 mg/L. All BOD values of the cold season with the range 3.7-5.9 mg/L were observed under the WHO standard limit 6 mg/L though the hot season within the range 2.5-7 mg/L surpassed the standard limit in two sites 4 and 4(a) and also the rainy season in all sites varied 7.8-17.5 mg/L. It showed that lower BOD and COD of the lake water than WHO limits could be observed at cold season due to the relation with low temperature while most of the sampling sites of hot and rainy seasons indicated higher BOD and COD amount than the WHO limits due to the relation with their high temperatures. Though the acceptable WHO guideline value of nitrate for drinking water is 50 mg/L, excess of 0.2 mg/L in the lake water indicate the possible eutrophic condition (WHO, 1992). Seven sampling points except site 3 in the cold season with the range 0.15-0.62 mg/L and three sampling points, site 1, 1(a) and site 3 expressed their exceeded levels in the hot season with the range 0.07-0.6 mg/L. All sampling points for the rainy season with the range 0-0.15 mg/L lie under 0.2 mg/L set by WHO. Due to the literature, the concentration of phosphate ranges 0.005-0.020 mg/L in most surface waters and higher than that support to the eutrophication (WHO, 1992). Most of the sampling points of three seasons (Tables 1, 2 and 3) showed exceeded phosphate level than WHO guideline limit. The lake has potential extent of nitrogen and phosphorous because a large outbreak of toxin producing cyanobacteria (blue green algae) in the whole lake, the excessive growth of aquatic plants, alkaline pH, oxygen depletion (DO) in bottom waters, decomposing macrophytes and algae in the sediment were also responsible for eutrophic condition. In addition, livestock and sewage from the agricultural run-off increased the amount of waste nutrients and then finally released the nutrients indirectly into the lake. Then more and more sedimentation set up and deposited especially in the north and south-edges of the lake which made the beds to rise up. Sediment caused water pollution, affected aquatic biota by smothering fish nursery area and wildlife by narrowing water channels and reducing lake basin area (Indawgyi Wildlife Sanctuary, 2018).

Among three seasons, the highest amount of total hardness 92 mg/L was observed at site 1(a) of the hot season with the range 62-92 mg/L and it was due to the evaporation of water by high temperature. All sampling points of three seasons showed the lower amounts of total hardness (Tables 1, 2 and 3) than the permissible limit, 500 mg/L prescribed by WHO for drinking water (WHO, 2017). The fresh water of the lake had not been detected the salinity. For all seasons, most of the sampling sites could be observed the concentrations of Fe under the guideline value 1.0 mg/L set by WHO for drinking water standard (WHO, 2017). The concentrations of Cu in all samples (Tables 1, 2 and 3) lie below the WHO guideline value 2.0 mg/L. The investigation of As pointed out as the lower levels in µg/L for all sampling points (Tables 1, 2 and 3) than WHO guideline value of drinking water

standard 0.01 mg/L. By comparison with WHO standard of Cr 0.05 mg/L, all sampling points contained the lesser amount (Tables 1, 2 and 3) than the standard values. Though all metal constituents showed with lower concentrations than the WHO limits, three heavy metals such as Hg, Cr and As could affect to the water quality with their impacts as possible as they contain. All EC values (Tables 1, 2 and 3) which lie under guideline limit 1.5 mmhos/cm set by WHO informed as the existence of low dissolved salts in the lake. Lake water also showed the lower TDS levels of all sampling points for three seasons (Tables 1, 2 and 3) than the WHO limit within 600-1000 mg/L. ORP indicates the state of oxidation and reduction process going on in water: a low value of ORP indicates high reduction while a high value for oxidation. The ranges were 153-293 mV in the cold season, 139-247 mV in the hot season and 216-442 mV in the rainy season respectively. Turbidity is caused by particular matter present in water such as clay, silt, colloidal particles, plankton and other microscopic organisms. In this research, higher amounts of turbidity units than the WHO standard limit 5 NTU were found in most of the sampling points of the cold, hot and rainy seasons were with the ranges 1.19-50.32 NTU, 1.81-8.22 NTU and 7.91-96.9 NTU but the outstanding values such as 96.9 NTU in site 1 of the rainy season maybe probably resulted by touching of detector to the bed. North-west corner of Indawgyi lake, site 1 and 1 (a) were especially suffering from the overloading of particulate matters because these two were very closed to the village and annual inflowing streams. This problem maybe probably the serious threat of pollution to the transparency of lake water in later. *Escherichia coli* was considered as the biological threat throughout the lake because it was found that 4-5 CFU/100 mL of all sampling sites (Tables 1, 2 and 3) along the west-lakeside from the north to the south due to direct latrine into the open water. *E. coli* in drinking water set by WHO is 0 CFU/100 mL (WHO, 2017). The result showed that it is necessary to carry out a proper water treatment for daily use and drinking water. Among three seasons, the rainy season does affect the most to the quality of lake water with the acidic pH, the highest turbidity, COD and BOD. The least affected season is the hot season which possesses the alkaline pH, the lowest turbidity, COD and BOD, the lowest contaminated mercury and chromium level than the other two seasons. Fish catches have decreased in the lake over the last five years of fisheries and late-rain in the rainy season were the current impacts to the environmental (Indawgyi Wildlife Sanctuary, 2018). It could be still noticed the relatively high transparency of the lake water about into 3.5m. But serious turbidity unit could replace the transparency of the lake water if the appropriate management was not applied to control the possible pollution sources in time. Natural aging process and filling to the lake with sediment slowly over time could support the eutrophication of the lake. The breeding of invasive species, water hyacinth and plants nutrients dominated many areas of the surface water and it could produce the reduction of the lake area for the aquatic biota. The use of detergents and soap, agricultural run-off, organic matter and sewage could increase the amount of nutrients and encourage the phenomenon of eutrophication. Starting point of plankton growth could be found on the surface water of the north-west part of the lake. In this area, reddish-yellow color water could be observed the whole year as shown in the Fig. 2. In conclusion, it could be said that physicochemical and biological properties of lake water quality was significantly suffered from the impacts of surroundings and needed to sustain its quality for the health of human and environmental.

## CONCLUSION

This research was conducted as the assessment of the water quality of Indawgyi lake, the biggest lake in Myanmar and performed some physical, chemical and biological examinations of the surface and depth-water. The results obtained of the present work showed their significant variation with seasonal fluctuations. The current situation and challenges of Indawgyi lake water were increasing with sedimentation, potential eutrophication and pollution of water quality by chemical, physical and biological contaminants. The main causes of these problems were probably excessive human activity such as domestic uses, agricultural runoff, recreation, tourism, fishing, nutrient submerged and floating

plants from the surrounding. The lake is now needed to be control for water quality and long-live of biodiversity especially for agricultural drainage, gold mining effluents, excess nutrients and sewage from direct toilets to prevent sedimentation and eutrophication of the lake and to protect the hazard of water pollution for health of populations from the surrounding area.

## **ACKNOWLEDGEMENTS**

We would like to thank to Department of Environmental Conservation, Myitkyina and Mohnyin and Wildlife Sanctuary, Indawgyi Lake for warm explanation, transportation, sampling, testing and giving permission to apply the laboratory.

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## **Experimental Study of Extension Impact on Farmers' KAP towards Sri Lankan Cassava Mosaic Disease Prevention in Cambodia**

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Received 12 January 2021   Accepted 23 June 2021   (\*Corresponding Author)

**Abstract** The Sri Lankan Cassava Mosaic Disease (SLCMD) poses a major threat to the cassava industry in Cambodia, as it can decrease cassava yield by up to 80 percent. As SLCMD has no clear prescription, and currently, the only remedy is pulling out and incinerating the infected stems. The government of Cambodia, through the General Directorate of Agriculture (GDA), has moved swiftly to sensitize farmers on the causes, effects, and prevention measures of SLCMD. The GDA has used various media and methods to disseminate knowledge on SLCMD. However, effective information dissemination methods for changing farmers' behaviors in terms of the knowledge, attitudes, and practices (KAP) still remain to be identified. In this study, we investigate the effectiveness of two knowledge dissemination methods, i.e., "single intervention"—distribution of printed educational materials (PEMs) and "multifaceted intervention"—distribution of PEMs combined with workshop training. The study was conducted in Battambang, north-west Cambodia over two periods, from June to October 2019, and from November 2019 to February 2020. In the first period of investigation, 468 farmers were randomly selected to participate in the study. We formulate the contents of the poster and workshop based on the "initial" KAP results where farmers had lower KAP. Then, all 468 farmers were divided randomly into three groups, namely "Control," "Treatment1," and "Treatment2." farmers in "Treatment1" were subjected to "single intervention" and those in "Treatment2" were subjected to "multifaceted intervention," while those in "Control" were subjected to no intervention. In the second period of investigation, the "second" KAP was analyzed to estimate the effectiveness of interventions applied in the first period. The results show that "multifaceted intervention" is the effective method to improve the KAP of farmers in

Cambodia. Our experience of running such farmers' workshops and the materials we developed could be useful to governments, non-governmental organizations, and commercial associations that are keen to mitigate the effects of SLCMD through appropriate interventions.

**Keywords** field experiment, extension, KAP, SLCMD, RCT

## **INTRODUCTION**

Cassava is the second major income source for Cambodian small-scale farmers after rice. In 2019, the number of small-scale cassava farmers in Cambodia was above 300,000 (Codes et al., 2019). From 2018 to 2019, land area under cassava cultivation increased from 611 thousand hectares to more than 624 thousand hectares (MAFF, 2019). In 2019, Cambodian cassava yield was, on average, approximately 27 tons per hectare. The total harvested area was approximately 504 thousand hectares (FAOSTAT, 2019). The corresponding benefit-cost ratio analysis shows that for every 1 riel invested in cassava farming, a profit of 1.31 riel was realized (Thav, 2017).

However, the increasing number of pests and cassava diseases poses a major threat to the quantity of cassava produce. This may in turn harm local food industries and consequently the national economies in cassava producing countries (Chanda et al., 2016). For example, cassava crops in Africa are being attacked by pests (such as the cassava green mite (CGM), the cassava mealybug, and the variegated grasshopper) and diseases (such as the cassava mosaic disease (CMD), the cassava brown streak disease (CBSD), and the cassava bacterial blight diseases) (Vanessa et al., 2011). In Africa, CMD is the most harmful of the cassava diseases. Data have shown that it can reduce cassava yield by up to 90 percent (Hahn et al., 1980). In Cambodia, the Sri Lankan cassava mosaic disease (SLCMD) is the most harmful. It was first discovered at a commercial farm in Ratanakiri province, Eastern Cambodia in May 2015 (Wang et al., 2016). By 2019, approximately 12 provinces had an outbreak of the SLCMD (MAFF, 2019).

Some of these outbreaks have been attributed to the whitefly and stem transmissions (Vanessa et al., 2011 & Minato et al., 2019). Currently, integrated insect pest and pathogen control methods are hardly used, owing to little sensitization of farmers on their availability and the trivial effort put by manufacturers into the manufacture of needed equipment. Biological methods seem to be the popular insect pest and pathogen control choice among farmers.

Therefore, there is an urgent need for improved uninfected planting tools (Nsiah-Frimpong et al., 2020). To address this situation in Cambodia, many strategies have been employed, including controlling the movement of planting materials, selecting healthy and uninfected seedlings, encouraging farmers to regularly check their fields, mapping the infected and non-infected areas, and improving communication between farmers and local agricultural extension agencies (MAFF, 2017, 2019). In addition, the general directorate of agriculture (GDA) has adequately sensitized farmers on SLCMD via field-day workshops, posters, and information leaflets. Further, the GDA has produced an educational video clip titled "managing the cassava mosaic disease," which can also be found on social media.

To cure infected cassava stems, the National University of Battambang, supported by the Japanese international cooperation agency and the Japan Science and technology agency, has propagated tissue culture of cassava (Tokunaga et al., 2020). Existing studies have recommended workshop training to improve the knowledge, attitudes, and practices (KAP) in managing the pests and diseases of cassava (Chikoti et al., 2016 and Nsiah-Frimpong et al., 2020). Disseminating the relevant knowledge to farmers could be the most splendid strategy for managing SLCMD (Houngue et al., 2018). However, there is no study that can help identify the information that the farmers are lacking. Further, and it has not been determined which of the information dissemination methods is more effective in changing the KAP of farmers. Therefore, in this study, we endeavor to identify the knowledge gaps and investigate the effectiveness of two knowledge dissemination methods, i.e., "single intervention"—distribution of

printed educational materials (PEMs) and “multifaceted intervention”—distribution of PEMs combined with workshop training.

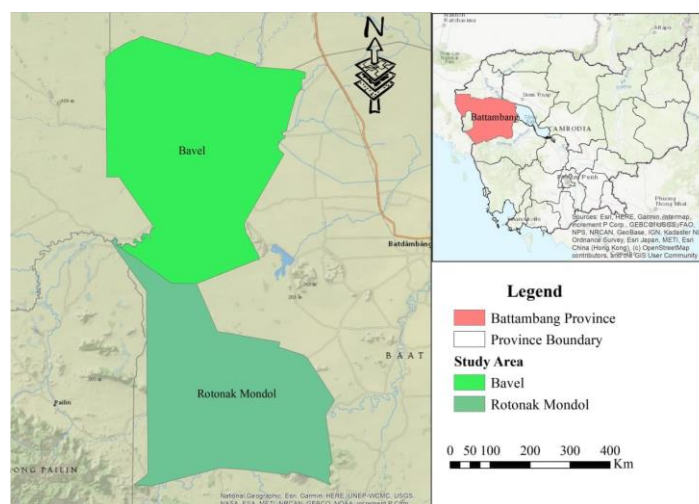
## OBJECTIVE

The main objective of this study is to evaluate the effectiveness of the current information dissemination methods compiled by the project in order to examine farmers’ behavioral change in terms of the KAP towards pest and disease control. We will determine if the information dissemination methods have been effective in discouraging the re-use of contaminated seedlings and suggest policy changes that will help mitigate further damage that may be caused by the SLCMD.

## METHODOLOGY

### Study Site

Battambang was selected as the study site. Battambang is the largest cassava producing province in northwest Cambodia. The total area under cassava cultivation in Battambang is 112,543 hectares which is ~ 18 % of the total area under cassava cultivation in Cambodia (MAFF, 2019).

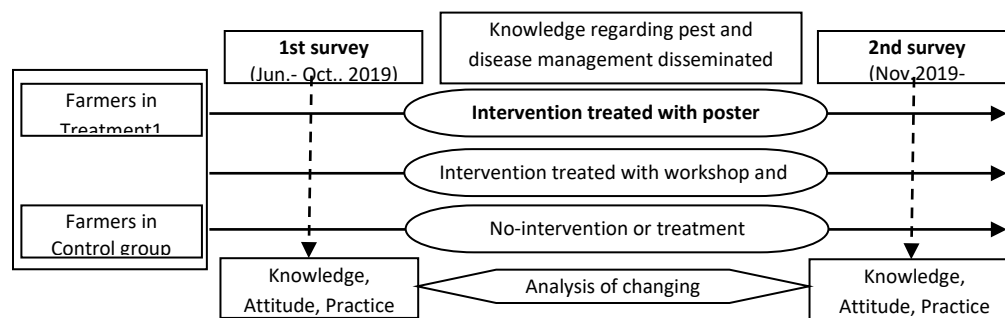


**Fig. 1 Study area in Battambang Province, Cambodia**

### Research Design and Data Collection

The study was conducted over 2 periods—from June to October 2019 and from November 2019 to February 2020 (Fig. 1). In the first period of the investigation, between June and August 2019, 468 farmers were randomly selected to participate in the study. Their “initial” KAP was analyzed, following which all the 468 farmers were divided randomly into 3 equal groups—“Control,” “Treatment1,” and “Treatment2,” using Stata version 16 (a statistic software). Randomization was evenly stratified at the commune level by gender, age, education, and knowledge level. Farmers in “Treatment1” received printed educational materials (PEMs) in the form of posters. Those in “Treatment2” received PEMs in the form of posters and were sensitized in educational workshops. Those in “Control” did not receive any sensitization material. In the second period of investigation, the “second” KAP was analyzed to estimate the effectiveness of interventions applied in the first period.

For this investigation, we received responses from 310 of the 468 research participants. This is because 158 farmers did not cultivate cassava during the second period of investigation.



**Fig. 2 Randomized Control Trial (RCT) design for Knowledge transfer**

**Table 1 Results of randomization at the commune<sup>6</sup> level**

Groups		Communes		Observations	Percentage (%)
Treatment1	Plov Meas	Sdao	Treng	231	49.35
Treatment2	Ampil 5 Derm	Kdol Taken	Reaksmey Sangha	157	33.54
Control	Khleang Meas	Onderk Herb		80	17.09

*Note: Data summarized by the authors*

## Intervention Design

### Single intervention - Printed educational materials (PEMs)<sup>7</sup>:

The PEMs distributed in the form of posters contained text, photos, illustrations, and spaces adorned in bright colors to attract the attention of readers. The majority of the information contained in the posters was about SLCMD. Specifically, the causes, effects, and prevention measures of SLCMD. Information on the transmission mechanisms of the disease was put in a conspicuous section of the poster. Information on the devastating effects of SLCMD in Cambodia and Africa was shown via images and text. SLCMD prevention and control measures were illustrated by cartoons, which made the poster more accessible. The designers of the posters took great care to make sure that information visualized by photos and cartoons could not be misunderstood or misinterpreted. The posters had a horizontal layout. The size of posters (A3) was carefully decided to enable farmers to easily hang them on walls and/or fold them in half during sessions of group/community information sharing.

### Multifaceted intervention:

The multifaceted intervention involved combined use of PEMs distributed in the form of posters (the same as used in the single intervention—PEMs) and a workshop. The workshop was held to improve the knowledge of farmers on pest and disease management. Information disseminated in the workshop was compiled based on pre-identified gaps in the farmers' understanding, awareness, and practices. The gaps were identified in the first survey. Similar to the poster's design process, the workshop content was created by the experts of agronomics from the National University of Battambang. The experts took into account the language, culture, and literacy of the targeted farmers. These farmers; social data were collected during the first period of our investigation. Moreover, the workshops included a Q&A session where misunderstandings of the audience were clarified. The audience was also allowed to share their experiences with SLCMD. We also used the workshops to collect more data on SLCMD by asking the farmers what they deal with in real life.

<sup>7</sup> Printed educational materials in the form of poster will be included in the Annex.

### Questionnaire:

The field survey was conducted in Rattanak Mondol, Reaksmeay Songha, Plov Meas, Sdao, Treng, Onderk Herb, Ampil 5 Derm, Kdol Tahern, and Kleang Meas communes (Table 1), and 468 samples were collected. There were 5 parts to the questionnaire: the first part was about the correspondents' socio-demographic status; we concentrated on the participants' knowledge, awareness, and practices in the second, third, and fourth parts, respectively; the final part focused on the farmers' understanding of willingness to pay for healthy cassava seedlings.

### Data analysis methods:

The data were analyzed using Stata version 16. From the ex-ante data (first survey) and the ex-post data (second survey), we scored the answers as 1 for correct and 0 for incorrect. For questions with multiple answers, if the respondents chose more than one answer, it would automatically be classed as incorrect and scored as 0. To estimate the effects of the two knowledge interventions (PEMs and multifaceted intervention), we used the differences between the treatment groups (Treatment 1 and Treatment 2) in the first and second surveys to compare with the difference in "Control" via t-test.

## RESULTS

The paired t-test was conducted to compare the differences between before and after treatments, namely difference-in-difference (DiD) among "Control" to "Treatment 1" and "Treatment 2." The differences in the means were calculated. Both DiD of mean differences of "Treatment1" versus "Control" and that of "Treatment2" versus "Control" showed statistical significance at a p-value of 0.01. Table 2 shows that improved knowledge of whitefly and SLCMD symptoms in "Treatment1" and "Treatment 2" versus "Control" was significantly different at a p-value of 0.01. Likewise, getting to know the name "whitefly" was significantly different at a p-value of 0.01 in "Treatment 2" and a p-value of 0.10 in "Treatment 1." Farmers' knowledge after receiving dissemination from both "single intervention" and "multifaceted intervention" increased at a p-value of 0.01.

**Table 2 T-test results of Farmers' Knowledge**

Variable	Definition of variable (Correct=1; incorrect=0)	Mean-Diff (2 <sup>nd</sup> – 1 <sup>st</sup> )			Coefficient	
		Control group (n=51)	Treatment1 group (n=165)	Treatment2 group (n=94)	Control vs. Treatment1	Control vs. Treatment2
KN_whitefly	Whitefly is an insect pest.	-0.039	0.151	0.351	0.143 ***	0.185 ***
KN_slcmd	Do you know SLCMD?	0.156	0.557	0.861	0.200 ***	0.363 ***
KN_whitename	Name of whitefly	0.140	0.234	0.554	0.068 *	0.226 ***
KN_whiteanswer	Whitefly causing the symptoms	0.039	0.327	0.489	0.304 ***	0.488 ***

\*\*\* Significant at 0.01 \*\* Significant at 0.05 \*Significant at 0.1 - Non-significant

As can be seen from the above table, before the distribution of PEMs and/or workshops, the majority of the respondents were not aware of the precariousness of getting their cassava seedlings from a neighbor or a middleman. Additionally, they were not aware of the benefits of getting their cassava seedlings from a certified healthy seedling distributor (Table 3). It means that farmers continue to face the risk of getting infected stems. On the other hand, when farmers are asked "I can do something to prevent (cassava) plants, Treatment1 seems to have more positive more than the farmers in the Control while farmers in Treatment 2 were not significantly different from that of Control. Moreover, farmers of both Treatment1 and Treatment 2, felt they need more authority advice regarding SLCMD and farmers' attitudes of both groups improved at a p-value of 0.01. Treatment 2 felt the importance of authority advice more than farmers in Treatment 1.



**Table 3 T-test results of Farmers' Attitude**

Variable	Definition of variable (Strongly disagree=1 to strongly agree=5)	Mean-Diff (2 <sup>nd</sup> – 1 <sup>st</sup> )			Coefficient	
		Control group (n=51)	Treatment1 group (n=165)	Treatment2 group (n=94)	Control vs. Treatment1	Control vs. Treatment2
AT_neighbor's stem	Neighbor's stem can reduce the risk of SLCMD.	-0.078	0.090	-0.510		
AT_middle man's stem	Middle man's stem can reduce the risk of SLCMD.	-0.745	-0.915	-0.765		
AT_certified healthy	Certified healthy's stem can reduce the risk of SLCMD.	-0.486	-0.236	-0.567		
AT_I_can_do_s.th	I can do something to prevent plant.	0.529	0.446	0.557	0.503 ***	
AT_authorityadvise	It is important to know SLCMD.	1.000	-0.496	-0.574	0.568 ***	0.891 ***

\*\*\* Significant at 0.01 \*\* Significant at 0.05 \*Significant at 0.1 - Non-significant

Table 4 indicates that the mean of purchasing seedlings for planting from a trusted source increased for both groups. On the other hand, the mean of timely spraying of insecticide to whitefly in neither group was significantly different. Finally, the mean of removing all cassava debris suspected of being SLCMD in Treatment1 versus Control was significantly different at a p-value of 0.1. Also, it was not significant in Treatment 2 versus Control, the direction was not to remove the debris. This might mean that farmers have gotten the wrong impression after receiving the contents in PEMs and workshops and can be a scope for improvement of the contents in the future training.

**Table 4 T-test results of Farmers' Practice**

Variable	Definition of variable (Never=1, Sometimes=2, Always=3)	Mean-Diff (2 <sup>nd</sup> – 1 <sup>st</sup> )			Coefficient	
		Control group (n=51)	Treatment1 group (n=165)	Treatment2 group (n=94)	Control vs. Treatment1	Control vs. Treatment2
PR_trusted sources	Purchasing cassava stem from trusted sources.	0.098	0.418	0.414	0.320 ***	0.317 ***
PR_insecticidewhitefly	Timely treatment of whitefly.	-0.294	0.000	-0.308	0.294	-0.014
PR_removed_debris	Removing all cassava debris suspected of being SLCMD	-0.254	-0.727	-0.276	-0.472 *	-0.022

\*\*\* Significant at 0.01 \*\* Significant at 0.05 \*Significant at 0.1 - Non-significant

## DISCUSSION

Our results show that the dissemination of information via the multifaceted intervention is the more effective method of increasing farmers' KAP. Previous studies had indicated that the majority of farmers who participated in workshop training became more aware of the cassava virus diseases (Eni et al., 2019). Nishiah-Frimpong et. al. also emphasizes the importance of training farmers on integrated methods of insect pest and disease control (2020). It is also possible that the workshops increased their outreach by extending the programs to the less literate farmers. In addition, other studies have posited that farmers are more susceptible to consume knowledge when it is disseminated by experts (Houngue et al., 2018).

However, we also found out that neither of the two methods sufficiently improved the attitudes of farmers toward disease preventive measures. When we asked whether they believe "certified healthy stem can reduce the risk of SLCMD," in the attitude question (Table 3), farmers in all groups slightly

shifted from disagreeing to agree; however, it was not statistically significant. On the other hand, when we asked their purchasing behavior in the practice question (Table 4), farmers indicated that they would purchase items for the next propagation from trusted sources. It could suggest that the majority of them did not believe that purchasing the seedlings from trusted sources can reduce the risk of SLCMD, and they would still face potential infection after purchasing healthy stems. On the other hand, farmers both in Treatment1 and Treatment2 would purchase stems for next propagation from trusted sources. It indicates that they are more aware of the potential risk of SLCMD even for healthy seedlings, but still purchasing stems from a trusted source is important to reduce the risk of getting stems infected from seedlings. Another reason could come from the fact that although multifaceted intervention enabled farmers to understand the importance of purchasing the seedlings from trusted sources, the majority of them did not believe that purchasing the seedlings from trusted sources can reduce the risk of SLCMD. Noticeably, the choice of cassava seedlings for the majority of the farmers depends on their agronomical traits, such as tuber yield, fast harvesting, resilience in the soil, and adaptability to drought (Bentley et al., 2017). Also, the reluctance of farmers to purchase seedlings from trusted sources can be attributed to the farmers realizing that purchasing seedlings from a trusted source cannot by itself reduce the risk of SLCMD transmission unless farmers implement regular monitoring and pest treatment management.

Moreover, the workshop disseminated information on well-known SLCMD symptoms and how to identify uninfected cassava seedlings for the next planting session. Farmers who could not come around to purchasing seedlings from trusted sources were taught how to propagate their own stems as an alternative mitigation measure of reducing the spread of SLCMD (Mulenga et al., 2016). The workshop with the poster group did significantly improve farmers' practices, for example, purchasing cassava seedlings from trusted sources. Our findings are consistent with those of a study conducted in Nigeria (Ebewore and Isiorhovoja, 2019), which acknowledged that training is a major source of information to cassava producers, and without training, only approximately 17.0% of the farmers will (N=569) practice disease management.

Further, the study revealed that although spraying insecticide the whitefly is crucial to curbing the spread of SLCMD, farmers were not spraying the insecticide. The two possible explanations are: (1) farmers have unsuccessfully tried to spray the insect before and had eventually given up; (2) farmers do not have sufficient finances to spray the insect. Previous studies showed that approximately 91.1% (N=90) of farmers were familiar with insect pests and diseases as causes of significant damage to the cassava crop, but they could not distinguish between the treatments of insect pests and diseases (Chikoti et al., 2016). From the results, it became clear the need to emphasize the importance of early treatment of whitefly, as it is the vector of SLCMD spread.

## CONCLUSION

The objective of this study was to estimate the effectiveness of knowledge dissemination methods in changing farmers' KAP towards pest and disease management. We sought to determine which method is effective in discouraging the re-use of contaminated seedlings. We also suggest policy changes that might help mitigate further damage due to the SLCMD. In general, we found that dissemination of information via workshop training, combined with the distribution of PEMs is an effective way of improving farmers' KAP and, consequently, prevent the spread of SLCMD. Nonetheless, as effective as workshop training combined with the distribution of PEMs was, this method did not sway farmers' KAP completely. In this regard, we suggest that for future training, trainers should emphasize the importance of early mitigation practices in minimizing yield loss. The emphasis is likely to further improve farmers' KAP. To do so, trainers should inform farmers of the actual yield loss per hectare they are likely to incur and consequent income loss if they neglect the early mitigation. Where possible trainers should give real-life examples that the farmers can relate to. This way, the farmers will see the

magnitude of the potential yield and income loss. Nevertheless, our experience of running such farmers' workshops and the materials we developed could be evolved and improved, and they could be useful to governments, non-governmental organizations, and commercial associations that are keen to mitigate the effects of SLCMD through appropriate interventions.

## ACKNOWLEDGEMENTS

This research was supported by the Science and Technology Research Partnership for Sustainable Development (SATREPS) in collaboration with the Japan Science and Technology Agency (JST, JPMJSA1508) and Japan International Cooperation Agency (JICA)

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## Evaluation and Preference Analysis of Improved Rice Genotypes in TharGaYa Village, Tharsi Township, Myanmar

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Received 22 December 2020    Accepted 24 June 2021    (\*Corresponding Author)

**Abstract** This study was conducted to identify high yielding improved rice genotypes which are adapted to the target location and accepted by local farmers. The experiment was conducted with a randomized complete block design with three replications, at TharGaYa village, Tharsi township, Myanmar. Sixteen improved rice lines and two check varieties were examined. Farmers voted for acceptance or rejection of individual varieties at the vegetative and pre-harvest stages and these votes were converted to a preference score. A significant difference among the genotypes tested occurred in yield per plant and its related characters. From the preference analysis, farmers and researchers showed highest preference for SM1/THY-DH-1-1 at the vegetative stage, followed by YAU-1214-183-3-1-2-1-1 and YAU-1201-90-2-4. At pre-harvest stage, farmers and researchers elected YAU-1201-90-2-4, followed by YAU-1214-183-35-1-1-1 and YAU-1214-183-3-1-1-1-1. According to the sensory evaluation test, the best line was YAU-1201-90-2-4, with second and third being YAU-1201-26-1-1 and YAU-1201-26-1-3. There was very strong agreement in preferences for the best performing lines shown by male and female farmers. However, a weak correlation between researchers and farmers preferences was identified. This seemingly indicates that farmers and researchers have different criteria in selecting good performing lines. Farmers have prioritized their own way of variety selection for their localities, using features such as uniformity, lodging, panicle length, effective tillering and earliness. Therefore, it is important to include farmers' preferences in any selection process to determine a suitable and acceptable variety.

**Keywords** correlation, preference score, sensory evaluation, variety selection

## INTRODUCTION

Rice is one of the most important cereal crops around the world. Myanmar is an agricultural country, and agriculture is the backbone of its economy. The agriculture sector contributed 27.5% of GDP; 13.7% of total export earnings; and employed 61.2% of the labor force in 2010 (MOALI, 2010). One fourth of Myanmar's total area is cultivated land. In Myanmar, rice production accounted for the majority of cropping area and it is also the major food source. Rice ecosystems in Myanmar are generally classified into seven categories: 1) Irrigated rice land, 2) Regular rain-fed lowland, 3) Drought-prone rain-fed lowland, 4) Deep water rice 5) Submerged rice land, 6) Sea water affected rice land and 7) Upland rice (MOALI, 2010). Since it is a staple crop, having high yielding varieties that are adaptable for different agro-ecosystems is crucial.

Plant breeders develop varieties through conventional breeding and release varieties that are most productive under ideal conditions; often they are not suitable for marginal farm conditions (Singh et al., 2014). Therefore, participatory varietal selection, facilitates development of varieties optimal for marginal soils and acceptable to farmers. Farmers' preferences represent a part of the genotype selection and testing process, in order to ensure the large-scale adoption of the selected genotype.

Participatory Variety Selection (PVS) can be used to identify farmer-acceptable varieties effectively, and thereby overcome the constraints that cause farmers to grow old or obsolete varieties, or late maturing varieties which are susceptible to drought (Joshi and Witcombe, 1996; Witcombe et al., 1996). PVS trials were conducted on farm under the supervision of farmers, and identification of advanced promising lines from the breeding program occurred. It is crucial for plant breeders to identify which genotypes farmers prefer, and which they dislike, and the reasons for these opinions, adjusted to local conditions. PVS is a rapid and cost-effective way of identifying farmer-preferred cultivars and whether a suitable choice of cultivars exists (Witcombe et al., 2008). Hence, research costs can be reduced and adoption rates increased since farmers participate in variety testing and selection (Joshi et al., 1995).

## **OBJECTIVE**

The experiment was conducted to identify high yielding and acceptable improved rice genotypes adapted to TharGaYa village, Tharsi Township, Myanmar through farmers' participation.

## **MATERIALS AND METHODS**

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications in farmers' fields in TharGaYa village, Tharsi Township, Myanmar. A total of 18 genotypes, including check varieties (Appendix 1), were raised in seedbeds and transplanted at 21 days after sowing, with a distance of 20 cm between rows and 20 cm within rows. All necessary precautions were taken to maintain uniform plant population in each treatment replication. The data recorded were 50 % flowering (DTF), plant height (PH), effective tiller per hill (ETPH), panicle length (PLen), spikelets per panicle (SPP), filled grain percent (FGP), 1000 grain weight (TGW) and yield per plant (YPP). These data were collected to know the farmer selected line(s) has short duration, medium or short plant height and high yield. These data were subjected to statistical analysis by using STAR software (STAR, 2014). Preference analysis through casting votes was conducted when most genotypes reached the active tillering and pre harvesting stages. In case of the selection of farmers, there were a lot of discussions and meetings with local staff from Department of Agriculture, key farmers and village authorities. After that, all the rice farmers who have different land access to grow rice for year-round cultivation were selected to participate. Additionally, voluntary farmers were asked to include to have a variety of preferences. In order to conduct the preference analysis, 61 farmers and 8 researchers were participated. In Myanmar, most of the farmers were males. Rahman et al., (2015) pointed that female farmer had different perceptions with males regards the selection process. In this case, the votes were separated from male and female farmers and identify whether their perceptions have somehow agree with each other.

A preference score was determined by using positive votes, negative votes and total votes cast (Paris, 2011). Firstly, the experimental plots were prepared with each line allocated with a bag for collection of ballots. Farmers were provided with four ballots to vote for two best and two worst lines. There were either positive or negative sign on one surface of each ballot and the possible reasons for their choice on one another. All the votes rooted from the quality of above-mentioned characters since the participants needed to mention the reason for their votes. To allow examination of farmer population' preferences, male farmers' ballots were blue, females' pink and researchers' green. It was

necessary to prepare separate ballot bags to collect the votes for each genotype. All the participants were asked to mention the reasons for their choices on the back of the ballots. Then preference scores for men and women were computed independently through counting positive and negative ballots collected in each ballot bag. The preference score was calculated as follows.

$$\text{Preference score} = \frac{\text{Number of positive votes} - \text{Number of negative votes}}{\text{Total number of positive and negative votes}}$$

Pearson's correlation was used to test the agreement of preferences between male and female farmers and between researchers and farmers.

## RESULTS AND DISCUSSION

### Yield and Yield Contributing Characters

Significant differences among the improved rice lines were observed in days to 50% flowering, effective tillers per hill, panicle length, spikelets per panicle, filled grain percent, 1000 grain weight and yield per plant (Table 1). Yield per plant of YAU-1215-183-3-4-1-1-1 line was highest among the tested lines, followed by YAU-1214-183-3-1-1-1-1 and YAU-1214-183-3-3-1-1-1. Yield per plant of the 9 improved rice lines were higher than those for the two local check varieties. However, effective tillers per hill was the highest in local check variety, ManawThuKha, followed by YAU-1215-183-3-4-1-1-1.

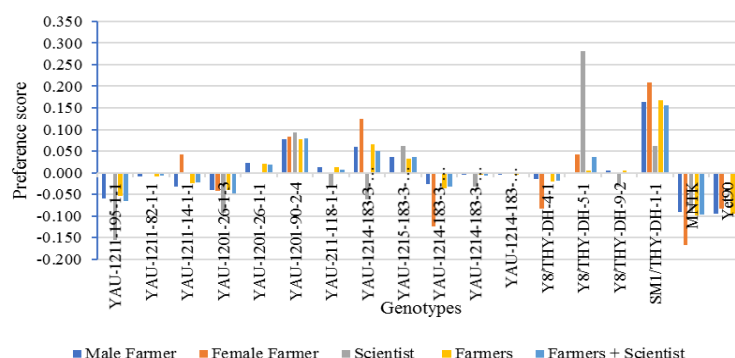
**Table 1 Mean performance of improved rice lines in TharGaYa village, Tharsi Township**

Improved Rice Lines	DTF (days)	PH (cm)	ETPH (no.)	PLen (cm)	SPP (no.)	FGP (%)	TSW (g)	YPP (g)
YAU-1211-195-1-1	99.33	107.27	14.27	22.23	149.62	52.49	19.19	20.73
YAU-1211-82-1-1	91.00	108.20	14.93	21.82	142.51	70.08	24.75	35.42
YAU-1211-14-1-1	104.33	118.80	13.13	21.85	147.43	53.51	20.97	21.70
YAU-1201-26-1-1	94.00	105.33	12.00	23.34	96.66	69.84	28.53	22.74
YAU-1201-26-1-3	99.33	107.87	14.20	22.22	124.08	58.65	25.20	25.97
YAU-1201-90-2-4	91.67	115.93	16.20	22.60	83.49	80.62	29.16	31.13
YAU-1211-118-1-1	94.00	94.20	15.47	25.57	116.71	71.49	26.61	33.97
YAU-1214-183-3-1-1-1-1	90.00	102.47	15.07	24.05	115.72	89.54	27.53	42.12
YAU-1214-183-35-1-1-1-1	91.67	107.93	16.07	23.39	98.00	88.13	27.61	38.01
YAU-1214-183-3-1-2-1-1	92.67	96.53	16.93	22.90	90.54	82.25	26.86	33.83
YAU-1215-183-3-4-1-1-1	95.00	96.33	18.60	26.86	120.01	83.02	27.53	48.73
YAU-1214-183-3-3-1-1-1	89.00	105.40	13.93	24.47	121.92	84.94	27.34	39.44
Y8/THY-DH-4-1	91.33	104.6	13.40	23.63	100.95	75.92	26.7	26.90
Y8/THY-DH-5-1	84.67	107.13	14.40	21.24	72.18	88.83	27.32	25.06
Y8/THY-DH-9-2	88.33	104.00	14.20	20.45	68.04	85.1	26.98	22.05
SM1/THY-DH-1-1	84.67	119.13	17.07	19.90	86.53	74.69	23.02	25.11
MNTK	98.67	101.00	19.13	19.80	102.09	73.18	18.55	26.23
Yet 90	67.67	96.13	17.67	21.29	64.11	90.94	24.59	25.00
Mean	91.52	105.46	15.37	22.65	105.59	76.29	25.47	30.23
CV	3.01	11.45	15.13	10.08	17.80	8.04	4.85	16.14
Pr (>F)	**	ns	*	*	**	**	**	**
LSD <sub>0.05</sub>	8.477	-	7.150	7.016	57.806	18.867	3.797	15.001

DTF = Days to 50% flowering, PH = Plant Height, ETPH = Effective tillers per hill, PLen = Panicle length, SPP = Spikelets per panicle, FGP = Filled grain percent, TSW = 1000 grain weight, YPP = Yield per plant

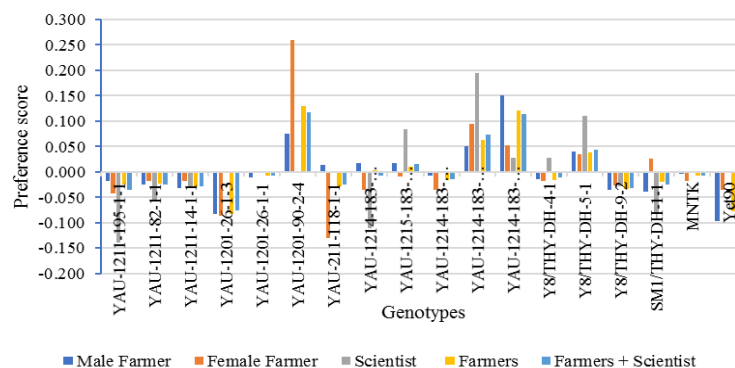
## Preference Analysis

The preference score of different improved rice lines are shown in Fig. 1. At the vegetative stage, both male and female farmers show preference for SM1/THY-DH-1-1, followed by YAU-1214-183-3-1-2-1-1 and YAU-1201-90-2-4. However, researchers selected the most preferable three improved lines in the following order: Y8/THY DH-5-1, YAU-1201-90-2-4 and SM1/THY-DH-1-1. The reason why farmers and researchers selected these lines as the most preferable are based on the uniformity in plant growth, the high to medium tiller numbers per hill and no incidence of pest infestation or diseases. The least preferred genotypes selected by the farmers were ManawThuKha, Yet90 and YAU-1211-195-1-1. In combining farmers' and researchers' preferences, SM1/THY-DH-1-1, YAU-1214-183-3-1-2-1-1 and YAU-1201-90-2-4 were the most preferable lines at the vegetative stage.



**Fig. 1 Farmers' and researchers' preferences for improved rice lines at vegetative stage in TharGaYa village, Tharsi Township**

At pre-harvest stage, the three most preferred lines selected by farmers were YAU-1201-90-2-4, YAU-1214-183-3-1-1-1-1 and YAU-1214-183-3-1-1-1-1 (Fig. 2). Farmers like these lines because they exhibit uniform growth, are lodging resistant, have long panicle length, have medium to highly effective tillers and display earliness. However, researchers selected YAU-1214-183-3-1-1-1-1, Y8/THY-DH-5-1 and YAU-1214-183-3-4-1-1-1 as the top three preferable lines. At the other extreme, YAU-1201-26-1-3, Yet90 and Y8/THY-DH-9-2 were the farmers' choice as the bottom three genotypes at pre-harvest stage. In all voting, YAU-1201-90-2-4, YAU-1214-183-3-1-1-1-1 and YAU-1214-183-3-1-1-1-1 were selected as the most preferable lines. Only one line; YAU-1201-90-2-4 was favored at the vegetative and pre-harvest stages. The other genotypes were preferred at only one of the stages considered in the selection process.



**Fig. 2 Farmers' and researchers' preferences for improved rice lines at pre-harvest stage in TharGaYa village, Tharsi Township**



According to Pearson's correlations, the results show significant and moderate positive correlation between male and female farmers' preference scores (Table 2). This means that, with  $r = 0.5861$  (at 5% level of significance), male and female farmers 'somewhat agree' in their preferences for the best performing genotypes. When farmers' preferences (both male and female) are compared with breeders' preferences, the correlation analysis shows a non-significant and slightly positive correlation ( $r = 0.311$ ). The results indicate that there is weak agreement between the farmers' preferences and the researchers' in selecting good performing genotypes.

**Table 2 Correlation analysis of preference scores between farmers (male and female) and researcher in TharGaYa village, Tharsi Township**

	Male farmer	Female farmer	Farmer	Researcher
Male Farmer	1	0.586*	0.930**	0.293
Female Farmer	0.586*	1	0.843**	0.256
Farmer	0.930**	0.843**	1	0.311
Researcher	0.293	0.256	0.311	1

\*, \*\* = significant at 5% and 1% level, respectively

The farmers' perception of good-performing varieties, as indicated by the preference score, is weakly associated with the researcher-calculated yields for the improved rice lines, with  $r = 0.345$  and non-significant (Table 3). This means that there is a somewhat weak agreement between the predicted yields and the resulting choices of the farmers based on their own set of criteria.

**Table 3 Correlation analysis of preference scores and yield in TharGaYa village, Tharsi Township**

	Yield per plant	Farmer
Yield per plant	1	0.345
Farmer	0.345	1

**Table 4 Sensory evaluation frequencies of rice varietal lines tested in TharGaYa village, Tharsi Township**

Improved Rice Lines	Bad	Fair	Good	Bad (%)	Fair (%)	Good (%)
YAU-1211-195-1-1	14	30	16	1.30	2.78	1.48
YAU-1211-82-1-1	20	27	13	1.85	2.50	1.20
YAU-1211-14-1-1	27	26	7	2.50	2.41	0.65
YAU-1201-26-1-1	12	31	17	1.11	2.87	1.57
YAU-1201-26-1-3	10	26	24	0.93	2.41	2.22
YAU-1201-90-2-4	7	19	34	0.65	1.76	3.15
YAU-1211-118-1-1	20	32	8	1.85	2.96	0.74
YAU-1214-183-3-1-1-1-1	21	24	15	1.94	2.22	1.39
YAU-1214-183-35-1-1-1-1	26	29	5	2.41	2.69	0.46
YAU-1214-183-3-1-2-1-1	21	29	10	1.94	2.69	0.93
YAU-1215-183-3-4-1-1-1	27	27	6	2.50	2.50	0.56
YAU-1214-183-3-3-1-1-1	19	26	15	1.76	2.41	1.39
Y8/THY-DH-4-1	35	19	6	3.24	1.76	0.56
Y8/THY-DH-5-1	35	21	4	3.24	1.94	0.37
Y8/THY-DH-9-2	31	25	4	2.87	2.31	0.37
SM1/THY-DH-1-1	18	28	14	1.67	2.59	1.30
MNTK	26	23	11	2.41	2.13	1.02
Yet 90	28	25	7	2.59	2.31	0.65

According to the result of sensory evaluation, the improved rice line with good eating quality was YAU-1201-90-2-4 (Table 4). Lines with fair eating quality were YAU-1211-118-1-1, YAU-1201-26-1-1 and YAU-1211-195-1-1. Lines with bad eating quality were identified as Y8/THY-DH-4-1, Y8/THY-DH-5-1 and Y8/THY-DH-9-2. Among the selected line at pre-harvest stage, only YAU-1201-90-2-4 had a good eating quality. The YAU-1214-183-3-1-2-1-1 line selected at vegetative stage and YAU-1214-183-35-1-1-1-1 line selected at pre-harvest stage both had a moderate eating quality. Therefore, the YAU-1201-90-2-4 line stands as the farmers' prefer improved rice line and needs to undergo further field testing.

## CONCLUSION

Significant differences exist among the tested genotypes and were found in yield per plant and its component characters. From the preference analysis, farmers and researchers selected SM1/THY-DH-1-1 as the best line at the vegetative stage followed by YAU-1214-183-3-1-2-1-1 and YAU-1201-90-2-4. The reason of selection of these lines are the uniformity in plant growth, the high and medium tillers number per hill and no incidence of pest and diseases. At the pre-harvest stage, farmers and researchers selected YAU-1201-90-2-4 as the best followed by YAU-1214-183-35-1-1-1 and YAU-1214-183-3-1-1-1-1. Farmers like these lines because they have growth uniformity, are lodging resistant, have long panicle length, have medium to highly effective tillers and display earliness. According to the sensory evaluation test, the best line is YAU-1201-90-2-4. This line is selected as the preferred line of farmers at the vegetative and pre-harvest stages. Therefore, YAU-1201-90-2-4 line ranks as the farmers' preferred improved rice line and needs to be tested again in field trials. There was a very strong agreement between male and female farmers' in their preference for the best performing lines at pre-harvest stage. However, only a weak correlation between researchers' and farmers' preferences has been identified. This indicates that farmers and researchers have different criteria in selecting what is a good performing line. Therefore, it is vitally important to include farmers' preferences in any variety selection process.

## ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to Japan International Cooperation Agency (JICA), Project for Capacity Development of Yezin Agricultural University for financial and technical support to conduct this study. Special thanks go to the farmers and staff from Department of Agriculture, Tharsi Township who participated in this research activities.

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### Appendix 1 Rice genotypes included in PVS

Pedigree source	Varietal code	Remarks
YAU-1211-195-1-1	G1	Pre-released, Salt Tolerance
YAU-1211-82-1-1	G2	Pre-released, Salt Tolerance
YAU-1211-14-1-1	G3	Pre-released, Salt Tolerance
YAU-1201-26-1-1	G4	Pre-released, Salt Tolerance
YAU-1201-26-1-3	G5	Pre-released, Salt Tolerance
YAU-1201-90-2-4	G6	Pre-released, Salt Tolerance
YAU-1211-118-1-1	G7	Pre-released, Salt Tolerance
YAU-1214-183-3-1-1-1-1	G8	Pre-released, High yield
YAU-1214-183-35-1-1-1-1	G9	Pre-released, High yield
YAU-1214-183- 3-1- 2-1-1	G10	Pre-released, High yield
YAU-1215-183-3-4-1-1-1	G11	Pre-released, High yield
YAU-1214-183-3-3-1-1-1	G12	Pre-released, High yield
Y8/THY-DH-4-1	G13	Pre-released, High yield
Y8/THY-DH-5-1	G14	Pre-released, High yield
Y8/THY-DH-9-2	G15	Pre-released, High yield
SM1/THY-DH-1-1	G16	Pre-released, High yield
MNTK	G17	Local Check, High yield
Yet 90	G18	Local Check, Short growth duration



## Rainwater Harvesting as a Mean for Water Conservation in Ovche Pole Region, Macedonia

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Received 30 December 2020    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Ovche Pole is an area in Macedonia located in the central part of the country. The region is the second largest grain producing area and agriculture is the main economic activity for most of the people there. From an agro-ecological perspective, larger part of the region is part of the sub-humid agricultural zone; however, there is an area that is identified as semiarid agricultural zone, too. In both agro-ecological zones, main problem and constraint for agricultural production are the dryness and low precipitation in the growing period. This problem is especially emphasized on the agricultural land without irrigation system. Therefore, this study analyzes rainwater harvesting approach as a mean for water conservation and improvement of agriculture productivity in the research area. The first objective of this study is to determine the runoff potential in the research area and the second is to identify suitable areas and measures for water harvesting. Remote sensing and geographic information system techniques were used to obtain, prepare and analyze input data. Because of simplicity and lack of hydrological data in the research area, SCS-Curve Number method was used for rainfall-runoff modelling. Hydrologic Soil Group (HSG) map was build using data obtained from the Macedonian Soil Information System (MASIS). Land use/land cover was prepared by combining supervised classification of Sentinel-2 satellite image and visual interpretation and editing to improve the classification. Slope map was generated from a 20-meter resolution DEM data obtained from the Agency for Real Estate Cadaster. Data of the annual average precipitation for the period between 1981 and 2010 was obtained from the National Hydrometeorological Service. Set of rainwater harvesting measures were selected as appropriate for the context of the research area. The site suitability for each rainwater harvesting intervention was determined by considering different varying parameters like runoff potential, slope, soil texture, land use, stream order of proximity to users. The results indicate that rainwater harvesting can be considered as a strategy for water conservation in the research area.

**Keywords** water conservation, rainwater harvesting, surface runoff, SCS-SN method, suitable site selection

## INTRODUCTION

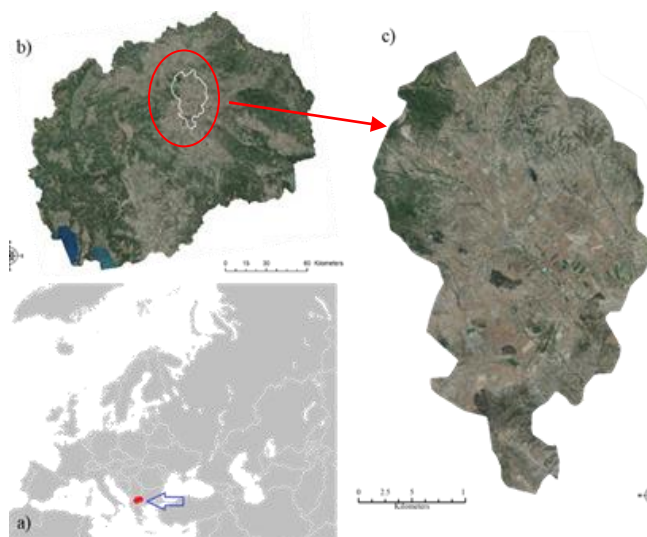
Having in mind the United Nation Convention for Combating Desertification (UNCCD) definition, approximately 40% of the global land can be considered as a type of dryland (WRI, 2012). Although there are many challenges, in these areas water is the key limiting constraint for agricultural and biological productivity. Irrigated areas to some extent can avoid this constraint, however rainfed agricultural land is dependent on water that comes as precipitation. To unlock the potential of small-

scale rainfed agriculture, investments in better water management need to be emphasized. In dry area water harvesting coupled with in situ water management as well as improved soil, nutrient and crop management have great potential. In humid areas, in situ water management technologies such as conservation agriculture based on no-till, mulching and crop rotation are generally more suitable and appropriate. Mekdaschi and Liniger (2013) define water harvesting (WH) as “The collection and management of flood water or rainwater runoff to increase water availability for domestic and agricultural use as well as ecosystem sustenance”. Although there are multiple benefits, there are some considerations that must be taken before water harvesting systems can be successfully implemented. Therefore, objectives of this study is to determine the runoff potential in the research area and to identify suitable areas and measures for water harvesting. In this line different methodologies and criteria for WH site and method selection were developed, however two main groups are defined-biophysical and socio-economic (Ammar et al., 2016). In his study Ammar (2016) identified three commonly most followed set of criteria for selection of suitable WH. Those are the sets proposed by: Integrated Mission for Sustainable Development (IMSD) in 1995, Dr. Theib Y. Owesi in 1998 and Food and Agriculture Organization of the United Nations (FAO) proposed in 2003. Since field surveying can be resource consuming, nowadays the assessment and selection of suitable WH sites and measures is assisted by remote sensing and geographical information systems, because when coupled they are cost-efficient and provide reasonably good possibility for analyzation of spatial data (Wani et al., 2009)

## METHODOLOGY

### Description of the Study Area

Ovche Pole is a plain located in central part of Macedonia that takes an area of 649 km<sup>2</sup>. It lies between latitude 41 59 30 N and 41 38 43 N and longitude 21 48 13 E and 22 01 28 E (Fig. 1). Agriculture is dominant economic activity in the region. Agricultural land takes 40,183 ha (62% of the study area), however much of the arable land is not irrigated. Forests cover 14,619 ha (22.5%) of which 2/3 are degraded, whereas 9,134 ha (14%) are pastures (CORINE LC/LU, 2012). The climate is modified warm continental with Mediterranean influence (Zikov, 1995; Filipovski et al., 1996). Considering the data between 1981 and 2010 the annual average precipitation in the region is 455 mm. From an agro-ecological perspective, larger area of the region is part of the sub-humid agricultural zone; however, there is an area that is identified as semiarid agricultural zone, too. In both agro-ecological zones, main problem and constraint for agriculture production are the dryness and low precipitation in the growing period (Aksoy et al, 2020).



**Fig. 1 Geographical position of the research area**

### Materials

Different data such as land use/cover data, Sentinel-2 satellite images, elevation model (DEM), climate data and soil data were used in this study analysis. Land cover/land use (LCLU) analysis was done by using CORINE Land Cover (CLC2018) data set from 2018, program coordinated by European Environmental Agency (EEA) and Sentinel-2 satellite image. Soil data were obtained from The Macedonian Soil Information System (MASIS). Watershed delineation, stream network, slope, topographic maps were generated using 20-meter resolution DEM obtained from the Agency for Real Estate Cadaster. Average year precipitation of 455 mm. was derived from climatic data for the period between 1981 and 2010 provided by the National Hydrometeorological Service (NHS).

## **Methods**

Assessment of the water harvesting potential and identification of suitable water harvesting sites was done by evaluation of parameters such as: surface runoff, land use/cover, slope, soil infiltration characteristics, stream order and proximity to users. The complete quantitative and qualitative data analysis in this research was done by remote sensing and Geographical Information System (GIS) techniques. To compute hydrological elements more accurately generation of Land Cover/Land Use map is required (Rana and Suryanarayana. 2020). In this study CORINE Land Cover 2018 (CLC2018) dataset for Macedonia together with Sentinel-2 satellite image were combined to create the LCLU map for the research area. The Sentinel-2 image captured on 20 August 2019 was used as a background information for additional interpretation and editing of existing CLC2018 vector layer. We only used the bands with 10-meter resolution (blue B2:490 nm, green B3:560 nm, red B4:665 nm, NIR B8:842 nm). As a result of this hybrid process, a new more precise and detailed land cover map was created. For the Hydrological Soil Group (HSG) analysis and map generation three raster files on sand, silt and clay content in soil were used. Each pixel is specified with a 100 meters resolution and a value that represents the content of specific soil fraction expressed in unit percent. Using raster calculator and reclassification function in ArcGIS Pro, the three layers were used to create new raster layer. In this raster each pixel was assigned a value that represented some soil texture class, following the USDA textural soil classification. Considering the infiltration rate of various soil texture each pixel was reclassified into A, B, C or D hydrologic soil group. Accordingly, the hydrologic soil group (HSG) map was prepared for the entire research area. The DEM data was used to generate the slope map and stream network map for the research area. Surface runoff is the primary component of every water harvesting system. In this study Conservation Service Curve Number (SCS-CN) methodology was used to calculate the runoff depth and generate run-off potential map for the research area. The SCS run-off equation is widely used in estimating direct run-off because of its simplicity, flexibility and versatility (Kumar and Jharya. 2016) and often used by researcher for watersheds that are ungauged, as it is the study area in this research. Although the method is designed for a single storm, it can be scaled to calculate the annual values for run-off of an area. Basically, the SCS-CN method depends on the relationship between the rainfall parameter (P) and the run-off depth (Q), simplified into the CN concept. The CN is a dimensionless run-off index determined based on Hydrological Soil Group (HSG), land use/land cover and Antecedent Moisture Conditions (AMC). The CN method can also reflect the effect of changes in land use on run-off. The CN values range between 1 and 100. Higher values of CN indicate higher run-off. In this study the runoff derived by SCS-CN method was expressed as runoff coefficient which represent the ratio between the runoff and rainfall. Based on the runoff coefficient a runoff-potential map was created in which the run-off coefficient is reclassified in four classes: low run-off potential, moderate run-off potential, high run-off potential and very high run-off potential.

## **Rainwater Harvesting Measures Selection and Site Suitability Analysis**

The rainfall, geology, physiographic, land use, climatic conditions and social set-up are the basic factors to be understood by the rainwater harvesters to choose the proper technique of rainwater management in a particular set-up identified by them. As first step in process, a set of RWH measures were identified and selected; in the second step, for each measure a set of site selection suitability criteria were compiled; and as last step, RWH site suitability analysis was performed. Selection of suitable RWH measures was done by reviewing studies, manuals and other on-topic literature. We identified to groups of measures. First group are measures that can be implemented in valley formations such as impermanent water streams and gullies, such as: ponds, gabion checks, boulder checks and brushwood checks. Second group of measures are those that can be implemented on the ridge formations. Here we identified revegetation, contour ditches and contour bunds as possible appropriate measure. In the second step, we compiled criteria for suitable site selection for each of the identified measure in the first step. The criteria for water harvesting site selection related to each water harvesting group of measure are presented in Table 1 and Table 2. According to the review study done by Ammar et al. (2016) on identification of suitable sites for water storage in arid and semi-arid regions, it was found out that the most common biophysical layers or criteria applied were slope followed by land use/land cover and soil type. In this study we selected runoff, land use/land cover, HSG, slope, stream network and distance to users play a critical role in rainwater harvesting site selection. These thematic layers were used as input data for a series of operations done in ArcGIS Pro that enabled identification of appropriate sites for each identified rainwater harvesting measure.

**Table 1 Criteria for selection of on-ridge rainwater harvesting measures**

	Water harvesting intervention	Runoff coefficient	Slope (%)	Land use	Hydrology Soil Group (HSG)
On-ridge interventions	Revegetation	>0.5	>25	Land principally occupied by agriculture with significant areas of natural vegetation; Pastures, Grasslands; Sparsely vegetated areas; Transitional woodland-shrubs	A, B, C, D
	Contour trenching	>0.5	10 - 25		A, B, C, D
	Contour bunds	>0.5	0-10		A, B, C, D

**Table 2 Criteria for selection of on-stream rainwater harvesting measures**

	Water harvesting intervention	Runoff coefficient	Slope (%)	Stream Order	Hydrology Soil Group (HSG)	Distance to users
On-stream interventions	Irrigation ponds	>0.5	0-5	1-2	C, D	< 250 meters from agricultural land
	Livestock ponds	>0.5	0-5	1-2	C, D	< 1000 meters from livestock barns
	Gabion checks	>0.5	0-5	1-2 (gullies)*	A, B, C, D	n/a
	Boulder Checks	>0.5	5 - 20	1-2	A, B, C, D	n/a
	Brushwood checks	>0.5	> 20	1-2	A, B, C, D	n/a

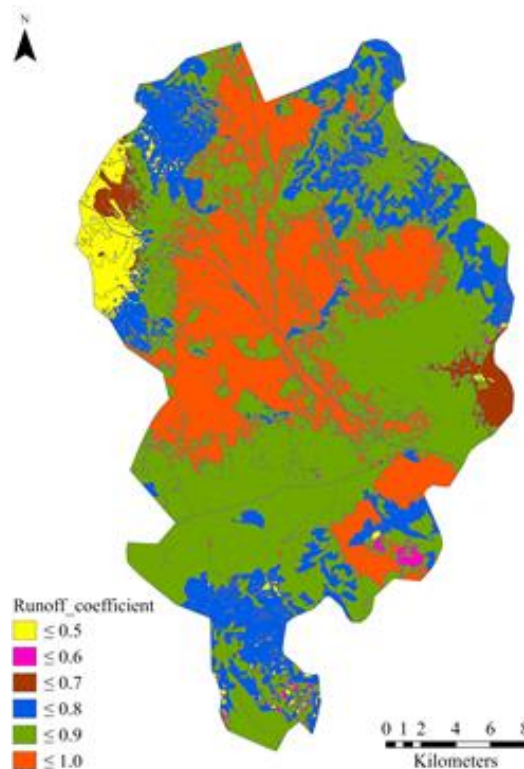
## RESULTS AND DISCUSSION

### Runoff Potential

The run-off potential of the area affects the recharge and movement of surface water and is one of the important parameters for rainwater harvesting. (Kumar and Jhariya, 2016). The run-off potential map (Fig. 2) was built based the SCS-CN method by integrating average annual rainfall for the period between 1981 and 2010, land use/land cover information, and distribution hydraulic soil groups in the research area. The runoff derived by SCS-CN method is a function of runoff potential which can be expressed in as runoff coefficient - ratio between the runoff and rainfall. To perform RWH site suitability analysis the run-off potential was reclassified as very high run-off potential, high run-off potential, moderate run-off potential and low run-off potential. Category description and distribution in the research area is presented in Table 3. Results show that 26.8% of the total research area has very high runoff potential and 66.8% has high runoff potential.

**Table 3 Runoff coefficient category description and distribution**

	Runoff coefficient	Description	Surface area (km <sup>2</sup> )	Percent of total area (%)
	$\leq 0.5$ (0.00 – 0.5)	Low runoff potential	23.25	3.6
	$\leq 0.6$ (0.51 – 0.6)	Moderate runoff potential	3.3	0.5
	$\leq 0.7$ (0.61 – 0.7)		14.6	2.3
	$\leq 0.8$ (0.71 – 0.8)	High runoff potential	115.3	17.9
	$\leq 0.9$ (0.81 – 0.9)		314.1	48.9
	$\leq 1.0$ (0.91 – 1.0)	Very high runoff potential	172.2	26.8






**Fig. 2 Runoff coefficient map of the research area**

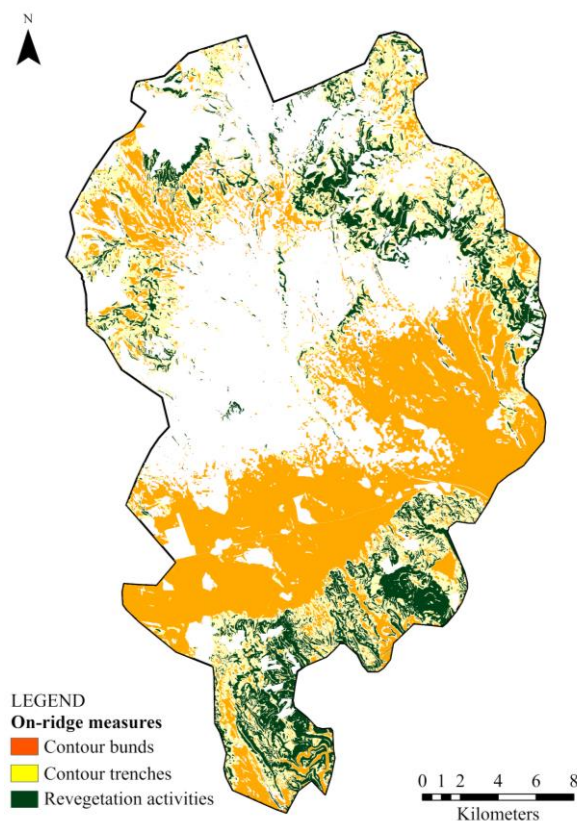


### Rainwater Harvesting Site Suitability Analysis

Rainwater harvesting site suitability analyses, based on the criteria given in Table 1 and Table 2 showed that identified measures can be applied on significant part of the research area. On-ridge measures can be implemented on 59% of the total area. Detailed information for each on-ridge measure is presented in Table 4, the distribution map is shown in Figure 4. Regarding the on-stream measures detailed analysis is presented in Table 5. and the distribution map in Figure 4. As an implementation unit for ponds and gabion checks is the number of pond-structures that can be build, whereas for brushwood and boulder check implementation unit is the length of stream sections appropriate for implementation.

**Table 4 On-ridge measure distribution analysis**

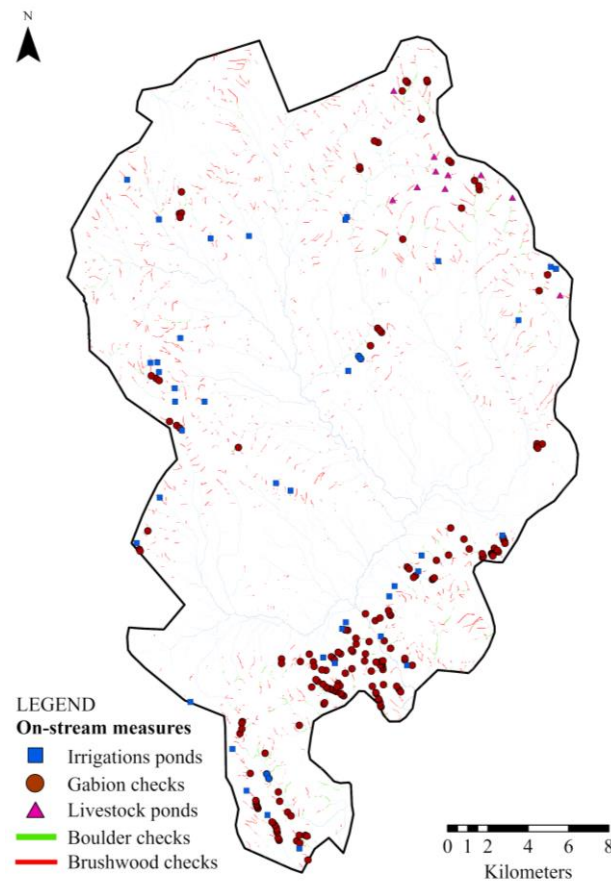
On-ridge measures			
Water harvesting intervention	Implementation area (km <sup>2</sup> )	Percent of total area (%)	
 Contour bunds	193	30	
 Contour trenches	109	17	
 Re-vegetation	77	12	



**Fig. 3 On-ridge measures distribution map**

**Table 5 On-stream measure distribution analysis**

On-stream measures	
Water harvesting intervention	Implementation units
Irrigation ponds	43 ponds
Livestock ponds	11 ponds
Gabion checks	183 checks
Brushwood checks	91 km (stream length)
Boulder checks	297 km (stream length)

**Fig. 4 On-stream measures distribution map**

## CONCLUSION

Ovche Pole region is part of the sub-humid and semiarid agricultural zone in Macedonia. Main constraint for agriculture production is dryness and low precipitation amounts during the growing season. This study explores the potential for implementation of rainwater harvesting measures as strategy for water conservation. The results show that 93.6% of the total area has potential to generate high and very high runoff. Rainwater harvesting site suitability analysis done in the study implies that

59% of the research area satisfy the criteria for implementation of the selected on-ridge RWH measures. Analysis shows there are significant number of potential sites suitable for implementation of on-stream RWH measures that can also significantly contribute to conservation of water resources in the research area.

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## Marketing Strategies and Grape Farmers' Welfare Improvement: Evidence from Afghanistan

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Received 7 March 2021    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** The role of marketing channels in agribusiness has been extensively studied in the last decade. While most studies have focused on the general impact of marketing channels on sales, the impacts of different choices of marketing channels on net returns has not been widely studied. Focusing on Afghan grape farmers, this paper studied the determinants of marketing channels, as well as the impact of marketing channel choices on net returns. The marketing channels considered in this paper were channels through farmer's organizations, local traders and farmers who do on spot selling. Using survey data from 150 grape farmers in Kabul, Parwan, and Kapisa provinces in Afghanistan, the results showed that grape farming experience, selling price, province, distance to markets and internet use were some of the factors that affected the choice of marketing channels. In addition, marketing channels through local traders had a positive impact on returns as compared to channels through farmer's organizations and on spot selling. The selectivity correction terms for all marketing channels were significant indicating the existence of selectivity bias from unobserved factors.

**Keywords** marketing channels, Afghan grapes, MNL model, BFG method, selectivity correction

## INTRODUCTION

Marketing channels act as a bridge between producers and consumers. According to Bowersox et al. (1986), marketing channels are mediators that are responsible for taking products through the marketing system. Marketing channels are purposed to reduce transaction costs and enhance competitiveness on the market. Therefore, marketing channels will ensure that products are at the right place, at the right time, hence poor choices in marketing channels would in turn result in unwanted costs.

It has been shown in literature that economic factors, social factors, and demographic factors are some of the determinants of marketing channels (Jari, 2009; Safi et al., 2019). Specifically, factors like access to information, the distance the nearest trading center, age, the level of education, income, experience and land holding have shown statistical significance in determining the choice of marketing

contracts (Jari, 2009; Safi et al., 2019). Furthermore, Chiang (2016) and Uematsu and Mishran (2011) reported that the choice of different marketing channels affects the net returns from farm produce.

Afghanistan, being one of the low income countries whose economy relies mainly on agriculture, still faces financial and agricultural related challenges in the grape industry (USAID, 2016). In addition, there is no proper marketing system for fruits, especially grapes in Afghanistan with less than 3 percent of grape farmers having access to formal marketing systems Afif (2015). This has led to 30-40% of the grapes getting destroyed due to poor marketing techniques with a lot of farmers opting for poppy farming. This research therefore aims at finding the determinants of marketing channels and the impact of marketing channels on net returns. Focusing on three major grape producing provinces, this research aims at drawing a light on the marketing channels farmers can opt for to maximize their returns.

## **DATA AND METHODS**

The study aims at finding factors that influence the choice of marketing channels by grape farmers in Afghanistan. Three marketing channels considered were marketing channels through contracts with farmer's organizations, contracts with local traders and on spot selling (farmers with no marketing contracts). The study focused on farmers cultivating grapes for commercial purposes. From the 7 main commercial grape growing provinces, a random sample of three provinces was drawn, leading to the study provinces of this research which are Kabul, Kapisa and Parwan. Using the adjusted Cochran's formula, a sample size of 164 was drawn and randomly sampled from a list of commercial grape farmers from the agricultural departments of Kabul, Kapisa and Parwan provinces. The study used a questionnaire which consisted of closed-ended questions and it captured information on farmer's demographic information, agricultural status and information on the marketing strategies, marketing channels and net returns. All data analysis was done in excel, SPSS and STATA.

### **Model Description**

Data was analyzed using a two stage model proposed by Bourguignon, Fournier, and Gurgand (2007) (hereinafter BFG), which controls for selectivity biasness. The first stage of the BFG method employs a multinomial logistic regression (MNL), which analyses the determinants of marketing channels. The second stage BFG uses the Linear Logit model to assess the impact of marketing channels on net returns while controlling for selectivity bias.

## **RESULTS AND DISCUSSION**

Data from 164 households was collected in which 14 households were dropped because of incompleteness, leaving the total number of households at 150. From the 150 farmer, 40 (26.67%) had contracts with farmer's organizations, 68 (45.33%) had contracts with local traders and 42(28.00%) did on spot selling. The mean age of farmers was 30.9 years and the average number of grape farming experience in years was 10.4.

Table 1 shows that internet use and farm distance have a significant positive impact on the choice of local traders. This means that farmers with access to intern and long farm distances respectively, are more likely to choose local traders. However, both internet use and farm distance do not have any impact on the choice of farmer's organizations. In addition, experience in grape farming contribute to choosing local traders, unlike farmer's organizations, which are negatively affected by grape farming experience. Furthermore, the negative marginal values of selling price and province indicate that an increase in selling price reduces the probability of farmers choosing local traders and farmers from Kabul are less likely to choose local traders unlike farmers from Parwan and Kapisa.

**Table 1 Determinants of marketing channels (First stage BFG)**

Variable	On spot selling		Channel with traders		Channel with farmers organization	
	Marginal effects	Z-value	Marginal effects	Z-value	Marginal effects	Z-value
Experience	0.008195	0.254	0.014967	1.75*	-0.02316	-3.69***
Sales volume	-7.00E-06	-0.71	9.67E-06	1.02	-2.67E-06	-0.57
Selling price	0.905188	0.147	-0.61295	-3.04***	0.707764	2.71***
Province	0.043652	0.46	-0.2764	-2.38**	0.232751	2.85***
Loans	0.0385	0.78	0.089493	1.5	-0.12799	-3.04***
Distance	-0.00532	-3.8***	0.004321	3.02***	0.001002	0.146
Internet	-0.26078	-2.87***	0.318437	3.25***	-0.05765	0.336
Training	-0.1369	-1.51	-0.00068	-0.01	0.137588	2.16**
Area	0.008411	0.25	-0.05485	-1.39	0.046441	1.87*
vehicle	-0.03844	-0.72	0.03992	0.69	-0.00148	0.963
Age	0.00373	0.97	-0.00321	-0.69	-0.00052	0.844
Education	-0.01832	-0.47	0.018808	0.44	-0.00048	-0.02

Source: own survey data. \*, \*\*, \*\*\* represent significance at 0.1, 0.05 and 0.01 significant levels, respectively. The base group is on spot selling.

Every increase in selling price and planting area increases the probability of farmers choosing farmer's organizations. On top of that, farmers from Kabul have a higher probability of choosing farmer's organizations unlike farmers from Parwan and Kapisa. Farmers who undergo training also have a higher probability of choosing farmer's organizations as compared to farmers without training. On the other hand, the significant negative value of access to loans indicates that farmers who take loans from farmer's organization are less likely to choose farmer's organizations as their marketing channels, as compared to the farmers with loans from friends, family and banks.

The results presented in Table 2 show that the selectivity terms are significant in the choice of channels through contracts farmer's organization, channels through contracts with traders and no spot selling. The selectivity coefficient of channels through contracts with farmer's organizations as compared to channels through contracts with traders is positive, meaning that the expected revenue for a farmer with a contract with farmer's organization will increase if they switched from farmer's organizations to local traders.

Likewise, the significant negative coefficient for on spot selling relative to contracts with farmer's organization indicate that the expected revenue for a farmer who does on spot selling will decrease if a farmer switched from on spot selling to having a contract with farmer's organization.

The results for the OLS indicate that farming vehicle has a positive significant impact on the net returns of farmers who choose farmers organizations. In addition, sales volume, internet use, planting area, loans and education have a positive impact on the net returns of farmers who do on spot selling. However, age has a negative impact on the net returns of farmers who do on spot selling. Furthermore, sales volume, training participation and education have a positive impact on the net returns of farmers with contracts with traders while planting area has a negative impact.

**Table 2 Impacts of marketing channels on net returns (second stage BFG)**

Variable	Channel with farmer's organization		On spot selling		channel with traders	
	coefficient	z-value	coefficient	z-value	Coefficient	z-value
Experience	-397.92	-0.11	-0.14	-0.25	-2731.29	-2.09
Volume	1.98	1.71	0.99	1328***	8.28	10.56***
Price	-138042.50	-0.27	-219.38	-0.94	138721.10	0.34
Province	-38662.15	-1.09	-1.23	-0.14	19460.29	0.83
Loans	2421.51	0.13	13.07	3.15***	-5447.20	-0.59
Distance	265.52	1.45	0.62	1.66	-263.05	-0.56
Internet	22279.50	0.97	42.17	1.90*	5689.33	0.17
Training	21013.61	1.05	7.86	1.00	35033.67	2.0*
Area	-559.30	-0.06	4.68	2.19**	-17112.90	-3.87***
Vehicle	3929.92	0.69*	4.99	0.89	-6764.24	-0.81
Education	7398.88	1.87	2.85	0.86**	16860.50	2.86***
Age	-659.71	-1.25	-0.50	-2.19*	971.28	1.28
_m1	23899.37	0.63	-104.36	-1.78*	166337.40	1.45
_m2	28801.98	0.39	-91.87	-2.5	140147.60	0.96
_m3	153988.40	2.12**	-19.51	-0.33	95157.10	1.85*
_cons	125075.60	0.61	43.42	0.48	-37630.50	-0.46

Source: own survey data. \*, \*\*, \*\*\* represent significance at 0.1, 0.05 and 0.01 significant levels, respectively.

## CONCLUSION

The study used the BFG methods to analyze the factors that influence the choice of marketing channels and the impact of marketing channels on net returns of grape farmers in Afghanistan. Focusing on three Afghan provinces, the results of the study showed that selling price, province, participation in training and planting area are some of the factors that influence the choice of marketing channels. In addition, the results showed that marketing channels through local traders would improve the overall net returns of farmers. However, future studies would incorporate management structures of farms, and focus on all commercial grape growing provinces in Afghanistan to give a clearer picture.

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# Effect of Temperature on the Cohort Life Table of Brown Planthopper (*Nilaparvata lugens* Stål) (Homoptera: Delphacidae)

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**Abstract** We investigated the effect of constant temperatures (24, 28, 32, and 36 °C) and room temperature on brown planthopper (BPH), *Nilaparvata lugens*, population dynamics; these data are useful for forecasting BPH outbreaks. Eggs laid by gravid females were incubated in test tubes at the treatment temperatures and observed for egg hatching. After hatching, first instar nymphs were collected using a camel-hair brush and incubated individually in new test tubes, each with a rice plant. The number of hatched and unhatched eggs were recorded. Nymphs were monitored daily for life table parameters until adult emergence; the longevity of adults were also recorded. From life table analysis, the greatest mortality occurred in the first nymphal instar, which resulted in type III survivorship curves. Moreover, at 36 °C, 100% mortality occurred at the egg stage. Brachypterous and Macropterous forms achieved high net reproductive rates at lower temperature regimes. The growth parameters of BPH decreased at 36 °C; temperatures above 32 °C were detrimental to the development of BPH. Results indicate that egg and nymphal stages were significantly affected by temperature. Egg hatch also decreased drastically with increased temperatures, especially at 36 °C.

**Keywords** constant temperature, *Nilaparvata lugens*, life table parameters, rice

## INTRODUCTION

Rice is an essential food crop and more than 90% of global production occurs in tropical and semi-tropical Asia (Food and Agriculture Organization, 2012). Each year, an additional 50 million rice consumers will be added to the world population, meaning that rice production must increase markedly (Heong and Hardy, 2009). In Myanmar, rice is the single most important crop, both as a staple and as a foreign exchange earner (Ministry of Agriculture and Irrigation, 2003).

Rice is susceptible to pests and diseases. From sowing to harvest, all parts of the plant are vulnerable to insect-feeding (Grist and Lever, 1969). Eight hundred species of insects attack rice crops, either in the field or during storage. Amongst these, one of the most economically important is the brown planthopper (BPH), *Nilaparvata lugens* (Stal.) (Homoptera: Delphacidae). BPH has become more problematic, posing a threat to rice production throughout South and South East Asia (Rao, 1950). In Myanmar, the first outbreak of BPH happened in 1970 at the Kyaukse Central Farm (Mandalay Division) and in Upper Myanmar (Myint, 1975). The BPH has since become a major pest of rice in Myanmar (Win et al., 2011).

BPH is present on rice during both dry and wet seasons. Biotic and abiotic factors affect survival, growth, development and multiplication of BPH; amongst these, temperature plays a significant role (abiotic factor). As a result of climate change, global mean temperatures are predicted to increase by 1.5 - 6.0 °C by the end of the 21st century (IPCC, 2001). This significant temperature increase will affect insect physiology, behavior and development as well as species distribution and abundance; there is already evidence for this in the increase in number of generations a year, increasing survival rates in winter, and the earlier appearance of some insects (Huang, et al., 2010).

## **OBJECTIVE**

This study aimed to investigate the effect of temperature on BPH life table parameters to provide the data needed for forecasting BPH outbreaks.

## **METHODOLOGY**

### **Study site:**

The experiments have been done at the JICA – ELB 1 Laboratory, Department of Entomology and Zoology, Yezin Agricultural University, Myanmar between August 2018 and January 2020.

### **Brown planthopper (BPH) rearing:**

The initial population of BPH was collected from rice fields at Yezin Agricultural University. To establish a colony adult was confined on 30-day old potted rice plants (susceptible variety Manaw thukha) in aluminum rearing cages in a screen house.

### **Life table study:**

There were four constant temperature treatments (24, 28, 32 and 36 °C), and a control maintained at room temperature (26.8-30.7 °C) which was recorded daily. Individual rice plants (tillering stage) were placed in test tubes with four gravid female BPH for the collection of eggs and sealed with a cotton wool plug. Replicate test tubes (n=5) were placed at each of the treatment, control temperatures and oviposition allowed to proceed for 24 h. After this time adult females were removed and the groups of eggs incubated at their allocated temperatures. Eggs were observed daily and, when they hatched, a camel-hair brush was used to transfer each first instar nymph individually into new test tubes, each with a new rice plant. Eggs continued to be observed and hatching first instars transferred into new test tubes until hatching stopped; the number of hatched and unhatched eggs were recorded at this point. The later instars were collected and transferred as the same way. Nymphs were monitored daily and the time to moult to each life stage recorded; they were provided with fresh plant materials as required. We recorded mortality ( $d_x$ ) daily until their death. As adults emerged, one male and one female were paired and moved to a new rice plant. The total numbers of laid eggs were recorded daily until females died.

### **Age specific life table construction:**

The life table was built by partitioning the life-cycle into distinct development stages (i.e. eggs, nymphs and adults) and recording the developmental time and survival or mortality of each stage. We calculated the pivotal age for the age class in units of time (days) ( $X$ ), number of alive at each life stage ( $n_x$ ), proportion of individuals surviving to the start of age interval  $x$  ( $l_x$ ), mean number of individuals alive during the age interval  $x$  to  $x+1$  ( $L_x$ ), number dying during the age interval  $x$  to  $x+1$  ( $d_x$ ), percent apparent mortality ( $100 q_x$ ), total number of age  $X$  units beyond the age ( $T_x$ ), mean expectation of life for individuals alive at the start of age  $x$  ( $e_x$ ), age-specific fertility, the number of living females born per female in each interval class ( $m_x$ ), net reproductive rate ( $R_o$ ), the innate capacity for an increase in numbers ( $r_m$ ), and the mean length of a generation ( $G$ ) from these data.

**Data analysis:**

ANOVA and comparison of means was done using LSD in the software program (SAS 9.1).

**RESULTS AND DISCUSSION****Life Table**

Life table analysis showed that a mean of 26.4% of 139.4 BPH eggs survived to adulthood when incubated at room temperature (control); this was 45.2% (of 143.0 eggs) at 24 °C, and 49.2% (of 113.8 eggs) at 28 °C. Win et al. (2011) found that 37.26% of 365 BPH eggs successfully emerged as adults in a field experiment where the temperature ranged between 23 °C and 33 °C.

**Table 1 Mean number and percentage of BPH alive at each life stage when reared at different constant temperatures**

Stages	Mean $\pm$ SE									
	Control		24 °C		28 °C		32 °C		36 °C	
	$n_x$	%	$n_x$	%	$n_x$	%	$n_x$	%	$n_x$	%
Egg	139.4 $\pm$ 3.9	100.0	143.0 $\pm$ 3.2	100.0	113.8 $\pm$ 1.1	100.0	132.4 $\pm$ 1.6	100.0	135.8 $\pm$ 2.7	100.0
First instar nymph	102.4 $\pm$ 6.2	73.5	137.0 $\pm$ 3.2	95.8	96.8 $\pm$ 1.5	85.1	107.4 $\pm$ 2.5	81.1	0.0 $\pm$ 0.0	0.0
Second instar nymph	77.6 $\pm$ 4.8	55.7	112.2 $\pm$ 3.1	78.5	77.4 $\pm$ 1.1	68.0	76.6 $\pm$ 1.8	57.9	0.0 $\pm$ 0.0	0.0
Third instar nymph	67.4 $\pm$ 3.8	48.4	99.0 $\pm$ 2.6	69.2	72.8 $\pm$ 0.9	64.0	56.4 $\pm$ 1.8	42.6	0.0 $\pm$ 0.0	0.0
Fourth instar nymph	57.2 $\pm$ 3.1	41.0	90.4 $\pm$ 2.6	63.2	67.2 $\pm$ 0.9	59.1	44.8 $\pm$ 1.3	33.8	0.0 $\pm$ 0.0	0.0
Fifth instar nymph	48.0 $\pm$ 2.7	34.4	80.0 $\pm$ 2.4	55.9	64.0 $\pm$ 0.8	56.2	22.6 $\pm$ 1.1	17.1	0.0 $\pm$ 0.0	0.0
Adult	36.8 $\pm$ 2.4	26.4	64.6 $\pm$ 2.2	45.2	56.0 $\pm$ 0.8	49.2	0.0 $\pm$ 0.0	0.0	0.0 $\pm$ 0.0	0.0

In our study high mortality occurred during the early immature stages and at 32 °C and 36 °C there was no survival to adulthood (Table 1). Begon and Mortimer (1981) reported that this low survivorship at higher temperatures is common in most insect species.

The highest mortality of any life stage (135.8 $\pm$ 2.7) was observed at 36 °C in the egg stage. Greatest mortality in first, second, third, fourth and fifth instar nymph were found at 32°C. At 36 °C, none of the nymphal instars survived (Table 2). Krishnaiah et al. (2005) reported that temperatures ranging between 25 and 30 °C were most favorable for BPH multiplication, and that the insect could not tolerate constant temperatures of 35 °C and higher. Rout and Jena (2012) found that BPH thrived and multiplied well at a temperature of 30  $\pm$  3 °C, and that 33°C and 35 °C were unfavorable for insect survival.

**Table 2 Mean number of BPH dying between age interval x and age interval x+1 (d<sub>x</sub>) when reared at different constant temperatures**

Stages	Mean $\pm$ SE				
	Control	24°C	28°C	32°C	36°C
	d <sub>x</sub>	d <sub>x</sub>	d <sub>x</sub>	d <sub>x</sub>	d <sub>x</sub>
Egg	37.0 $\pm$ 2.4	6.0 $\pm$ 0.7	17.0 $\pm$ 0.9	25.0 $\pm$ 1.7	135.8 $\pm$ 2.7
First instar nymph	24.8 $\pm$ 1.5	24.8 $\pm$ 0.8	19.4 $\pm$ 0.7	30.8 $\pm$ 0.8	0.0 $\pm$ 0.0
Second instar nymph	10.2 $\pm$ 1.1	13.2 $\pm$ 0.5	4.6 $\pm$ 0.3	20.2 $\pm$ 0.6	0.0 $\pm$ 0.0
Third instar nymph	10.2 $\pm$ 0.8	8.6 $\pm$ 0.3	5.6 $\pm$ 0.2	11.6 $\pm$ 0.6	0.0 $\pm$ 0.0
Fourth instar nymph	9.2 $\pm$ 0.5	10.4 $\pm$ 0.5	3.2 $\pm$ 0.2	22.2 $\pm$ 0.5	0.0 $\pm$ 0.0
Fifth instar nymph	11.2 $\pm$ 0.4	15.4 $\pm$ 0.2	8.0 $\pm$ 0.1	22.6 $\pm$ 1.1	0.0 $\pm$ 0.0

The highest net reproductive rate (175.6) of the brachypterous form was observed at room temperature (control), followed by (168.2) at 24 °C, (166.8) at 28 °C and (74.6) at 32 °C, respectively. The lowest net reproductive rate (32.6) was found at 36 °C. The intrinsic rate of natural increase ( $r_m$ ) was 0.6, 0.5, 0.7, 0.9 and 0.8 for control, 24 °C, 28 °C, 32 °C and 36°C, respectively (Table 3). The net reproductive rate of BPH was higher at lower temperature regimes. Manikandan et al. (2015) found that the net reproductive rate of BPH was greater at 30 °C (39.95) than at 36 °C (8.84).

**Table 3 Life table parameters of brachypterous forms at different temperature**

Parameter	Formula	Brachypterous forms				
		Control	24 °C	28 °C	32 °C	36 °C
Mean length of a generation (G)	$G = \sum l_x m_x x / \sum l_x m_x$	8.5	9.8	7.4	4.7	4.4
Instantaneous rate	$r_m = \log_e(R_o) / G$	0.6	0.5	0.7	0.9	0.8
Net reproductive rate	$R_o = \sum l_x m_x$	175.6	168.2	166.8	74.6	32.6
Gross reproductive rate	$\sum m_x$	175.6	168.2	166.8	74.6	32.6

The highest net reproductive rate (204.0) of macropterous forms was found at room temperature (control), followed by 142.6 at 28 °C, 102.4 at 24 °C and 93.6 at 32 °C. The lowest net reproductive rate (29.2) was found at 36°C. The intrinsic rate of natural increase ( $r_m$ ) was 0.6 in both the control and at 24 °C, 0.6 at 28 °C, 0.8 at 32 °C and 0.9 at 36 °C (Table 4). Similar results were reported for BPH by Manikandan et al. (2015) who reported that the net reproductive rate was greater at lower temperature regimes (30.0 °C) than at higher temperature regimes (36.0 °C).

Developmental stages of BPH varied in length and time at different temperatures. The mean number of first instar nymph was highest at 24 °C with the value of (137.0  $\pm$  3.2) and lowest at 28 °C (96.8  $\pm$  1.5). The second instar nymph was highest at 24 °C (112.2  $\pm$  3.1) and lowest at 32 °C (76.6  $\pm$  1.8). The third instar nymph was highest at 24 °C (99.0  $\pm$  2.6) and lowest at 32 °C (56.4  $\pm$  1.8). The fourth instar nymph was highest at 24 °C (90.4  $\pm$  2.6) and lowest at 32 °C (44.8  $\pm$  1.3). The fifth instar nymph was highest at 24 °C (80.0  $\pm$  2.4) and lowest at 32 °C (22.6  $\pm$  1.1). Adults had the highest at 24 °C (64.6  $\pm$  2.2) and no adults emerged adult at 32 °C. All eggs died at 36 °C (Table 5). This shows that, overall, 24 °C is the most favorable temperature for development of each life stage. Bae and

Pathak (1970) reported that there was no apparent difference in rate of growth of nymphs when reared at 25 °C or 29 °C but at 33 °C no nymphs survived beyond second instar.

**Table 4 Life table parameters of macropterous forms at different temperature**

Parameter	Formula	Macropterous forms				
		Control	24 °C	28 °C	32 °C	36 °C
Mean length of a generation (G)	$G = \sum l_x m_x X / \sum l_x m_x$	8.8	7.5	8.9	6.0	3.8
Instantaneous rate	$r_m = \log_e(R_o) / G$	0.6	0.6	0.6	0.8	0.9
Net reproductive rate	$R_o = \sum l_x m_x$	204.0	102.4	142.6	93.6	29.2
Gross reproductive rate	$\sum m_x$	204.0	102.4	142.6	93.6	29.2

**Table 5 Life table of developmental stages of BPH at different temperature**

Stages	Mean $\pm$ SE					P- value
	control	24 °C	28 °C	32 °C	36 °C	
Egg	139.4 $\pm$ 3.9	143.0 $\pm$ 3.2	113.8 $\pm$ 1.1	132.4 $\pm$ 1.6	135.8 $\pm$ 2.7	<.0001
First instar nymph	102.4 $\pm$ 6.2	137.0 $\pm$ 3.2	96.8 $\pm$ 1.5	107.4 $\pm$ 2.5	0.0 $\pm$ 0.00	<.0001
Second instar nymph	77.6 $\pm$ 4.8	112.2 $\pm$ 3.1	77.4 $\pm$ 1.1	76.6 $\pm$ 1.8	0.0 $\pm$ 0.00	<.0001
Third instar nymph	67.4 $\pm$ 3.8	99.0 $\pm$ 2.6	72.8 $\pm$ 0.9	56.4 $\pm$ 1.8	0.0 $\pm$ 0.00	<.0001
Fourth instar nymph	57.2 $\pm$ 3.1	90.4 $\pm$ 2.6	67.2 $\pm$ 0.9	44.8 $\pm$ 1.3	0.0 $\pm$ 0.00	<.0001
Fifth instar nymph	48.0 $\pm$ 2.7	80.0 $\pm$ 2.4	64.0 $\pm$ 0.8	22.6 $\pm$ 1.1	0.0 $\pm$ 0.00	<.0001
Adult	36.8 $\pm$ 2.4	64.6 $\pm$ 2.2	56.0 $\pm$ 0.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.00	<.0001

## CONCLUSION

This study showed that population growth parameters were positively correlated with temperature up to 28 °C but negatively correlated at 32 °C and above. At 36 °C, survival of all growth stages decreased. Thus, temperatures above 32°C are detrimental to the development of BPH and no adults emerged at 32 °C and 36 °C. The most favorable temperature for BPH development was 24 °C for every life stage. According to results, BPH's net reproductive rate was greater at lower temperature regimes (control, 24 and 28 °C) than at higher temperature regimes (32 °C and 36 °C). No eggs hatched into nymph at 36 °C. BPH development was good at control, 24 °C and 28 °C although fitness was reduced at 32 and 36 °C. In the life table analysis, the highest mortality occurred in the first nymphal instar. The survivorship curve indicated a modest rate of mortality during the early life stages that gradually decreased as individuals reached adulthood; this followed a type III survivorship curve. Temperature had a direct effect on development, survival and reproduction of BPH.

Brown planthopper have high reproductive rate, so critical monitoring and forecasting of the brown planthopper is very necessary for timely control management. The research finding will provide valuable information to take monitoring program for control of BPH population outbreak according to the climate change.

## ACKNOWLEDGEMENTS

The authors would like to thank the generous financial and technical training support provided by the Japan International Cooperation Agency (JICA) Project for Capacity Development of Yezin Agricultural University (YAU-JICA TCP).

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# Effects of adding Rice Husk Biochar on GHG Emission and Compost Quality during Cow Manure Composting

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Received 31 December 2020    Accepted 30 June 2021    (\*Corresponding Author)

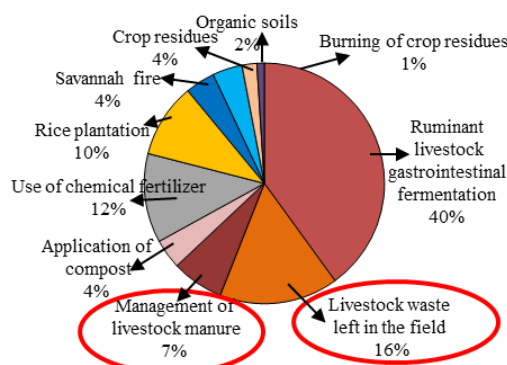
**Abstract** Agriculture is a major anthropogenic source of Green House Gases (GHG) and contributes 24% of total GHG emitted (IPCC, 2014). Livestock sector forms a significant part of agriculture and is responsible for major GHG emission. According to FAO, 2016, livestock manure contributes 23% to total GHG emitted in agricultural sector.  $N_2O$ ,  $CO_2$  and  $CH_4$  are important GHG emitted during management of livestock manure. Against the background of global warming, there is increased motivation in reducing GHG emission. Therefore, this study deals to see the effects in  $N_2O$ ,  $CO_2$  and  $CH_4$  emission by adding 5%, 10% and 15% of rice husk biochar during cow manure composting. In addition, degradation rate and C/N ratio of manure samples were analyzed for determining the final compost quality. Subsequently, a composting experiment was conducted using rice husk biochar and gases emitted were measured periodically with static gas chamber method. The experimental results indicated that biochar was effective in reducing  $N_2O$  and  $CO_2$  emissions. The cumulative emission of  $N_2O$  was reduced by 40% in 5% treatment, 46% in 10% treatment and 60% in 15% treatment. Similarly, there was decrease of  $CO_2$  by 69% in 5% treatment, 68% in 10% treatment and 48% in 15% treatment. Biochar's well developed pore structure and adsorption capacity reduced the gas emission. Furthermore, addition of biochar enhanced degradation rate and better C/N ratio. Biochar addition provided necessary nutrients and habitat, facilitating growth of various microorganisms. The results of  $CH_4$  emission did not show any significant difference between the treatments. The obtained results indicate that rice husk biochar addition is beneficial in reducing GHG emission and improving compost quality with faster degradation and better C/N ratio.

**Keywords** GHG emission, cow manure composting, rice husk biochar, compost quality

## INTRODUCTION AND BACKGROUND

As the human civilization has evolved, the global climate change has been continuously changing. In recent years, global climate change issues and its relation to human activities has gathered widespread attention. IPCC, 2007, IPCC, 2013, states that global warming is significantly affected by human activities. Furthermore, these reports states that fossil fuel use and land use change are the major contributor of GHGs. AFLOU, which represents agriculture, forestry and other land uses is a major anthropogenic source of GHGs emission and contributes 24% of total GHG emitted (IPCC, 2014). GHGs are mainly composed of  $CO_2$  (76%),  $CH_4$  (16%),  $N_2O$  (6%) (IPCC, 2013). Agricultural activities contributes 20% of  $CO_2$ , 70%  $CH_4$  and 90% of  $N_2O$  (Cole et al., 1997; Yousefi, Damghani and Khoramivafa, 2016). Livestock sector forms a significant part of agriculture and is responsible for major GHGs emission. FAO, 2016, reports that management of livestock manure contributes 23% to

total GHGs emitted in agricultural sector. Generally, livestock manure has been used as a soil amendment in the form of compost. Composting is an efficient way of manure management, but also releases GHGs.  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{CH}_4$  are important GHGs emitted during composting of livestock manure. In recent years, biochar has gained significant interest in various environmental applications. Biochar is a product of pyrolysis characterized with microscopic structure having large surface area and has affinity to absorb nutrients, ions etc. (Dünisch et al., 2007; Major et al., 2009), and has been applied in soil to maintain soil fertility and reducing GHG emission (Van Zwieten et al., 2015). Due to its unique properties, it is an efficient, cost effective and environmentally friendly material for diverse purposes. Against the background of global warming, there is increased motivation in reducing GHG emissions. Therefore, this study focus to see the effects of adding rice husk biochar during cow manure composting in reducing GHG gases emission and better compost quality.

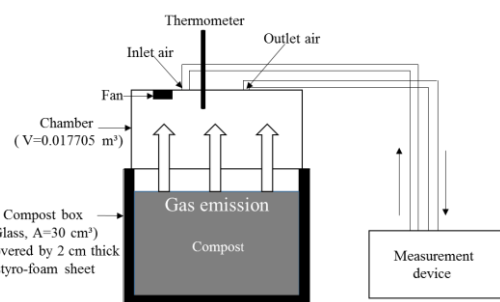


**Fig. 1 GHGs contributor in agricultural sector (FAO, 2016)**

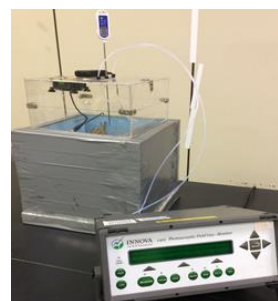
## METHODOLOGY

### Compost Box and Gas Chamber

A glass container of dimension  $30\text{ cm}^3$  was used as a compost box. The box was covered with styro-foam sheets of thickness 2 cm on five sides for minimizing the heat loss (Fig. 2) during composting process. A static gas chamber made up of acrylic sheet was used to measure the gas flux. Acrylic sheet was used as it is inert to the target gases. A digital thermometer and a fan were attached inside the chamber for measuring temperature and uniform circulation of air during measurement of gas respectively (Fig. 2 and 3). The volume of air inside the chamber was calculated as  $0.017705\text{ m}^3$ .



**Fig. 2 Schematic diagram of composting box and gas flux analysis**



**Fig. 3 Apparatus used during gas flux measurement**



## Conditions of the Experiment

The composting experiment was performed from 8 August 2019 to 7 October 2019 in Laboratory of Land and Water Use Engineering, Tokyo University of Agriculture. The average temperature was 23.0°C during the experiment period. Cow manure, rice straw and litter was used as main components for composting. The nitrogen content in cow manure and rice husk biochar was 23695 mg/kg and 1241 mg/kg respectively at the start of the experiment. Rice husk biochar was added in three different variations (Table 1). These variations were made to see the most effective composition. The initial water content of the composting material was set at 70±2% for all the treatments.

**Table 1 Composition of treatments**

Composition	Control	5% Treatment	10% Treatment	15% Treatment
Cow manure (g)	3200	3200	3200	3200
Litter + rice straw (g)	1800*	1800*	1800*	1800*
Rice husk bio-char (g)		250*	500*	750*

Note: \* represents air-dry weight basis

## Gas Flux Measurement

Gas flux was measured using static gas chamber method with photoacoustic spectrometer. The spectrometer used in this study was INNOVA 1412 Photoacoustic Field Gas Monitor (Fig.3). Gas flux was calculated using linear aggression method showed in Equation 1 (Minamikawa et al., 2015).

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{T} \quad (1)$$

Where,

F is gas flux (mg m<sup>-2</sup> hr<sup>-1</sup>),

ρ is density of gas (kg m<sup>-3</sup>) where density of NH<sub>3</sub> is 0.772 kg m<sup>-3</sup> and of N<sub>2</sub>O is 1.96 kg m<sup>-3</sup>,

A is bottom surface area of chamber (m<sup>2</sup>),

V is volume of air inside the chamber (m<sup>3</sup>),

Δc/Δt is average increase rate of gas density inside the chamber (10<sup>-6</sup> m<sup>3</sup> m<sup>-3</sup> hr<sup>-1</sup>),

T is average temperature inside the chamber (K)

## Determination of Degradation Rate

Loss of organic content during a composting process is a measure of biological decomposition and is expressed in the form of degradation rate. The degradation of composting substrates as a function of time follows first order kinetics (Haug 1993, Kulcu and Yaldiz, 2004) and can be expressed as following,

$$\left\{ \frac{OM}{OM_0} \right\} = -kt \quad (2)$$

Where,

OM is mass of biodegradable organic matter at any time of composting,

OM<sub>0</sub> is initial mass of biodegradable organic matter,

t is time in day,  
 $k_t$  is reaction rate constant in days<sup>-1</sup>

### Compost Sampling and Analysis

Compost was sampled at 5, 10, 20, 30, 40 and 60 days to analyze its quality. Temperature of the compost pile was measured using Custom CT-0580 data logger and digital thermometer on daily basis. Organic content was analyzed using Ignition loss method. Carbon content was determined by using the formula  $C=0.580 \times IL$ . Total nitrogen was analyzed by absorption spectroscopy using HC-1000 (Central Science Corp.) as measurement device (Mihara and Ueno, 2000).

## RESULTS AND DISCUSSIONS

### Biochar Effect on Nitrous oxide Emission

Composting of high organic content materials has been shown to produce  $N_2O$  by nitrification and denitrification under aerobic and low oxygen conditions respectively. Fig. 4 shows the periodic change in emission between the treatments. The cumulative gas emission (Fig. 5) was lowered by 40%, 46.4% and 60.4% in 5%, 10% and 15% biochar added treatments compared to control. The result observed is supported by that of Jeffery et al., 2015, which states that addition of biochar can decrease the emission of nitrous oxide. Although the mechanisms of  $N_2O$  formation during composting process of animal manure is not studied in details,  $N_2O$  emission is mainly associated with nitrification and denitrification process (Sanchez-Monedero et al., 2010). These microbial process is regulated by the quantity of mineral nitrogen, presence of carbon sources and oxygen concentration in composting material. Wang et al., 2013, showed 31% lower emissions in biochar treated piles and linked this result in reduction to change in the abundance and composition of denitrifying bacteria. It has been well reported that addition of biochar increases pile porosity, thus increasing oxygen content, weakening denitrification enzyme activity and inhibiting denitrification reaction under anaerobic conditions (Singh et al., 2010).

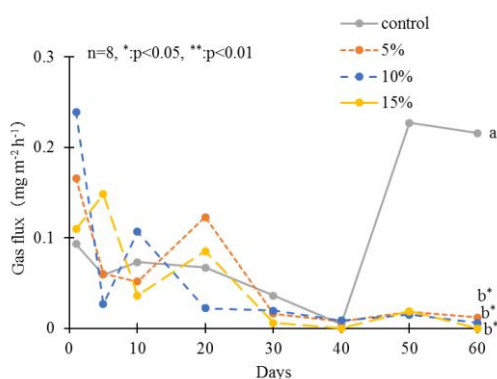


Fig. 4 Periodic change in  $N_2O$  flux

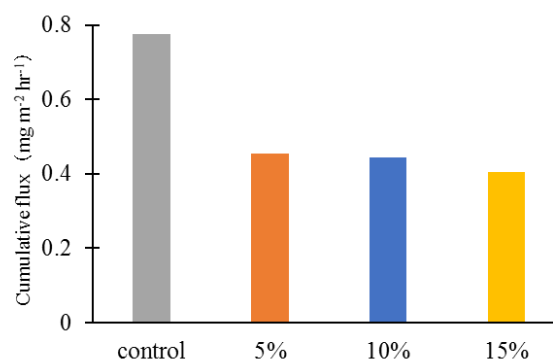
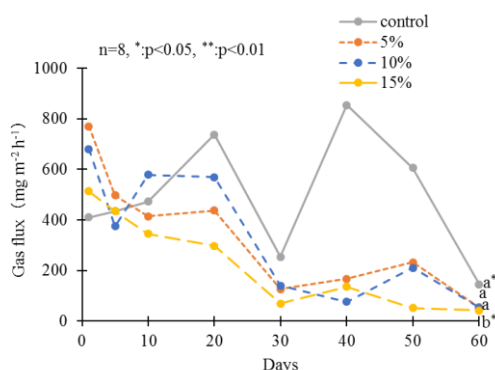


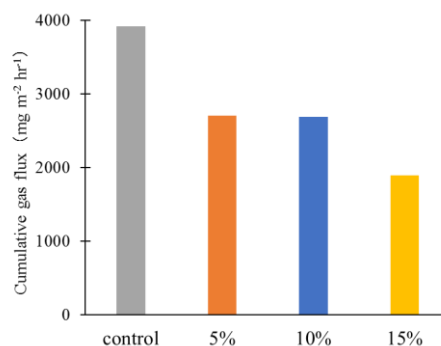
Fig. 5 Cumulative gas flux of  $N_2O$

### Biochar Effect on Carbondioxide Emission

The result of periodic changes in  $CO_2$  flux is shown in fig 6. The concentration of  $CO_2$  was highest in control amongst the treatments which coincides to the studies from Awasthi et al., 2017 and Jiang et al., 2016, according to which biochar reduces  $CO_2$  emission. The periodic change in  $CO_2$  flux showed an initial rise and fall at the later stage of the composting process, which was consistent with change in temperature.



**Fig. 6 Periodic change in CO<sub>2</sub> flux**

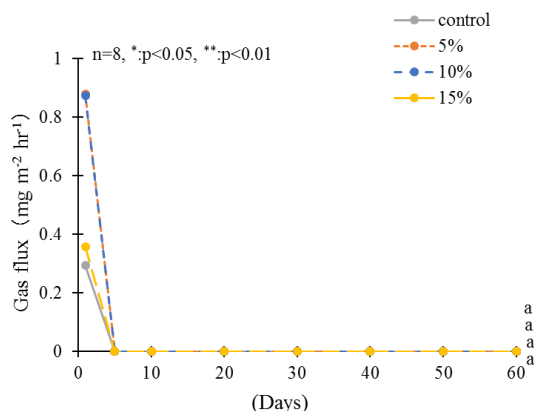


**Fig. 7 Cumulative gas flux of CO<sub>2</sub>**

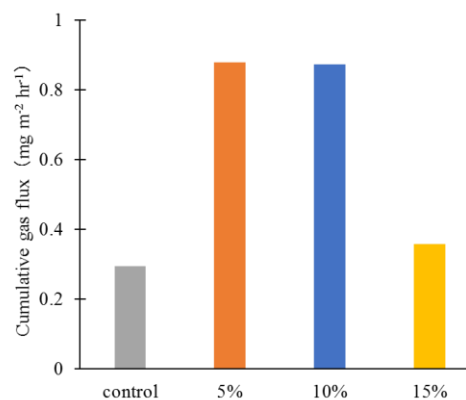
The cumulative gas flux (Fig. 7) showed that there was decrease of CO<sub>2</sub> by 69% in 5% treatment, 68% in 10% treatment and 48% in 15% treatment. According to the obtained results, biochar added treatments could reduce the emission of CO<sub>2</sub>. This result coincides to the ones reported by Ngo et al., 2013, which states that biochar could increase carbon sequestration of exogenous organic matter when applied in compost mixtures.

### Biochar Effect on Methane Emission

Fig. 8 shows the results of periodic change of CH<sub>4</sub> flux and fig. 9 shows the cumulative gas flux emission. CH<sub>4</sub> emission was observed in early stage of the composting period for all the treatments with biochar added treatments having higher emission compared to control. The emission was seen during first week of the experiment with no emission observed after a week for all the treatments. Although, studies have shown that biochar addition enabled to inhibit CH<sub>4</sub> emission in cow manure (Sonoki et al., 2013) and municipal solid waste composting (Vandecasteele et al., 2013), similar results could not be observed in our study. CH<sub>4</sub> production during composting is mainly associated to anaerobic processes.



**Fig. 8 Periodic change in CH<sub>4</sub> flux**

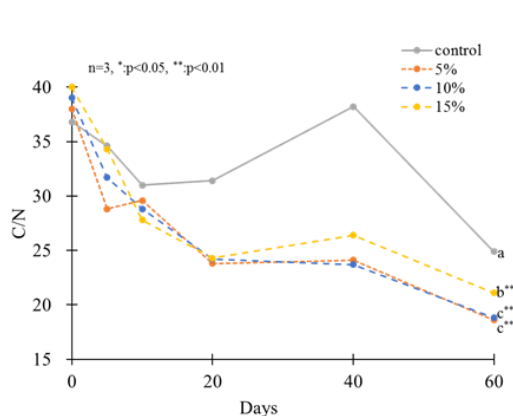


**Fig. 9 Cumulative gas flux of CH<sub>4</sub>**

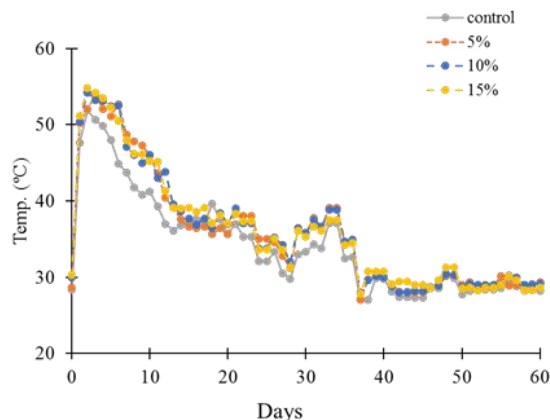
According to Sanchez et al., 2015, favourable conditions for CH<sub>4</sub> emission can be found in the early stages of composting process, where large amount of nutrients and organic matter are available which enhances microbial growth, depleting the oxygen levels in the pile. Referring to the results of temperature, (fig. 11) where biochar added treatments showed higher temperature gain, shows increased microbial activity, creating anaerobic environment. To support this, general bacteria count in the early stage of composting showed higher microbial presence in biochar added treatments.

## Biochar Effects on Quality of Compost

C/N ratio and temperature profile are often used as a criteria for determining the quality of compost. C/N ratio is used as an indicator of compost stability and nitrogen availability. Whereas, increased temperature shows higher microbial activity and faster decomposition of organic substrates. The periodic change of C/N ratio showed better values in biochar added treatments compared to control (Fig. 10).



**Fig. 10 Periodic change in C/N ratio**



**Fig. 11 Periodic change in temperature**

Better C/N ratio amongst the biochar added treatments compared to control may be due increased surface area and moisture content favouring microbial activity for degradation and humification of organic material. In addition, biochar increased sorption of nitrogenous compounds in its microspores, providing microorganism with sufficient nitrogen for their metabolism. Temperature results (Fig. 11) showed rise in temperature in early stages of composting, followed by fall after one week and stabilising around 30 °C at later stage of composting. Biochar added treatments had higher temperature gain during early stages of composting compared to control due to faster degradation due to reduced heat loss as addition of biochar filled the air pores (Zhang and Sun, 2014). Khodadad et al., reports that adding of biochar increases the relative abundance of Actinobacteria, which are generally able to degrade more organic material, resulting in heat generation.

## Biochar Effect on Degradation Rate

The results of degradation rate of organic substrates is shown in table 2. The highest biodegradability after 5 days of composting was seen in 15% treatment with rate constant of  $0.034 \text{ d}^{-1}$ , followed by 5% treatment with rate constant of  $0.032 \text{ d}^{-1}$ , followed by 10% treatment with reaction rate of  $0.031 \text{ d}^{-1}$ , with the least in control having  $0.013 \text{ d}^{-1}$  of reaction rate. For 60 days of composting, the degradability was in order of 10% treatment with  $0.0056 \text{ d}^{-1}$ , 15% treatment with  $0.0054 \text{ d}^{-1}$ , 5% treatment with  $0.0052 \text{ d}^{-1}$ , followed by control with  $0.0045 \text{ d}^{-1}$ . The obtained results shows that adding of biochar enhances decomposition of organic matter and degradation rate of the composting substrates. The degradation rate was higher during early and decreased with time. The results of increased degradation in biochar added treatments also agree to that of Zhang and Sun, 2014, which states that biochar amendment increased composting rate, increasing temperatures due to denser substrates with filled pore spaces reducing the heat loss that occurs because of greater air space. Moreover, adding of biochar increased the oxygen uptake, moisture retention and increased the microbial activity resulting in better degradation. Steiner et al., 2015, biochar is a highly porous material having high capacity to

retain excess water, improve aeration conditions and provide suitable conditions for microorganisms, promoting microbial activity and increasing organic matter degradation.

**Table 2 Degradation rate at 5 and 60 days passed**

Treatments	5 days $k_t$ (d <sup>-1</sup> )	60 days $k_t$ (d <sup>-1</sup> )
Control	0.013 a	0.0045 a
5%	0.032 b <sup>**</sup>	0.0052 a
10%	0.031 c <sup>**</sup>	0.0056 a
15%	0.034 c <sup>**</sup>	0.0054 a

Note: Significance difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$

## CONCLUSIONS

This study was conducted to see the effects of adding rice husk biochar in reducing GHG emission and enhancing final compost quality during cow manure composting. According to the results of this study, addition of rice husk biochar was efficient in reducing GHG emission and significantly enhancing degradation and mineralization of organic substrates during cow manure composting. In the given conditions, addition of biochar was effective in decreased N<sub>2</sub>O and CO<sub>2</sub> emission. However, no significance difference was seen in CH<sub>4</sub> emission. On the other hand, compost quality with better degradation and nitrogen mineralization was observed. Although, 15% treatment (750 g in 5 kg) had highest reduction of GHG emission and enhanced compost quality, relatively small amount of 5% (250 g in 5 kg) treatment was also effective. Considering these findings, rice husk biochar could be an effective and beneficial practice for management of cow manure for reducing GHG emission and better quality of cow manure compost.

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## Conservation and Ecological Threats of Agarwood (*Aquilaria* sp.) on Leyte Island, Philippines

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Received 30 December 2020   Accepted 30 June 2021   (\*Corresponding Author)

**Abstract** *Aquilaria* is a genus of about 20 species distributed mainly in the Indo-Malesian region. The genus *Aquilaria* belongs to the Thymelacaceae family of Indo-Malayan trees known for producing the world's most expensive oils, which are naturally occurring throughout the Philippines. *Aquilaria* is well known for the production of agarwood which is a highly wanted forest product of substantial economic value. In the Philippines, there is limited published information on the physical condition, habitat structure, and ecological threats of *Aquilaria*, which is a crucial factor to determine the optimum requirements to develop *Aquilaria* production system. Hence, this study was conceptualized to assess the habitat structure of *Aquilaria* as well as its conservation and ecological threats in the wild. Assessment, surveys and interview were conducted to gather on-site atmospheric data as well as ecological threats of *Aquilaria* in the wild. Soil collection was also gathered to analyzed soil properties. The results revealed that there were two species of *Aquilaria* documented in the study site, namely: *A. malaccensis* and *A. cumingiana*. Moreover, the soil chemical properties are not significant between different topographic expositions, but it is acidic and generally have low nutrient status. Furthermore, the conservation and ecological threats documented in the study site are unsustainable harvesting, a massive collection of regenerant, and inflecting damage of *Aquilaria* by punching nails or drilling holes in the trunk of standing mature tree. Therefore, it is highly recommended that *Aquilaria* should be protected, particularly *A. malaccensis*, since it is rare and considered a new record of occurrence to Leyte Island as one of the major findings of the study. The establishment of *Aquilaria* based production system is necessary as an option to reduce the rampant illegal poaching of agarwood in the wild but should be established on private land and registered with the Department of Environment and Natural Resources (DENR).

**Keywords** *Aquilaria*, topographic expositions, ecological threats, habitat structure

## INTRODUCTION

*Aquilaria* is a genus of about 20 species (International Plant Names Index, 2015), distributed mainly in the Indo-Malesian region. Agarwood also known as *gaharu* in the South East Asia, *oud* in the Middle

East, *chen xiang* in China, *jinkoh* in Japan and *agar* in India is a highly valuable aromatic dark resinous heartwood of *Aquilaria* species (Liu et al., 2017). The genus *Aquilaria* belongs to the Thymelacaceae family of Indo-Malayan trees known for producing the world's most expensive oils (Lee & Mohamed, 2016). Fernando et al. (2018) reported that *Aquilaria apiculata*, *A. filarial*, *A. malaccensis*, *A. brachyantha*, *A. cumingiana*, *A. urdanetensis*, *A. citrinaecarpa*, and *A. parvifolia* are just eight out of the 24 species of agarwood oil-producing trees in the world that are naturally occurring in the Philippines. In Leyte, Biliran, Samar, and Negros, *Aquilaria* sp. is locally known as *lapnisan* and *laneti*.

Agarwood is traded in several raw forms, ranging from large sections of the trunk to finished products such as incense and perfumes (Barden et al., 2000). They reported that natural stocks are largely depleted by overexploitation, and the demand for agarwood is higher. Foreign nationals are now searching agarwood in the Philippines, specifically Leyte, Biliran, Samar, and Negros. There have been reports in the field that sometimes these nationals are impatient to find *Aquilaria* since it was reported by Abdin (2014) that global agarwood prices can be ranging from US\$ 20 – 6,000 per kilogram for the wood chips depending on its quality or US\$ 10,000 per kilogram for the wood itself. There was a destructive exploitation of agarwood that has badly affected the wild population of all *Aquilaria* species due to widely used as therapeutic perfumes, traditional medicine, religious purposes and aromatic food ingredient (Tan et al., 2019).

There is a scarcity of information on the physical condition, habitat structure and ecological threats of *Aquilaria* which is crucial factor to determine the optimum requirements to develop *Aquilaria* based production system. This study was conceptualized to assess the habitat structure of *Aquilaria* and its conservation and ecological threats in the wild.

## OBJECTIVES

1. To characterize the habitat condition and structure of *Aquilaria* on Leyte Island; and
2. To determine the ecological threats of *Aquilaria* in the natural habitat.

## METHODOLOGY

### Selection of the Study and Sampling Site

The study was conducted at the selected site of Leyte Island. The major criteria in selecting the sampling site were the presence of *Aquilaria* in the area which was selected through a preliminary survey by directly collecting information from reliable individuals (i.e., poachers, a buyer of agarwood) further identify the exact location of *Aquilaria* trees in the wild. Furthermore, the study site was selected based on the incidence of rapid and over-harvesting of the *Aquilaria* matured trees and regenerating, respectively, during the pre-assessment.

### Data Collection

The atmospheric data, i.e., light intensity (lux), temperature (°C), and relative humidity (%) were collected on-site to determine the climatic factor that may affects the habitat structure of *Aquilaria* under natural condition. The data was collected in each exposition at an interval of two hours, starting from seven o'clock in the morning until five o'clock in the afternoon.

Soil samples were collected in the study site. The soil samples were then brought to the laboratory to analyze soil chemical properties such as soil pH, soil organic matter, total nitrogen, and available phosphorus.



To determine ecological threats, an actual field survey and photo documentation of poached *Aquilaria* trees were done. Likewise, an interview was conducted to collect information from seedling-growers, poachers, and agarwood buyers.

### Data Encoding and Statistical Analysis

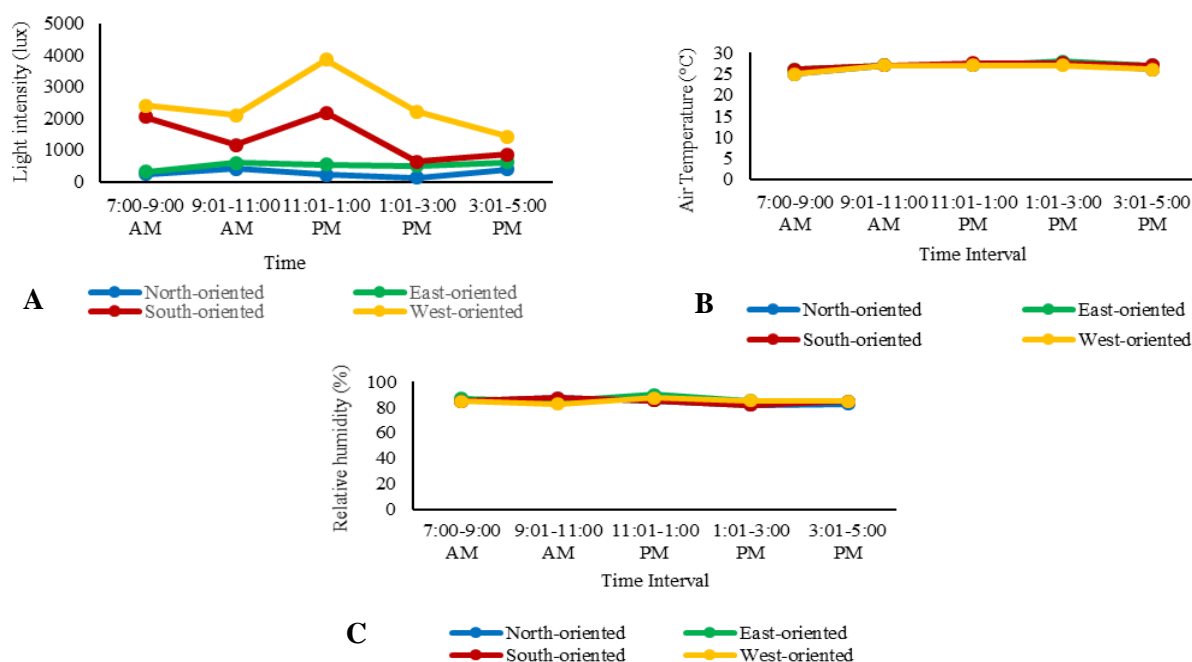
All data gathered were collated, encoded, and summarized using an electronic spreadsheet editor, Microsoft Excel 2013. The data were analyzed using the Statistical Package for Social Science (SPSS version 20).

The atmospheric data were analyzed descriptively. The variability of the mean of soil chemical properties was analyzed using the one-way analysis of variance (ANOVA). Moreover, in a case where the significant variations at  $p \leq 0.05$  was identified, Tukey and Least Squares Differences (LSD) was carried out to compare means.

## RESULTS AND DISCUSSION

### Microclimatic Conditions in the Study Site

The data on atmospheric parameters such as light intensity, air temperature, relative humidity, and rainfall are presented in the figure below to describe the on-site condition of the study area.



**Fig. 1 Microclimatic conditions between different expositions of the study site at different time intervals**

Results show that west-oriented topographic exposition had higher light intensity compared to other topographic expositions (Fig. 1A). Meanwhile, the average temperature at all periods of data collection had an increasing trend from seven o'clock in the morning until three o'clock in the

afternoon but had a slight drop at five o'clock in the afternoon (Fig. 2B). On the other hand, the result of the relative humidity had a slight variation (Fig. 1C).

### Soil Chemical Properties

Table 1 shows the chemical properties of the study site. Statistical analysis showed no significant difference between soil chemical properties of the study site between different topographic expositions. Soil pH was very strongly acidic, and soil organic matter content was moderate. The total nitrogen in the study site is medium, as interpreted by Landon (1991). According to Krofranek et al. (2007), total N is deficient mostly in the tropics since it undergoes various losses such as leaching and volatilization. The results on total N can therefore, be attributed to the lower soil organic matter in the study site.

**Table 1 Soil chemical properties within study site between different topographic expositions**

Expositions	pH	Soil Organic Matter (%)	Total Nitrogen (g/kg)	Available Phosphorus (mg/kg)
North-oriented	4.90 ± 0.18	4.27 ± 0.63	0.21 ± .03	0.96 ± 0.16
East-oriented	4.85 ± 0.18	4.11 ± 0.63	0.21 ± .03	0.59 ± 0.16
South-oriented	4.90 ± 0.18	4.46 ± 0.63	0.22 ± .03	0.92 ± 0.16
West-oriented	4.74 ± 0.18	4.01 ± 0.63	0.20 ± .03	0.63 ± 0.16

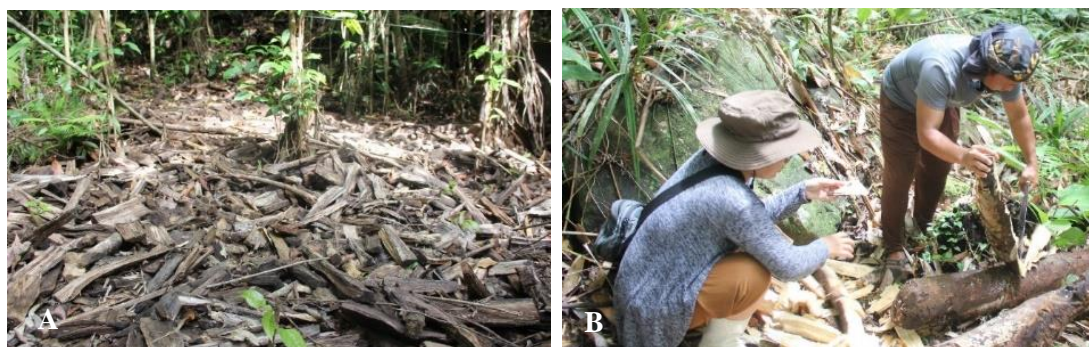
On the other hand, according to the sufficiency ranges of soil of Landon (1991) the available P in the study site is very low.

Therefore, the result of this study supports the findings of Piamonte et al. (2014), where they found out that the soil in Mt. Nacolod Mountain Range (Abuyog-Silago-Hinunangan-St. Bernard-Libagon-Mahaplag) is classified as Ultisols, which is characterized as acidic and generally have low nutrients status.

### Conservation and Ecological Threats

#### Unsustainable harvesting:

In the study site, five illegally-cut matured *Aquilaria* were documented and recorded. Meanwhile, a community resident began harvesting *Aquilaria* in the forest in June 2019. According to the respondent, since it was difficult for them to identify the exact *Aquilaria* trees, they would randomly cut trees without properly distinguishing them and chopping them into pieces (Fig. 2A). Until eventually, traders from other countries (China and Taiwan) and other places within the Philippines (Mindanao and Samar) came and trained them to identify and distinguish the *Aquilaria* tree.



**Fig. 2 Local practice in harvesting *Aquilaria* in the study site**

The traders instructed them that a matured *Aquilaria* tree that looks rotten or unhealthy has most likely agar in it, whereas those that look firm and healthy are not good. This information was confirmed by the study of Rasool and Mohamed (2016) that agarwood formation is often linked to the physical wounding or damage of *Aquilaria* trees caused by thunder strike, animal grazing, and pest and disease infestations.

According to the respondents, *Aquilaria* is cut into pieces from trees to reveal the resinous product (Fig. 2B). Apparently, poachers are chopping down trees faster than they can grow, including forest across neighboring towns. According to Ng et al. (1997), not all *Aquilaria* trees produce agarwood, estimating that only approximately 10% of wild *Aquilaria* spp. produce resin.

Furthermore, respondents stated that they are more eager to search for agarwood than work as a hired labor or harvest copra because of its immense value. They further explained that they earn higher income from agarwood than farming and working as laborers. Harvesting *Aquilaria* and selling agarwood enable them to triple their income, which consequently improved their living condition. For instance, most of the respondents could buy new motorbikes, repair or enhance their houses and buy property (such as land).

#### Massive collection of regenerant:

Based on the interview results, in 2019, when *Aquilaria* became widely known, the value of seedling rose to 500 to 1000 Pesos. The seedling, particularly from *Aquilaria malaccensis* (Fig. 3A), generates higher income since wildlings are abundant in the forest. According to Chua (2008), *Aquilaria malaccensis* produces seeds after 7-9 years, while some other species produce seeds only once in their life cycle.



**Fig. 3 *Aquilaria malaccensis* seedlings (A) and *Aquilaria cumingiana* seeds (B) owned by one of the respondents**

As for *Aquilaria cumingiana* regenerant (Fig. 3B), only few are being propagated after collecting seeds. According to the seedling growers, its value is worth 40,000 Pesos per kilo. The seedling grower clarified that none of the seeds survived due to insufficient knowledge on how to properly propagate *Aquilaria* seeds and the appropriate time of collecting the matured fruits.

#### Inflecting damage to the matured *Aquilaria* trees:

In the study site, landowners and poachers have created an illegal method of inflecting matured *Aquilaria*. This is by punching nails or drilling holes on the trunk of standing matured *Aquilaria* (Fig. 4). They believe that by damaging the trunk using nails, it will gradually trigger infection and eventually develop agar inside. This method is widely visible on the study site.

Moreover, according to Ng et al. (1997), the age of the tree, differences in the tree caused by seasonal variation, environmental variation and genetic variation of *Aquilaria* spp. may also play an essential role in agarwood formation.



**Fig. 4 Locally practiced method of inflecting (by punching nails and drilling holes at the trunk) matured *Aquilaria* species in the community**

## CONCLUSION

The results show that west-oriented topographic exposition had higher light intensity, while the average temperature during the conduct of the study had an increasing trend from seven o'clock in the morning until three o'clock in the afternoon but had a slight drop at five o'clock in the afternoon. The result of the relative humidity showed that the east-oriented and north-oriented topographic location got the highest and lowest average of 86.29% and 84.26%, respectively.

The soil chemical properties are not significant between different topographic expositions. However, the soil on the study site is acidic and has a generally low nutrient status. The ecological threats recorded in the study site were unsustainable harvesting, a massive collection of regenerants, and inflecting damage of *Aquilaria* by punching nails or drilling holes in the trunk of a mature standing tree.

Therefore, it is highly recommended that *Aquilaria* should be protected particularly *Aquilaria malaccensis* since it is rare and is considered a new record of occurrence to Leyte Island. The establishment and development of an *Aquilaria*-based production system are necessary as an alternative option to reduce the rampant illegal poaching of agarwood in the wild. Furthermore, the *Aquilaria*-based production system should be established in private land and registered with the Department of Environment and Natural Resources.

## ACKNOWLEDGEMENTS

We extend our gratitude to the Institute of Tropical Ecology and Environmental Management, Visayas State University, Baybay City, Leyte and to the Municipality of Abuyog, Leyte, for the great support for this study.

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## Effects of Air Injection and Iron Oxide Pellet Addition on Hydrogen Sulfide Removal and Biogas Production

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Received 22 January 2021    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Hydrogen sulfide ( $H_2S$ ) in biogas resulting from anaerobic digestion process is unwanted trace compound, because it is toxic and can corrode biogas engine. This study aimed to compare the 2 desulfurization methods, air injection and iron oxide pellets addition, on  $H_2S$  removal and quantity and quality of biogas. The experiment was carried out with two floating drum digesters ( $1\text{ m}^3$  each) constructed at Royal University of Agriculture, Phnom Penh, from January to August 2020. Three levels of air injection at 2%, 4%, and 6% of  $O_2$  regarding the daily biogas production and iron oxide pellets application at 1 kg, 2 kg, and 4 kg per  $\text{m}^3$  of biogas were applied to remove  $H_2S$  in biogas from different raw materials of pig manure and food waste. The amount of daily biogas production was quantified by gas flow meter, also gas quality was measured using a GEM5000 gas analyzer. The experimental results indicated that food waste had higher daily biogas production comparing to pig manure in both desulfurization methods. Biogas from food waste increased from  $544\text{ L d}^{-1}$  without iron oxide pellets addition (0 kg) to  $657\text{ L d}^{-1}$  with 4 kg iron oxide pellets addition; and to  $566.5\text{ L d}^{-1}$  with 2% of injected  $O_2$ . To the contrary, desulfurization for pig manure with 2% of  $O_2$  and 1 kg of iron oxide showed high daily biogas yield of  $348\text{ L d}^{-1}$  and  $340\text{ L d}^{-1}$ , respectively. For raw materials of

pig manure, in both desulfurization methods, had higher  $\text{CH}_4$  content than food waste. Air injection was more effective in  $\text{H}_2\text{S}$  removal than iron oxide for both substrates, but higher level of  $\text{H}_2\text{S}$  reduction was observed with pig manure. Accordingly, it was concluded that desulfurization methods with air injection and iron oxide pellets addition were effective in biogas production as well as  $\text{H}_2\text{S}$  removal, but a clear trend appeared in the raw material of food waste.

**Keywords** floating drum digester, anaerobic digestion, food waste, pig manure,  $\text{H}_2\text{S}$  removal

## INTRODUCTION

Biogas is a product generated from the anaerobic digestion of organic substances by appropriate microorganisms through four metabolic stages; namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Dumont, 2015). It is considered one of the renewable energy sources that can provide both heat and electricity for use in households, in farms, or in industries. Gas compositions contained in biogas include methane ( $\text{CH}_4$ ), carbon dioxide ( $\text{CO}_2$ ), and other trace elements such as ammonia ( $\text{NH}_3$ ), water vapor ( $\text{H}_2\text{O}$ ), and hydrogen sulfide ( $\text{H}_2\text{S}$ ). The majority of them are  $\text{CH}_4$  (60-70%) and  $\text{CO}_2$  (30-40%) (Okoro and Sun, 2019), but the only energy source is  $\text{CH}_4$ , which has both benefits and drawbacks. If released into the atmosphere unused,  $\text{CH}_4$  is a greenhouse gas which has 28 times more powerful than  $\text{CO}_2$ , which actually accelerates global warming (National Geographic, 2019). When burned or used for internal combustion engine, its harmful effects are reduced and at the same time, its beneficial energy can be harnessed. Nevertheless, the presence of  $\text{H}_2\text{S}$  in biogas may cause problems in terms of health hazard and economic aspects (Pinate et al., 2017).

$\text{H}_2\text{S}$  is an unwanted gas mixed in biogas because it is toxic to humans at low concentrations and corrosive to engines. High concentrations of  $\text{H}_2\text{S}$  can corrode engines or metal parts, and lead to faster degradation of engine lubricant oil. A maximum recommended level for generator operation ranges from 200 to 500 ppm (Rodriguez et al., 2014). If it is used without treatment, oil lubricants must be changed more often, or the lifespan of a generator is reduced, resulting in high investment and operating costs. Furthermore,  $\text{H}_2\text{S}$  limits for gas heating boilers, combined heat and power (CHP), fuel cells, and national gas upgrade are 1,000, 1,000, 1, and 4 ppm, respectively (Choudhury et al., 2019). However, different substrates used for biogas production produce different concentrations of  $\text{H}_2\text{S}$ . Substrates that contain high-level protein produce higher  $\text{H}_2\text{S}$  content than those contain carbohydrate and lipid due to the presence of more sulfurous elements in it. Biogas produced from organic waste may contain  $\text{H}_2\text{S}$  in the range of 10-20,000 ppm, but about 10-40 ppm is found in biogas produced from sewage and 50-300 ppm from landfill (Dumont, 2015). Moreover, Huertas et al. (2020) stated that  $\text{H}_2\text{S}$  produced from organic waste such as pig manure can be as high as 30,000 ppm.

To reduce its concentration to an acceptable level, desulfurization techniques can be applied physically, biologically, and chemically. Biologically removal is done by air injection into digester with regulated amounts of oxygen ( $\text{O}_2$ ) between 0.3 and 3% of produced biogas; however, this reaction may result in sulfur build-ups in the digester space (Hines et al., 2019). Many kinds of chemical methods have been studied. Pinate et al. (2017) proposed a small batch test for  $\text{H}_2\text{S}$  removal by absorbent granules soaked in ferric chloride ( $\text{FeCl}_3$ ) and in sodium hydroxide solution ( $\text{NaOH}$ ), the removal efficiency was around 90%. Zulkeflia et al. (2016) stated that  $\text{H}_2\text{S}$  removal could be done by activated carbon soaked in  $\text{NaOH}$ , potassium hydroxide ( $\text{KOH}$ ), and potassium carbonate ( $\text{K}_2\text{CO}_3$ ), but added that the last chemical element was the most effective. Biological  $\text{H}_2\text{S}$  removal methods are also increasing popular and considered highly effective. Those biological methods include biofilters, biotrickling filters, bioscrubbers, and activated sludge (Barbusiński and Kalemba, 2016). However,  $\text{H}_2\text{S}$  removal techniques are limited, or have not been well documented in Cambodia, which makes it difficult for promotion of biogas use inside the country. Therefore, proposing an applicable desulfurizing technique is highly valuable for economic reasons.

Pig manure is a major waste from animal productions in Cambodia. According to MAFF (2020), the number of pigs was around 2.18 million heads in 2019 of which more than half raised in commercial farms. On the other hand, kitchen waste amounted from 63.30% to 80.46% of all solid wastes from households (Sour, 2017). Proper management of these two wastes by converting them to biogas will provide both environmental and economic benefits.

## OBJECTIVE

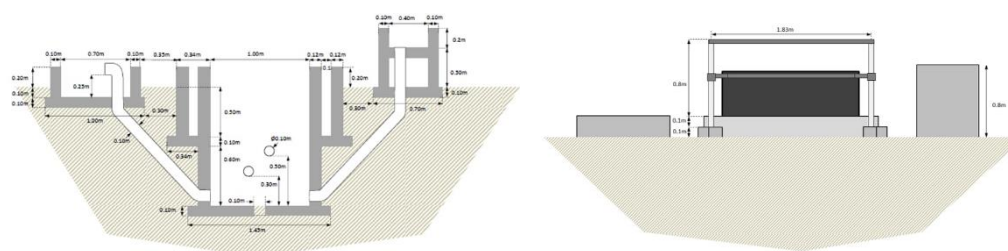
The objectives of this study were (1) to compare the effects of air injection and iron oxide pellets on H<sub>2</sub>S removal and quantity and quality of biogas produced from two different substrates, food waste and pig manure; and (2) to identify relations between biogas production with CH<sub>4</sub> content in the two substrates.

## METHODOLOGY

This research was conducted at the pilot biodigesters belonging to the Biogas Technology and Innovation Center (BTIC), at the Royal University of Agriculture, Cambodia, starting from January to August 2020. Two floating drum biodigesters (1 m<sup>3</sup> each) were used for this experiment. Two different substrates, food waste and pig manure, were daily fed into the systems. At its full production, biogas was then treated by two desulfurizing methods: air injection and iron oxide pellets. Air injection was applied directly into the gas holders (the floating drum) on top of the digesters at O<sub>2</sub> levels of 2%, 4%, and 6% of daily produced biogas. Meanwhile, iron oxide pellets were placed in a gas treatment container through which raw biogas was passed through and treated before being quantified. The levels of iron oxide pellets were applied at 1, 2, and 4 kg m<sup>-3</sup> of daily produced biogas. Effectiveness of the desulfurizing methods was also determined by comparing the results with raw untreated biogas.

## Materials

Two floating drum biodigesters used in this experiment had concrete bases and walls constructed under the ground. The whole volume was 1 m<sup>3</sup>, one third of which was for gas storage. The gas holders were high-grade polyethylene plastic water tanks that were cut in half and used as covers for the digesters. They can move up and down, depending on the biogas pressure produced inside.



**Fig. 1 Technical drawing of a floating drum biodigester used in this experiment**

Substrates used in this experiment were food waste and pig manure. Their physical and chemical properties were analyzed in advance. Still, there might be some variations due to everyday fresh collection of the substrates. To reduce errors, substrate collection was done at the same place throughout the experimental period.

Food waste was daily collected from the university canteen. It contained 21.9% dry matter, 1.4%



ash, 5.6% protein, 3.2% lipid, and a C/N ratio of 12.6:1. For high efficiency, bones and other organic materials that were slowly biodegradable were removed. Before being fed into the digester, food waste was thoroughly mixed with water at a ratio of 1:1, or 5 kg each. Pig manure used in this experiment was collected from two small-scale pig farms nearby the university. It contained 25% dry matter, 8.1% ash, 10.4% protein, 0.5% lipid, and a C/N ratio of 6:1. It was freshly collected, mixed with water, and fed into the system on daily basis at a ratio of 1:1, or 5 kg each.

### Sampling Methods

Feeding time was done in the morning and repeated regularly every day for 30 days, which was assumed as a minimum hydraulic retention time (HRT) for the digesters. Then, volume of untreated biogas was measured for 7 days to determine average daily biogas production, followed by air injection, which lasted 7 days for each O<sub>2</sub> level. In total, the whole process of air injection lasted one month. After this process was completed, the untreated biogas was measured another 7 days before application of iron oxide pellets in filter for H<sub>2</sub>S removal. Experiments with each level of iron oxide lasted 7 days, which took 1 month in total. The iron oxide pellets used in the experiment were proprietary ones which had been imported from China.

To measure the quality of biogas produced from the two substrates, a GEM5000 gas analyser was utilized. It was a product supplied by Geotech, UK, and was capable of measuring biogas quality such as CH<sub>4</sub>, CO<sub>2</sub>, and O<sub>2</sub>, all in percentage; and H<sub>2</sub>S in ppm with the maximum concentration of 5,000 ppm. Biogas quality was measured three times a day for each treatment after it was treated with air injection a day before, or went through the desulfurizing container on the day of the measurement.

To quantify daily biogas production, a gas flow meter was used. When the biogas was used for boiling water, the flow meter started to record the consumption at the average outdoor temperature of 28-33 °C.

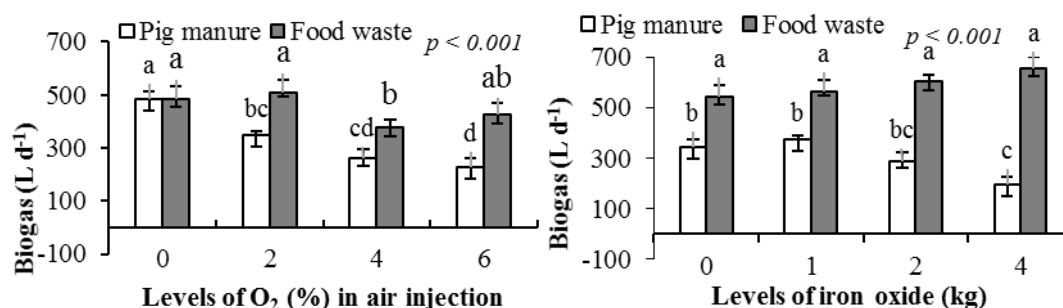
### Data Analysis

Analysis of Variance (ANOVA) were used for the data analysis, and when significant differences were observed, a post-hoc LSD-test was then used to show significant differences among the treatments at  $\alpha = 5\%$ . Besides that, the data for air injection and iron oxide pellets were presented separately because the experiments were done separately.

## RESULTS AND DISCUSSION

### Daily Biogas Production

Daily biogas production was compared among the two substrates, when treated with air injection at different levels of O<sub>2</sub> (Fig. 2a). Significant differences were detected among the treatments, and it was observed that biogas produced from food waste was higher than from pig manure. Daily biogas quantity was similar for both substrates, when measured without application of air injection. It was about 484 L d<sup>-1</sup>. For food waste, daily biogas production increased slightly with 2% of injected O<sub>2</sub> to almost 500 L d<sup>-1</sup>, but decreased continuously at O<sub>2</sub> levels of 4% and 6%. Similar results were obtained for pig manure. Daily biogas quantity decreased more and more, as levels of air injection increased. Daily biogas production from pig manure was 384, 260, 227 L d<sup>-1</sup> at injected O<sub>2</sub> levels of 2%, 4%, and 6%, respectively. This finding indicates that putting more air into the digester may affect biogas production because its ideal condition is anaerobic. Hines et al. (2019) proposed a maximum air injection level of 3%.

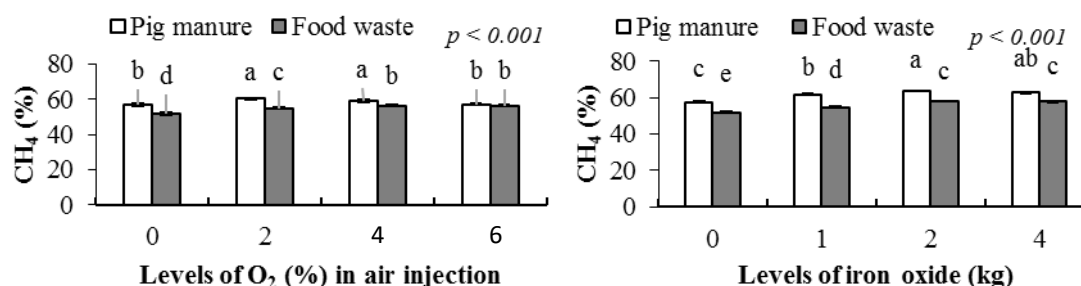


**Fig. 2 Comparison of biogas production with air injection (a) and with iron oxide pellet (b)**

Daily produced biogas quantity was compared among the two substrates when using iron oxide pellets (Fig. 2b). It was not affected by different levels of pellet, but by different substrates. Higher daily biogas production was observed with food waste at all levels of applied iron oxide pellets. Its average value was 585 L d<sup>-1</sup>. In contrast, daily biogas production from pig manure was quite low, averaging 320 L d<sup>-1</sup>. Moreover, it was surprising to see the lowest daily biogas production with 4 kg of iron oxide pellets. The reason why biogas quantity was higher for food waste used as a substrate because it contained more lipid. Lipid has more carbon atoms than protein, thus producing more biogas (Morales-Polo et al., 2018).

### Methane Content

CH<sub>4</sub> content contained in biogas was compared among the two substrates with air injection at different levels (Fig. 3a). It was found that there were significant differences among the treatments and CH<sub>4</sub> content was higher for pig manure, when compared with food waste. CH<sub>4</sub> fluctuated for pig manure at different levels of inject O<sub>2</sub>; however, the average value ranged from 57 to 61%. Meanwhile, CH<sub>4</sub> content for food waste increased, as the levels of O<sub>2</sub> increased. The highest value was only around 56%. Similar results were found for food waste when iron oxide pellets were applied at different levels (Fig. 3b). CH<sub>4</sub> increased, as the amounts of pellets increased for both food waste and pig manure. Still, the average value was 60% for pig waste and 56% for food waste.

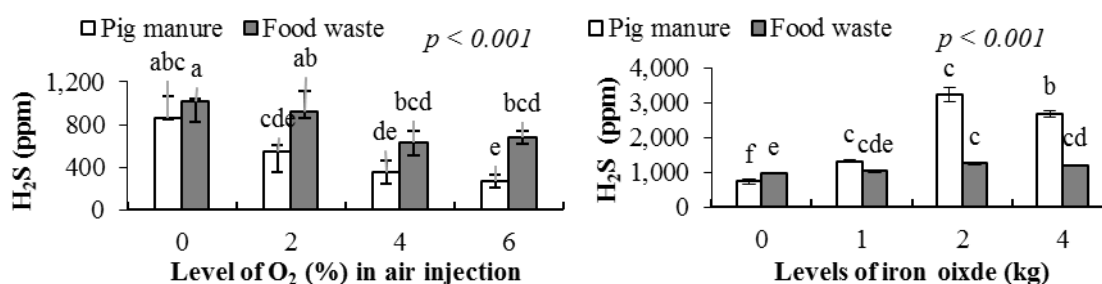


**Fig. 3 Comparison of CH<sub>4</sub> content with air injection (a) and with iron oxide pellet (b)**

### Hydrogen Sulfide Content

H<sub>2</sub>S content was compared between the two substrates under different levels of injected O<sub>2</sub> (Fig. 4a). Significant differences in H<sub>2</sub>S was observed among the treatment, and the higher level of O<sub>2</sub> was injected, the more H<sub>2</sub>S was reduced. Greater H<sub>2</sub>S reduction was observed with the use of pig manure. Without air injection, the level of H<sub>2</sub>S was 840 ppm and 1,000 ppm for pig manure and food waste, respectively. this level decreased sharply for pig manure, as levels of injected O<sub>2</sub> increased. H<sub>2</sub>S

content was 522 ppm at O<sub>2</sub> level of 2%, and decreased to 351 ppm at 4% and then to 271 ppm at 6%. For food waste, H<sub>2</sub>S content decreased from 923 ppm at O<sub>2</sub> level of 2% to 626 ppm at 4%, and then slightly increased to 674 ppm at 6%. Higher levels of injected O<sub>2</sub> reduced H<sub>2</sub>S, but also affected overall biogas production (Fig. 2a). Thus, it should be applied at recommended levels only. Nevertheless, different levels of pellets did not affect H<sub>2</sub>S content, but different substrates did (Fig. 4b). It was observed that H<sub>2</sub>S content for pig manure was higher than for food waste, and varied greatly from about 800 to over 3,000 ppm, though treated with high levels of iron oxide pellets. Such unusual increase was due to using pig manure from a different, as the first one emptied the barns. However, a tendency for H<sub>2</sub>S removal was observed at 4 kg of pellets, as H<sub>2</sub>S decreased to above 2,500 ppm. For food waste, iron oxide did not affect H<sub>2</sub>S content, and its average ranged from 1,000 to 1,100 ppm.



**Fig. 4 Comparison of H<sub>2</sub>S reduction by air injection (a) and by iron oxide pellets (b)**

## CONCLUSION

Pig manure and food waste were fed into two floating drum digesters for biogas production and inspection of biogas quality, along with H<sub>2</sub>S removal techniques by air injection and ferrous oxide pellets. It was observed that food waste produced more biogas than pig manure, but less CH<sub>4</sub> content. H<sub>2</sub>S contents were similar for both substrates, and the use of air injection tends to be more effective than ferrous oxide in this study.

## ACKNOWLEDGEMENTS

The study was made possible thanks to the project “Reduction of Greenhouse Gas Emission through Promotion of Commercial Biogas Plant in Cambodia” implemented by United Nations Industrial Development Organization (UNIDO) that not only provided funding, but also continuously assisted in strengthening the research team.

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## Changing Spent Mushroom Substrate into a Quality Vermicompost

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Received 3 March 2021    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Safety food and healthy food gain widespread popularity nowadays. In Thailand, mushroom consumption is increasing because of its high protein content and role as an effective stimulant of various immune system functions. As mushroom farmers' respond to this demand, the need for sustainable waste management of mushroom cultivation has become important. Therefore, the study examined the growth and reproduction of earthworm and chemical properties of *Eudrilus eugeniae* vermicompost cultured under different ratio of spent mushroom substrate. The study found that application of bedding with spent mushroom substrate at the ratio 60:40 gave the highest growth rate of earthworms (2.5%) and had the highest increase in nutrient content (Total N, P, K, Mg, Ca) and had the highest decrease in the value of pH, electrical conductivity, organic carbon and C/N ratio significantly. Based on these results, application of bedding with spent mushroom substrate at the ratio of 60:40 seemed to be the most suitable for vermicompost production. In general, recycling of spent mushroom substrate through vermicomposting may reduce the environmental stress and can produce organic fertilizers with better chemical and biological properties.

**Keywords** spent mushroom substrate, mushroom, earthworm, vermicompost, vermicompost quality

## INTRODUCTION

Safety food and healthy food gain widespread popularity nowadays. The effect to mushrooms consumption is increasing because of mushroom are a healthy food. The world production of *Pleurotus ostreatus* was about 4.1 million tons in the year 2019 (FAO, 2020). In Thailand has the potential to produce mushrooms in the industrial sector. The export data of mushroom products in 2011 showed that the total export volume was 939 tons, increasing from 2008 with 781 tons of export volume. The total mushroom production is 120,000 tons per year (Chanyuth, 2018).

The production 1 kg of mushrooms generates about 5 kg of spent mushroom substrate (Hrebeckova et al., 2020). The spent mushroom substrate (SMS) is a by-product generated from mushroom production, which contains a large amount of fungal mycelium and extra-cellular lignocellulosic enzymes along with various organic substances (carbohydrates, proteins, and fats), as well as a considerable quantity of inorganic nutrients such as ammonium nitrate, superphosphate, and potassium salts, (Lou et al., 2017; Gong et al., 2019). In the year 2017 there was about 20.5 million tons of SMS generated from *Pleurotus ostreatus* cultivation around the world. (Huan-Na et al., 2017; Wang et al., 2020). In Thailand found about 1.1 million tons of SMS in the year 2020. These SMS are

commonly sent to landfill or openly burnt at farms and could not be reused in the next cultivation due to the possibility of contamination which will consequently affect the mushroom production. As mushroom farmers' respond to this demand, the need for sustainable waste management of mushroom cultivation has become important.

Vermicomposting is a sustainable technique to convert organic wastes into a nutrient-rich humus-like product, important substances that are beneficial to plants such as plant growth promoters. through the joint action of earthworms and microorganisms under aerobic conditions (Edwards and Burrows, 1988). In addition, it can be easily done with low investment and high benefits. Can do both at the family and farm system. Therefore, this method is interesting for reduce SMS or organic waste and leading to produce organic fertilizers.

## OBJECTIVE

This research aims to study the growth and reproduction of earthworm and chemical properties of *Eudrilus eugeniae* vermicompost cultured under different ratio of spent mushroom substrate.

## METHODOLOGY

### Materials for Vermicomposting

The SMS was obtained from the Sufficiency Mushroom Learning Center, Tha Phra, Mueang, Khon Kaen, Thailand. Its is waste from the cultivation of *Pleurotus ostreatus*. The SMS containing sawdust (80-90%), rice bran (5-10%), rice broken (1%), lime (2%), gypsum (0.5%), sodium sulfate (0.2%) and pumice (2%), at 65-70% humidity. All samples are dried in the shade for 7 days to feed earthworms and bedding was obtained from faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand including soil, cow dung, rice husk ash and vegetable waste at ratio 4: 3: 2: 1 dry weight respectively (Iwai et al., 2011).

### Treatments and Experimental Design

In this study, the earthworm *Eisenia eugeniae* (density about 20 pcs/kg) was selected for vermicomposting because it tolerates a wide range of pH, temperature and moisture content. Plastic buckets of the diameter size 50 cm × height 18 cm were used to accommodate 4 kg of feed materials. Each container had a 0.2 m<sup>2</sup> of exposed surface covered with mosquito net to prevent hindrance from small insects and other microorganisms which could hamper the findings of this study. Punch holes in the bottom of the tank for good drainage and air. The materials used cultivating earthworms were cultured under bedding (B) with SMS in 6 different ratios were 100: 0, 80:20, 60:40, 50:50, 40:60 and 20:80 respectively. The study planned a Completely Randomized Design (CRD) there were 6 treatments and 3 replications with 45 days period.

### Measurements

The number of earthworms were monitored at the beginning and the end of process following the methods of Gong et al. (2019). The electrical conductivity (EC) and pH were determined in an aqueous solution (sample: water, 1:10) using a conductivity meter and a pH meter (Unuofin and Mnkeni, 2014). The wet oxidation method of Walkley (1934) was used for determination of organic carbon (OC). Total nitrogen was analyzed following a modified semi-micro Kjeldahl method. For the determination of total phosphorus, potassium, magnesium and calcium 0.2 g of dry sample was digested with 70% (v/v) sulfuric acid and 65% (v/v) hydrogen peroxide. Total phosphorus was measured by

Spectrophotometric molybdovanadophosphate method. Total potassium was determined using a flame photometer. Total Calcium and Magnesium measured with atomic absorption spectrophotometer. (AOAC, 2000). C/N ratio values were calculated and analysed.

### Statistical Method Analysis

The reported results are the mean of three replicates with standard error (SE). Chemical properties were analyzed by repeated measures analysis of variance (ANOVA). Mean values were compared by Tukey's HSD method. Differences were considered significant, only when P values were lower than 0.05.

## RESULTS AND DISCUSSION

### Earthworm Growth and Reproduction

During the 45 day of vermicomposting, The number of juvenile earthworm significantly increased in all treatments ( $p \leq 0.05$ ) and adult earthworm increased in B+SMS 50:50 and B+SMS 60:40 (Table 1).

**Table 1 The growth and reproduction of earthworm at the beginning and the end of the vermicomposting process by using *Eudrilus eugeniae* in different ratio for 45 days**

Parameter	The number of adult earthworm			Juvenile earthworm
	Initial	Final	Percent change (%)	
B + SMS 100:0	80 $\pm$ 0.00	77 $\pm$ 1.11	-3.75 $\pm$ 1.38	4 $\pm$ 1.56b
B + SMS 80:20	80 $\pm$ 0.00	77 $\pm$ 2.00	-3.75 $\pm$ 0.63	49 $\pm$ 11.78ab
B + SMS 60:40	80 $\pm$ 0.00	82 $\pm$ 0.89	2.50 $\pm$ 1.11	101 $\pm$ 7.00a
B + SMS 50:50	80 $\pm$ 0.00	83 $\pm$ 1.56	3.75 $\pm$ 0.63	49 $\pm$ 14.00ab
B + SMS 40:60	80 $\pm$ 0.00	76 $\pm$ 4.22	-5.00 $\pm$ 1.88	69 $\pm$ 11.78ab
B + SMS 20:80	80 $\pm$ 0.00	80 $\pm$ 0.00	0.00 $\pm$ 0.00	62 $\pm$ 2.44ab
F -test	ns	ns	ns	*

ns = non significantly different \*, \*\* = significantly different at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively. Similar letter within the same column indicates no significant difference by Tukey's HSD test. The numerical value of  $\pm$  shows standard error. B = Bedding (soil: cow dung : rice husk ash: vegetable waste; 4:3:2:1 ), SMS = spent mushroom substrate.

These changes in amount of earthworm may have reflected the greater availability of food at the start of the incubation and the reduction of food over time. The substrate ratios added significantly altered *Eisenia eugeniae* number during the vermicomposting. The maximum individual number of earthworm and maximum individual growth rate significantly higher in all bedding with SMS addition treatments than in the control. Among all substrates tested, the growth rate of earthworm had the highest increase in the B+SMS 60:40 and B+SMS 50:50. However, low quality organic waste may cause earthworms to lose weight and increase mortality during vermicomposting. (Gong et al., 2019).

### Physiochemical Change during Vermicomposting Process

The pH, EC, organic carbon and C/N ratio value at the end of the process significantly decreased in all treatments ( $p \leq 0.05$ ). (Table 2) The decreasing of pH in the early stage can be attributed to the degradation of the organic compounds, which leads to the generation of ammonia, and the subsequent decrease in pH might be due to the production of intermediate organic acids (Hanc and Chadimova, 2014). The B+SMS 60:40 treatment had the highest decrease in the value of EC was 1.67 dS m<sup>-1</sup>

(43.85%). The decreasing of EC during vermicomposting confirms by Lou et al. (2017) which spent mushroom compost for 12 weeks using earthworms *Eisenia andrei* and *Eisenia fetida*. It had (8580  $\mu\text{S cm}^{-1}$ ) decreased about 40% when compared to week zero (14,650  $\mu\text{S cm}^{-1}$ ).

**Table 2 Chemical characterization of materials at the beginning and at the end of the vermicomposting process using *Eisenia eugeniae***

Parameter		B + SMS 100:0	B + SMS 80:20	B + SMS 60:40	B + SMS 50:50	B + SMS 40:60	B + SMS 20:80	F- test
pH	Initial	7.69 $\pm$ 0.11b	8.06 $\pm$ 0.07a	7.80 $\pm$ 0.08ab	7.53 $\pm$ 0.10bc	7.51 $\pm$ 0.01bc	7.36 $\pm$ 0.05c	**
	Final	7.64 $\pm$ 0.02a	7.56 $\pm$ 0.03ab	7.35 $\pm$ 0.06ab	7.30 $\pm$ 0.18ab	7.30 $\pm$ 0.04ab	7.24 $\pm$ 0.11b	*
	Change (%)	- 0.65 $\pm$ 1.22b	- 6.20 $\pm$ 0.79a	- 5.77 $\pm$ 0.56a	-3.05 $\pm$ 1.20ab	-2.80 $\pm$ 0.70ab	-1.63 $\pm$ 1.00b	**
EC (dS m <sup>-1</sup> )	Initial	2.55 $\pm$ 0.05b	2.48 $\pm$ 0.11b	2.96 $\pm$ 0.16ab	2.57 $\pm$ 0.11b	3.14 $\pm$ 0.17a	3.09 $\pm$ 0.13a	**
	Final	2.27 $\pm$ 0.15ab	1.97 $\pm$ 0.13ab	1.67 $\pm$ 0.09b	1.57 $\pm$ 0.23b	1.83 $\pm$ 0.08b	2.84 $\pm$ 0.52a	**
	Change (%)	-11.03 $\pm$ 1.13c	-20.30 $\pm$ 3.54bc	-43.85 $\pm$ 3.93a	-39.17 $\pm$ 6.36ab	-41.59 $\pm$ 3.43a	-23.69 $\pm$ 1.13bc	**
Organic carbon (%)	Initial	3.41 $\pm$ 0.19d	5.12 $\pm$ 0.04d	11.74 $\pm$ 1.24b	8.46 $\pm$ 0.64c	17.28 $\pm$ 0.18a	18.27 $\pm$ 1.29a	**
	Final	2.69 $\pm$ 0.33d	3.49 $\pm$ 0.27d	4.62 $\pm$ 0.16c	5.85 $\pm$ 0.16b	6.95 $\pm$ 0.22b	18.16 $\pm$ 0.52a	**
	Change (%)	-20.63 $\pm$ 3.71b	-31.76 $\pm$ 5.75b	-60.14 $\pm$ 3.07a	-30.31 $\pm$ 6.25b	-59.77 $\pm$ 1.37a	- 0.24 $\pm$ 4.76c	**
Total nitrogen (g kg <sup>-1</sup> )	Initial	1.17 $\pm$ 0.02	1.28 $\pm$ 0.02	1.17 $\pm$ 0.02	1.40 $\pm$ 0.00	1.52 $\pm$ 0.02	1.41 $\pm$ 0.02	ns
	Final	1.87 $\pm$ 0.02b	2.45 $\pm$ 0.02ab	3.03 $\pm$ 0.02a	2.10 $\pm$ 0.02b	2.33 $\pm$ 0.02ab	1.75 $\pm$ 0.00b	**
	Change (%)	61.11 $\pm$ 7.41ab	97.22 $\pm$ 12.50ab	166.67 $\pm$ 2.38a	50.00 $\pm$ 12.50b	55.00 $\pm$ 13.33b	30.56 $\pm$ 12.50c	**
Total phosphorus (g kg <sup>-1</sup> )	Initial	0.36 $\pm$ 0.00a	0.34 $\pm$ 0.00ab	0.30 $\pm$ 0.00c	0.30 $\pm$ 0.00c	0.32 $\pm$ 0.00bc	0.25 $\pm$ 0.00d	**
	Final	0.79 $\pm$ 0.00a	0.64 $\pm$ 0.00b	0.64 $\pm$ 0.00b	0.69 $\pm$ 0.00b	0.65 $\pm$ 0.00b	0.54 $\pm$ 0.00c	**
	Change (%)	119.63 $\pm$ 5.63ab	83.56 $\pm$ 3.74c	113.63 $\pm$ 3.05ab	124.85 $\pm$ 6.79a	100.09 $\pm$ 7.78bc	113.09 $\pm$ 3.12ab	**
Total potassium (g kg <sup>-1</sup> )	Initial	2.19 $\pm$ 0.01ab	2.10 $\pm$ 0.01ab	1.83 $\pm$ 0.01b	2.05 $\pm$ 0.01ab	2.28 $\pm$ 0.01a	2.02 $\pm$ 0.01ab	ns
	Final	2.68 $\pm$ 0.01	2.97 $\pm$ 0.02	2.69 $\pm$ 0.02	2.46 $\pm$ 0.01	2.89 $\pm$ 0.01	2.44 $\pm$ 0.01	ns
	Change (%)	22.81 $\pm$ 8.81	42.11 $\pm$ 12.74	48.70 $\pm$ 12.58	20.35 $\pm$ 2.74	27.10 $\pm$ 3.04	20.78 $\pm$ 0.57	ns
Magnesium (g kg <sup>-1</sup> )	Initial	1.02 $\pm$ 0.00d	1.35 $\pm$ 0.01cd	1.52 $\pm$ 0.01cd	1.65 $\pm$ 0.02bc	2.18 $\pm$ 0.02b	3.26 $\pm$ 0.02a	**
	Final	1.21 $\pm$ 0.01d	1.49 $\pm$ 0.00cd	1.67 $\pm$ 0.02cd	1.97 $\pm$ 0.02c	2.52 $\pm$ 0.01b	3.68 $\pm$ 0.02a	**
	Change (%)	18.96 $\pm$ 0.15	11.07 $\pm$ 2.26	10.26 $\pm$ 3.85	21.07 $\pm$ 1.79	16.54 $\pm$ 5.74	13.84 $\pm$ 1.03	ns
Calcium (g kg <sup>-1</sup> )	Initial	0.80 $\pm$ 0.01d	1.00 $\pm$ 0.00cd	1.06 $\pm$ 0.01cd	1.28 $\pm$ 0.00c	1.79 $\pm$ 0.01b	2.80 $\pm$ 0.02a	**
	Final	0.88 $\pm$ 0.01d	1.09 $\pm$ 0.00cd	1.41 $\pm$ 0.01bcd	1.53 $\pm$ 0.01bc	1.91 $\pm$ 0.01b	3.96 $\pm$ 0.02a	**
	Change (%)	9.51 $\pm$ 4.69c	9.30 $\pm$ 3.13c	34.23 $\pm$ 4.38ab	19.40 $\pm$ 3.55b	7.05 $\pm$ 3.05c	42.40 $\pm$ 3.36a	*
C/N ratio	Initial	29.92 $\pm$ 4.75c	40.61 $\pm$ 5.23c	102.45 $\pm$ 5.04ab	60.45 $\pm$ 4.59bc	115.04 $\pm$ 9.83a	135.68 $\pm$ 9.07a	**
	Final	14.63 $\pm$ 2.41c	14.32 $\pm$ 0.76c	15.46 $\pm$ 1.95c	28.49 $\pm$ 4.19b	30.02 $\pm$ 3.11b	103.77 $\pm$ 2.94a	**
	Change (%)	-50.01 $\pm$ 5.83b	-63.86 $\pm$ 5.95ab	-84.50 $\pm$ 2.96a	-52.60 $\pm$ 8.07b	-73.80 $\pm$ 2.54ab	- 20.00 $\pm$ 6.47c	**

ns = non significantly different \*, \*\* = significantly different at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively. Similar letter within the same column indicates no significant difference by Tukey's HSD test. The numerical value of  $\pm$  shows standard error. B = Bedding (soil: cow dung : rice husk ash: vegetable waste; 4:3:2:1 ), SMS = spent mushroom substrate. (-) = The percent change of nutrient decreased.

The SMS is comprised of easily degradable carbon substrates which tend to benefit the microbial growth, resulting in a greater organic matter decomposition rate. From the result, had the highest decrease in the percent change of organic carbon (60.14%) and C/N ratio (84.50%) at the ratio 60: 40 of bedding with SMS. The organic carbon loss is likely due to the mineralization of the organic matter



through time (Gong et al., 2019). Elvira et al. (1998) reported that during composting there was a loss of 20-43% of organic carbon in the form of carbon dioxide. Due to microbial respiration and from the ingestion of organic matter. The longer duration either for vermicomposting or composting indirectly enhanced the quality of final end product as a result of the loss of carbon and increased nitrogen content (Kaviraj and Sharma, 2003).

The nitrogen, phosphorus, magnesium and calcium content at the end of the process significantly increased in all treatments ( $p \leq 0.05$ ). (Table 2) While, the percent change of potassium had the highest increased at the ratio 60:40 of bedding with SMS (48.70%). But The potassium content non significantly increased at the end of the process due to microorganisms present in vermicompost utilize bound nutrients in vermicompost for their survival. This results in increased microbial activity, which leads to quicker immobilization of nutrients, causing decreases potassium content in vermicomposting. The percent change of nitrogen had the highest increased at the ratio 60:40 of bedding with SMS (166.67%) due to the high growth rate of earthworm. Causing bacteria to play a role in the mineralization process. Moreover, the excretion of mucus, fluids, enzymes and dead tissue during digestion processes containing nitrogen effect the total nitrogen content increased (Suthar, 2007; Lou et al., 2017). Application of bedding with SMS at the ratio 50:50 gave the highest percent change of phosphorus (124.85%) and magnesium (21.07%) because of earthworm like a media to increased phosphatase enzyme content, which increased content by waste from the gut becomes earthworm effect to increased phosphorus content in vermicompost (Gong et al., 2019). The calcium content had the highest increase in vermicompost from bedding and SMS at the ratio 20:80 was  $3.96 \text{ g kg}^{-1}$ . Increasing SMS rate will be higher of calcium content. This is because in the process of mushroom production are added lime, gypsum, salt and pumite to be used as food and adjusted the acidity and alkalinity for good mushroom growth. This results cause a large amount of calcium in waste from mushroom cultivation.

## CONCLUSION

Waste from mushroom cultivation could improve vermicompost quality. It is a biological method that relies on earthworms farming to convert organic matter into high quality organic fertilizer. It was found that the spent mushroom substrate contained high protein and carbon content as a food source for earthworms. It can increase the amount of nutrients and microorganisms that are beneficial to plants. Application of bedding with spent mushroom substrate at the ratio of 60:40 seemed to be the most suitable for vermicompost production in term of plant nutrition and earthworm production. In general, recycling of spent mushroom substrate through vermicomposting may reduce the environmental stress and can produce organic fertilizers with better chemical and biological properties.

## ACKNOWLEDGEMENTS

We would like to acknowledge the scholarship from Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2019, Integrated Land and Water Resource Management Research and Development Center in Northeast Thailand and Japanese Chamber of Commerce, Bangkok Scholarship 2019 and Department of Soil Science and Environment, Faculty of Agriculture, Khon Kaen university, Khon Kaen, Thailand for facility supported this research.

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## Study on Use of Plant Based Material as a Fertilizer

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Received 23 November 2020    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Soil is the mixture of mineral, organic material, living organisms, air and water that together support the growth of plant life. Fruit peels contain potassium, vitamins, minerals and some essential elements which enhance the growth of plant. Generally, fruit peels are thrown in garbage and it goes to solid waste dumping site. That causes odour problem due to degradation of peel content in dumping site. The present study deals with the utilization of different fruit peels such as orange peels and banana peels as fertilizer added to soil. The soil sample was collected from Shar-Taw-Lay village, Amarapura Township, Mandalay, Myanmar. Three soil samples were investigated by addition of organic waste fruit peel powder of orange and banana. Soil sample, S<sub>1</sub> (2000 g soil) as control, soil sample, S<sub>2</sub> (2000 g soil + 500 g orange peel powder) and soil sample, S<sub>3</sub> (2000 g soil + 500 g banana peel powder) were fertilized soil. The physicochemical properties of three soil samples such as pH, electrical conductivity, organic matter and texture were determined. The elemental composition of three soil samples was measured by applying EDXRF. The content of N, P, K nutrients of three soil samples was determined by chemical instrumental method. Different fruit peel powders add soils to compare the plant growth were investigated. The chemical fertilizers can be replaced by the fruit peel powder to protect the soil from the infertility. This fruit peel materials have not cost bearing and thus aids in converting this waste into a usable resource.

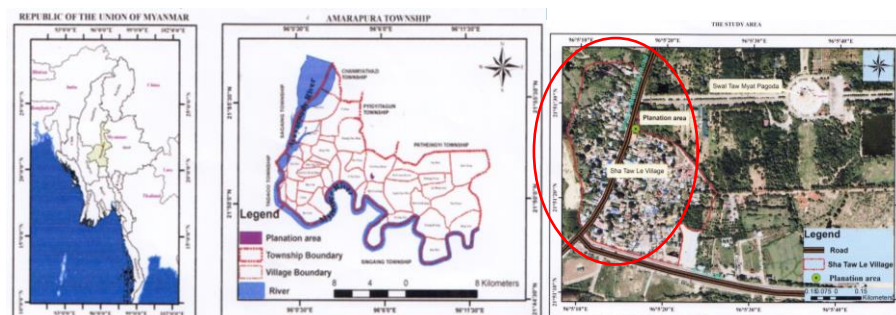
**Keywords** fertilizer, fruit peel powder, nutrient, soil

## INTRODUCTION

Soil is important because it provides the nutrients that plant need to grow. Without soil, there would be no plants, without plants, there would be no foods, without food, animals could not survive. Soil is relating with fertilizer. Soil is needed the fertilizer that is one of the sources for plant growth. There are two types of fertilizer used in agriculture, organic fertilizer and inorganic fertilizers. Organic fertilizers are fertilizers derived from animal or vegetable matter (eg. compost, manure). Inorganic fertilizers are produced or artificially in a chemical refinery. Fertilizers typically provide in varying proportions. Good fertility is fundamental to success full plant growth, and the application of fertilizers and manures is an essential graining act activity. The maintenance of adequate levels of nutrients in soil is essential for healthy plant growth; cheapest and harmless materials can be used for the plant growth. Fruits contain a high amount of antioxidants that are beneficial to our health in many ways. The fruit peels have nutrients like potash, iron, zinc, calcium, citrate content etc. In addition, among the many fruit sample, orange and banana are traditional food, main consumed for Myanmar people and well cultivated for everywhere in Myanmar. Therefore, fruit sample orange and banana were chosen in this research.

## OBJECTIVE

The present work was to investigate how the changes in physical and chemical properties by adding banana peel powder and orange peel powder to the soil from Shar-Taw Lay village, Amarapura Township, Mandalay, Myanmar. Discard substance of orange peel powder and banana peel powder into usable product as fertilizer that cheapest and harmless material can be used for the plant growth.



**Fig. 1 Map of study area**

## METHODOLOGY

The soil samples were collected from Shar-Taw Lay village, Amarapura Township, Mandalay, Myanmar. These soil was chosen for this study because these soil was supplied by natural fertilizer such as cowdung, dry peat leaves before harvested. These selected soil sown so many crops and paddy. The soil samples were dug the depth 20 cm from the earth surface in the study area and were put in thick plastic bags. The soil normally contain clay, pebbles, root, rock pieces, these soil is sieved by using 2 mm sieve to remove the pebbles, root, rock pieces. The soil samples were air-dried and were crushed into fine powder by using grinder. This dried soil was packed in sterile polythene bags and sent to soil quality center, Department of Agricultural Research, Yezin, Nay-Pyi Taw, Myanmar.

### Collection and processing of fruit peel:

Generally fruit peels are collected from fruit juice vendors or thrown in garbage by peel household. Discard fruit peels (orange peel and banana) are cut into small pieces and dried in air. After drying of these peels into powder by using blander.



**Fig. 2 Different fruit peel and fruit peel powder**

### Preparation of fruit peel powder in soil:

Orange peel powder (500 g) and soil (2000 g) mixed with 300 mL of distilled water as soil sample, S<sub>2</sub> were added to the pot. The amount of water to add was calculated by field capacity method. The fertilized materials were weighed every 2 weeks and the loss of weight was re-filled with water. Banana peel powders were prepared for the above as soil sample S<sub>3</sub> and without peel powder as soil sample S<sub>1</sub>. After composting 45 days, these two soil samples sent to soil quality center, Department of Agricultural Research, Yezin, Nay-Pyi-Taw, Myanmar.



Control soil sample  $S_1$   
(2500 g)



Soil sample-  $S_2$   
Soil 2000 g + 500 g orange peel powder



Soil sample- $S_3$   
Soil 2000 g + 500 g banana peel powder

- (1) pH, 1 : 2.5 soil: Water suspension method, pH meter (F-51 HORIBA)



- (2) Electrical conductivity (EC), Saturation extract method, conductivity meter (DS-51 HORIBA)



- (3) Organic matter, Tyurin's method, Analytical balance



- (4) Soil texture, pipette method, Analytical balance



- (5) Moisture, Gravimetric method, Temperature controlled oven



- (6) Total N, Kjaldehl distillation method, Gerhardt Vapodest20s



- (7) Total P, Molybivanado phosphoric acid method, UV-visible Spectrophotometer Jenway 6305



- (8) Total K, Wet digestion with  $\text{HNO}_3$  :  $\text{HClO}_4$  (4 :1), Atomic Absorption Spectrophotometer, NOV AA-400



- (9) Elemental percent composition, powder-pellet, EDXRF



**Fig. 3 Control soil and fruit peel powder in soil analytical item, analytical method, apparatus used**

In this study, the pH, electrical conductivity (EC), organic matter, texture, moisture, total N, P, K and Elemental percent composition of control soil sample S<sub>1</sub>, orange peel powder soil sample S<sub>2</sub>, banana peel powder soil sample S<sub>3</sub> were measured at Department of Agricultural Research, Yezin, Nay-pyi-daw, Myanmar.

The pH of three soil samples were measured by water suspension method using pH meter. Electrical conductivity (EC) was also measured by saturation extract method using conductivity meter. Organic matter of soil samples S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> were determined Tyurin's method using Analytical balance and also measured soil texture by pipette method. Moisture for three soil samples were known by Gravimetric method using temperature controlled oven. Three soil samples S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> were measured by Kjaldehl distillation method for Total N, Molybivanado phosphoric acid method for Total P and wet digestion with HNO<sub>3</sub> : HClO<sub>4</sub> (4 : 1) for Total K by respective apparatus and also determined elemental percent composition by EDXRF.

#### Pot experiment of chili plants and pea plants:

Effects of prepared fertilized soil sample with chili and pea plants were investigated by pot experiment.

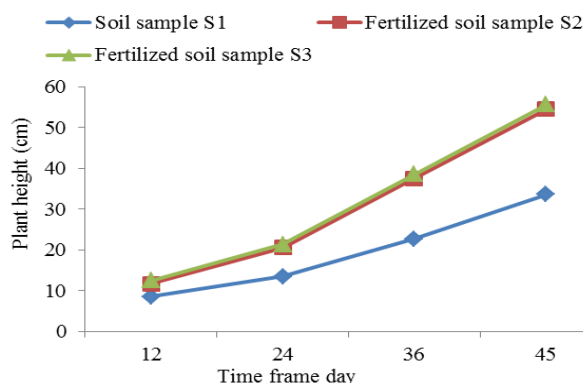
#### Pot experiment of chili plants:

Duration from time of emergence of chili plant to the end of time period was 45 days work. According to result, increase the time period with increasing of plant growth of fertilized soil sample S<sub>2</sub> and S<sub>3</sub>.

## RESULTS AND DISCUSSION

**Table 1 Result of growth length of chili plant on control soil and prepared fertilized soil**

Time frame day	Plant height (cm)		
	Soil sample S <sub>1</sub>	Fertilized soil sample S <sub>2</sub>	Fertilized soil sample S <sub>3</sub>
12	8.6	11.7	12.5
24	13.5	20.6	21.4
36	22.7	37.5	38.5
45	33.6	54.5	55.6



**Fig. 4 The plot of growth length of chili plant cultivated on prepared fertilized soil sample versus time frame day**

**Table 2 Physicochemical properties of soil sample S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>**

No	Soil sample	pH	Organic matter (%)	EC (dS/m)	Texture	Moisture
1	Control soil sample, S <sub>1</sub>	8.1	2.0 %	0.18	sandy loam	1.4 %
2	Soil + Orange peel powder soil sample, S <sub>2</sub>	7.2	11.6 %	0.39	sandy loam	2.4 %
3	Soil + Banana peel powder soil sample, S <sub>3</sub>	7.8	13.2 %	0.38	sandy loam	2.8 %

From the above Table, the pH of control soil sample was slightly higher than soil sample S<sub>2</sub> and S<sub>3</sub>. Soil organic matter decline in many agroecosystems occur because losses of carbon through oxidation and erosion by intensive cropping are not compensated by carbon inputs through the return of plant biomass. Organic matter reduction is in turn, association with the soil structure degradation. Organic matter of soil sample S<sub>2</sub> and S<sub>3</sub> were higher than control soil sample S<sub>1</sub>. Neither strong acidic nor strong basic of three soil samples were found. Organic matter plays a major role in moisture retention, helping crops withstand drought contributes to the chemical and biological properties of the soil and also a source of and exchange for nutrients. The soil sample consists of 61% of sand, 26% of silt and 13% of clay. The texture class of soil sample was sandy loam. Electrical conductivity (EC) is a measure of the salt concentration in the soil solution. Increasing electrical conductivity of control soil sample, S<sub>1</sub> than soil sample S<sub>2</sub> and S<sub>3</sub>. The moisture of control soil sample S<sub>1</sub> (1.4%), soil sample S<sub>2</sub> (2.4%), soil sample S<sub>3</sub> (2.8%) were observed.

**Table 3 pH of orange peel powder and banana peel powder**

No	Fruit peel powder	pH	N	P	K
1	Orange peel powder	5.6	7.2	2.3	1.2
2	Banana peel powder	7.7	4.6	3.7	2.8

By the experiment, it can quantify how much amount of different peel powder required for the particular soil. The orange peel powder was used to decrease the pH of the soil and the banana peel powder was used to increases the pH of the soil.

Citrate peel powder like, orange peel powder, reduces salinity of soil and alkaline peel powder like, banana peel powder reducing acidity content present in soil. According to result, orange peel powder has higher nitrogen content (7.2 mg/g), whereas banana peel powder has higher quantity of phosphorous (3.7 mg/g) and potassium (2.8 mg/g).

**Table 4 Major nutritive value of soil sample S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>**

No	Soil sample	Total N	Total P	Total K
1	Control soil sample, S <sub>1</sub>	0.09%	0.11%	0.31%
2	Soil + Orange peel powder soil sample, S <sub>2</sub>	0.18%	0.40%	1.01%
3	Soil + Banana peel powder soil sample, S <sub>3</sub>	0.20%	0.35%	1.03%

The major nutritive value of prepared fertilized soil sample S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> were determined. The primary nutrient (N, P, K) are mainly necessary for plants. The total nitrogen value of three soil samples ranged from 0.09%, 0.18% to 0.21%. It was found that nitrogen content of three soil samples were increased and that causes soil fertility.

The total phosphorus values ranged 0.11%, 0.40%, 0.35%. Phosphorus concentration may increases as composting. Total potassium content ranged 0.31%, 1.01%, 1.03%. According to this



results, potassium content of prepared fertilized soil sample  $S_2$  and  $S_3$  are higher. Therefore it is suitable for crops and vegetable growing. By comparing these results, we can conclude that increased N, P, K value with increasing fertility of soil, soil microorganism, plant growth and maximum nutrient content.

The element composition of prepared fertilized soil sample were determined by EDXRF at Department of Agricultural Research, Yezin, Nay Pyi Taw, Myanmar. According to table, all elemental composition of soil sample  $S_2$  and soil sample  $S_3$  are higher than control soil sample  $S_1$ . In this elemental composition,  $S_2$  and  $S_3$  fertilized soil sample contained highest amount of Si, Al, Fe and high amount of Ca, K. And then, meidum for Ti, Mn and low mineral levels are observed in Zn, Pb, Cr. The high elemental content of  $S_2$ , and  $S_3$  fertilized soil sample show increasing pest and pathoger resistance, drought and heavy metal tolerance, and stability and yield of agricultural corps.

**Table 5 Determination of elemental composition of soil sample  $S_1$ ,  $S_2$  and  $S_3$**

Soil sample	Si	Al	Fe	Ca	K	Ti	Mn	Zn	Pb	Cr
Control soil sample $S_1$	30.726%	5.528%	2.059%	2.359%	1.243%	0.456%	0.084%	0.028%	0.018%	0.006%
Orange soil sample, $S_2$	47.100%	15.200%	18.300%	6.470%	8.630%	1.450%	0.217%	0.153%	0.186%	0.0889%
Banana soil sample, $S_3$	46.500%	15.000%	17.000%	7.190%	8.730%	1.580%	0.276%	0.148%	0.180	0.0663%

#### Application of fruit peel powder in soil:

Soil samples  $S_1$ ,  $S_2$ ,  $S_3$  were filled with each pot. Pea seeds and chili seeds were sown in various pots and water was poured every day. Control and two different peel powder was used to compare the plant growth.

After 45 days, check the growth of pea plant and chili plant compare with the plant in control soil. Length of the plant was very less in control soil than the orange peel powder applied soil. Size of the leaves was also increased in the plants which were grown on banana peel powder with soil. Length of peas and chili plant were also bigger than control.



**Fig. 5 Different in growth of chili plant**  
a) control soil and b) soil + banana peel



**Fig. 6 Different in growth of pea plant**  
a) control soil and b) soil + banana peel

## CONCLUSION

In this study, the plant base materials such as orange peel and banana peel were used as natural fertilizer. The physicochemical changes during the combination of orange peel powder or banana peel powder to soil were determined at enhancing properties of soil. The orange peel powder was used to



decrease the pH of the soil and the banana peel powder was used to increase the pH of the soil. The pH of control soil samples is slightly higher than soil sample S<sub>2</sub> and S<sub>3</sub>. It was found that, organic matter, electrical conductivity (EC), of orange peel powder soil sample S<sub>2</sub> and banana peel powder soil sample S<sub>3</sub> were higher than control soil sample S<sub>1</sub>. Furthermore, total N, P, K value of soil sample S<sub>2</sub> and S<sub>3</sub> were also found higher than control soil sample S<sub>1</sub> as well as elemental composition. So, the peel powder can be feasibly used as a soil fertilizer, improving soil morphology, as micronutrient supplement (Zn, Fe, Ca) and also for horticultural purposes. The plant growths of different fruit peel powder add soil were more than control soil. The organic waste such as orange and banana fruit peel powder were used as natural fertilizer. By using this orange peel powder and banana peel powder as fertilizer, it can be reduced to fruit peel waste. Soil application of compost from organic residues, such as animal manures, sewage sludge, household wastes, represents a management strategy that could counteract depletion of organic matter in soils. Besides, organic residues recycling and further use in soils represents an attempt to alleviate the serious environmental problems caused by residue accumulation. The use of compost in soils requires that it achieves an adequate degree of maturity, which implies a stable organic matter content and the absence of phytotoxic compounds and plant or animal pathogens. Mineral fertilization provides readily available nutrients for plant growth; however, it does not contribute to improve soil physical condition. For the enhancement of the growth of plant and vegetables in home gardens, banana peel powder and orange peel powder were applied as soil conditioner and its response was measured by using some parameters.

## **ACKNOWLEDGEMENTS**

I express my gratitude to Rector Dr. Aung Aung Min and Pro-rectors Dr. Si Si Khin and Dr. Tint Moe Thu Zar, Yadanabon University, for their permission to do this research. I also wish to express many thanks to Professor Dr. Hlaing Hlaing Myat, Head of Chemistry Department, Yadanabon University for her kind permission to carry out this work and her interest, constructive comment and suggestions and we also thank the ethical research committee of department of chemistry, Yadanabon University. I am also thanks to Dr. Ni Ni Tint, Department of Agricultural Research, Yezin, Nay Pyi Taw, Myanmar.

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## Effect of Chicken Manure and Chemical Fertilizer Applications on Growth and Yield of Rice (*Oryza sativa* L.)

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Received 30 December 2020   Accepted 30 June 2021   (\*Corresponding Author)

**Abstract** The field experiment was conducted at Yezin Agricultural University Farm, Yezin, Nay Pyi Taw, during July to November wet season, 2020, to investigate the effects of chicken manure and chemical fertilizer applications on growth, yield and yield components of rice. The experimental design was a randomized complete block (RCB) design with four replications. The four treatments were T1 (control, no application), T2 (80 N, 20 P, 32 K) kg ha<sup>-1</sup> (recommended rate), T3 (5 ton ha<sup>-1</sup> of chicken manure), and T4 (2.5 ton ha<sup>-1</sup> of chicken manure + recommended rate of fertilizer). Urea, triple superphosphate and muriate of potash were used as the N, P, and K sources and chicken manure was applied basally. The Sinthukha rice variety was tested. The plant growth parameters were recorded at biweekly intervals and the yield and yield components data were also collected at harvest time. The results show that the combined application of organic manure and recommended rate of fertilizer (T4 treatment) increased the number of panicles hill<sup>-1</sup>, number of spikelets panicle<sup>-1</sup>, filled grain percentage, and harvest index in comparison to other treatments. Moreover, the highest grain yield (6.87 ton ha<sup>-1</sup>) was observed in T4 treatment and the minimum grain yield (6.09 ton ha<sup>-1</sup>) was found in T1. Among the four treatments, T2 showed the second highest yield of rice in this study. The combined application of chicken manure and chemical fertilizer (T4) increased grain yield up to 12% over control. The application of chemical fertilizer only (T2) increased yields 7% more than the control. The application of chicken manure (T3) resulted in a 6% yield increase compared to the control. The application of chicken manure and chemical fertilizer (T4) increased yield per hectare by up to 6-12% more than the control. Therefore, the combined application of chicken manure and chemical fertilizer had the greatest effect on improving the yield of Sinthukha rice variety and maintaining soil sustainability.

**Keywords** chicken manure, chemical fertilizer, rice yield

## INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of about 3 billion people, nearly half the world's population, who depend on rice for survival. In many countries, rice accounts for more than 70% of human caloric intake and represents the main source of protein for poor people in all developing countries (Sharma, 2014). Rice cultivation is the principal activity and source of income for millions of households around the world, and several countries in Asia and Africa are highly dependent on rice as a source of foreign exchange earnings and government revenue (FAO, 2014).

Myanmar is the world's sixth-largest rice producing country (Maclean et al., 2013). Rice is grown during the monsoon and summer seasons in Myanmar's four growing zones designated as delta, dry zone, coastal zone, and mountainous areas (Linn and Maenhout, 2019). The total area sown to rice in Myanmar was 7.22 million hectare (ha) with an annual production of 28.01 million metric ton and an average yield of 3.92 MT ha<sup>-1</sup> in 2018-2019 (MOALI, 2019).

Due to the increasing cost of chemical fertilizers, depletion of soil micronutrients, environmental and health hazards, the use of organic manure in farming has attracted much attention (Ramesh et al., 2005). The adequate fertilizers in rice are fundamental to achieve great productivity. On the other hand, animal manures and green manures are little used as a source of nutrients in rice crops (Schmidt and Knoblauch, 2019). Organic manures are excellent fertilizers, containing nitrogen, phosphorus, potassium and micronutrients essential for the healthy growth of plants. Furthermore, it has numerous benefits due to the balanced supply of nutrients including micronutrients. In agricultural fields, organic manure that is manufactured from animal byproducts has been utilized to overcome environmental pollution and plant productivity reductions that result from the constant utilization of chemical fertilizers (Han et al., 2016).

Organic manure such as poultry manure increases the organic matter (OM) content of soil and in turn releases the plant nutrients in a form available for use (Magkos et al., 2003). It contains essential nutrient elements associated with high photosynthetic activity and thus promotes root and vegetable growth (John et al., 2004). Previous studies have shown that the integration of inorganic fertilizer with organic manure has been more beneficial than the use of either mineral fertilizer or organic manure alone, especially in intensive agricultural production. Therefore, the integrated use of both organic manure and chemical fertilizers can provide the best approach in providing greater stability in production and improving soil fertility status (Islam et al., 2011).

## OBJECTIVES

The present study was conducted to investigate the effect of applications of different combinations of chicken manure and chemical fertilizer on growth, yield and yield components of rice and to ascertain the most favorable application rate to improve yield of rice.

## MATERIALS AND METHODS

The experiment was conducted in the Yezin Agricultural University Farm Yezin, Nay Pyi Taw, during 2020 wet season, from July to November. Yezin is located at 19° 52' N and 96° 37' E with an altitude of 103 meters above sea level. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments and four replications. The individual plot sizes were (5 x 5) m<sup>2</sup>. Double bands provided separation between plots. Treated plots were separated about 1m from surrounding fields to prevent contamination which may have an effect on treatments, such as any mixing of

fertilizers during irrigation. The treatments were T1 (control, no fertilizer application), T2 (80 N, 20 P, 32 K) kg ha<sup>-1</sup> (recommended rate), T3 (5 ton ha<sup>-1</sup> of chicken manure), T4 (2.5 ton ha<sup>-1</sup> of chicken manure + fertilizer at the recommended rate). Urea, triple superphosphate and muriate of potash were used as N, P and K sources. Chicken manure and triple superphosphate and potash fertilizers were applied basally. Urea fertilizer was applied equally at three different stages (basal, active tillering and panicle initiation). The soil texture of the experimental field was sandy loam, had a moderately acid (pH 5.8), was low in organic matter (1.09 %), had medium levels of available nitrogen (87 ppm), was slightly low in available phosphorus (3.5 ppm), low in available potassium (104 ppm) and low in available sulphur (36 ppm).

Sinthukha rice was the variety tested. The twenty day old seedlings were transplanted and spaced at 20 cm × 20 cm. Irrigation was provided during the growing season as necessary. Weed control and other management procedures were regularly undertaken, especially at the early stages of growth. Harvesting was at 98 days after transplanting (DAT).

Plant growth parameters such as plant height and number of tillers per hill from five randomly selected sample hills in each plot were collected from 14 DAT to 98 DAT at biweekly intervals. The yield and yield components were recorded at harvesting stage and grain yield was measured from a 1 m<sup>2</sup> centrally located area in each plot.

The collected data were analyzed statistically using Analysis of Variance (ANOVA) and mean values were compared by least significant difference (LSD) at 5% probability level. All statistical analyses used statistix 8.0 software.

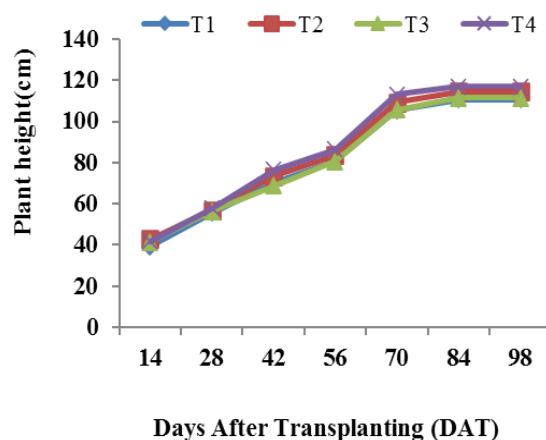
## RESULTS AND DISCUSSION

Plant height was measured starting from 14 DAT to 98 DAT at biweekly intervals (Fig.1). Plant heights were increased significantly under all treatments, from 14 DAT to 42 DAT. There was no significant difference in plant heights in all treatments at 28 DAT, 56 DAT, 70 DAT, 84 DAT and 98 DAT. The tallest plants (116.91 cm) grew subject to T4 treatment, followed by T2 treatment (114.55 cm), and T3 treatment (111.59 cm) at 98 DAT. The shortest plant was found in T1 treatment (110.53 cm). These differences may be due to the effects of the combined applications of organic manure and chemical fertilizer, releasing macronutrients and micronutrients to the plants.

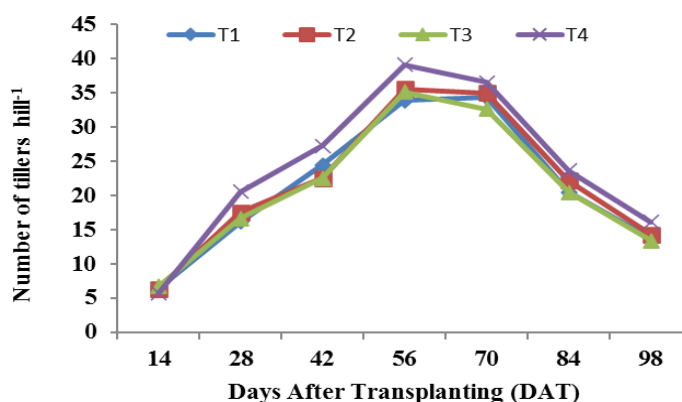
The number of tillers per hill was recorded from 14 DAT to 98 DAT at biweekly intervals (Fig. 2). The number of tillers per hill did not increase significantly with the application of chicken manure and chemical fertilizer application at the different growth stages of rice. The highest number of tillers per hill occurred in T4 (16.15) followed by T2 (14.25), T3 (13.75) and the lowest value was in T1(13.40) at 98 DAT. It was similarly reported by Nayak et al. (2007), that a significant increase in effective tillers hill<sup>-1</sup> was obtained with the applications of organic manure and chemical fertilizer.

The effect of chicken manure and chemical fertilizer application on yield and yield components are described in Table. 1. There was no significant variation in number of panicles per hill for each treatment. The number of panicles per hill ranged from 10.80 to 12.55. The maximum number of panicles per hill occurred in T4 (12.55) followed by T2 (12.50) and T3 (11.35) and the minimum number of panicles per hill was in T1 (10.80). The greatest increase in the number of panicles per hill occurred with the combined application of chicken manure and chemical fertilizer.

There was not a significant variation in panicle length among the different treatments. The panicle length ranged (ranges) from 22.78 to 23.92 cm. The highest panicle length (23.92cm) was observed in the T4 and the lowest panicle length (22.78 cm) was in T1. The increase in panicle length in response to combined application of organic and inorganic fertilizers (T4 treatment) may be due to the greater availability of macronutrients as well as micronutrients (Awan et al., 2011).



**Fig. 1 Influence of chicken manure and chemical fertilizer applications on mean values of plant height of rice**



**Fig. 2 Influence of chicken manure and chemical fertilizer applications on mean values of number of tillers hill<sup>-1</sup> of rice**

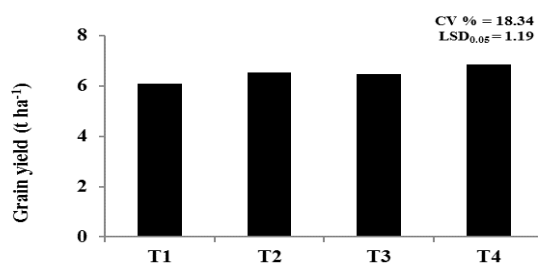
There was no significant difference in number of spikelets panicle<sup>-1</sup> among the different fertilizer treatment methods. However, the number of spikelets panicle<sup>-1</sup> ranged from 145.60 to 158.40, with the highest number of spikelets panicle<sup>-1</sup> occurring in T4 (158.40), followed by T2 (152.40), T3 (146.15), and the lowest number of spikelets panicle<sup>-1</sup> was in T1 (145.60) where there was no fertilizer applied. Increases in spikelet panicles<sup>-1</sup> may be due to the application of chicken manure and chemical fertilizer. The maximum spikelets panicle<sup>-1</sup> occurred with the addition of organic matter to the soil and this might be due to the availability of macro as well as micro plant nutrients (Siavoshi et al., 2011).

The filled grain percent ranged from 65.61% to 80.10%. The maximum filled grain percentage was shown in T4 (80.10%), followed by T2 (78.10%), T3 (76.48%) and the minimum value occurred in T1 (65.61%). The previous study reported that the combined application of manure and fertilizer significantly increased the number of filled grains panicle<sup>-1</sup> (Satyanarayana et al., 2002).

The 1000-grain weight ranged from 20.48 g to 22.49 g. The highest 1000-grain weight was in T4 (22.49 g), followed by T2 (21.88 g) and T3 (21.45 g). The lowest 1000-grain weight was in T1 (20.48 g). This result was in conformity to the findings of Yang et al. (2004) who recorded that 1000-grain weight was increased by the application of chemical fertilizer and organic manure.

The highest grain yield (6.87 ton ha<sup>-1</sup>) was in T4, followed by T2 (6.53 ton ha<sup>-1</sup>), T3 (6.49 ton ha<sup>-1</sup>), with the lowest grain yield (6.09 ton ha<sup>-1</sup>) in T1 treatment where no fertilizer was applied (Fig.3). The T4 treatment (combined application of chicken manure and chemical fertilizer) increased grain

yields by up to 12%, over those in the control. The chemical fertilizer application in the T2 treatment increased yields 7% more than in the control. The application of just chicken manure increased yield by 6% compared to the control. To sum up, the application of chicken manure and chemical fertilizer increased the grain yield 6-12% over the control, and also increased the straw yields of rice. It is clear that organic manure in combination with inorganic fertilizers increased the vegetative growth of plants and thereby increased the straw yield of rice (Rahman et al., 2009). The chemical fertilizer offers nutrients which are readily soluble in soil solution and thereby instantly available to plants. Nutrient availability from organic sources is due to microbial action and improved physical condition of soil (Sarker et al., 2004). Several authors have shown that organic amendments play a crucial role in both short-term nutrient supply and long-term build-up of soil quality in flood-irrigated rice crops (Nishikawa et al., 2014). Moreover, organic manures increased soil nutrient availability, improved soil structure and root development and increased soil water availability (Han et al., 2016).



**Fig. 3 Influence of chicken manure and chemical fertilizer applications on mean values of grain yield of rice**

**Table 1 Effect of chicken manure and chemical fertilizer applications on mean values of yield and yield components of rice**

Treatments	Number of panicles hill <sup>-1</sup>	Panicle length (cm)	Number of spikelets panicle <sup>-1</sup>	Filled grain %	1000-grain weight (g)	Harvest index
T1	10.80	22.78	145.60	65.61	20.48	0.40
T2	12.50	23.37	152.40	78.10	21.88	0.42
T3	11.35	23.22	146.15	76.48	21.45	0.41
T4	12.55	23.92	158.40	80.10	22.49	0.43
LSD <sub>0.05</sub>	3.35	1.56	23.56	18.89	2.30	0.07
Pr>F	ns	ns	ns	ns	ns	ns
CV%	17.94	4.15	9.78	15.73	6.63	10.86

ns = not significant

There was no significant variation in harvest index with different fertilizers application (Table 1). The harvest index ranged from 0.40-0.43. The maximum harvest index occurred in T4 (0.43), followed by T2 (0.42), T3 (0.41) and the minimum value was in T1 (0.40).

## CONCLUSION

The application of chicken manure and chemical fertilizer influences the growth, yield and yield components of rice. The largest differences that occur in growth parameters are in plant height, and number of tillers and these occur where chicken manure and chemical fertilizer (T4) are used.

Moreover, the T4 treatment results in the highest yield and yield components. The chicken manure released enough nutrients that resulted in increase in growth and yield of rice and it also improved the soil properties, which in turn resulted in better growth and yield. Additionally, the organic fertilizer can be a better supplement of inorganic fertilizer to produce higher growth and yield. The lowest values for plant growth and yield parameters are recorded in T1 where no fertilizer was applied. Only use of chemical fertilizer is not judicious for producing any crop in agriculture. Therefore, it can be concluded that combined application of organic manure and chemical fertilizer is necessary for crop production as well as maintaining soil fertility in study area.

## ACKNOWLEDGEMENTS

The authors acknowledge the Japan International Center for Agricultural Sciences (JIRCAS), for the financial support allowing the completion of this research.

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## Characterization and Diversity of Selected Maize (*Zea mays* L.) Genotypes Using Qualitative Traits

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Received 22 December 2020    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Phenotypic traits qualitatively and quantitatively are helpful as a preliminary evaluation of maize genetic diversity and provided practical and critical information required characterizing genetic resources. To find out the varietal characterization and grouping with similarity, fifty maize genotypes including hybrids and inbreds were examined in this experiment. This experiment was carried out in a randomized complete block design with three replications during wet season from June to October, 2019, at Maize and other Cereal Crops Research Section, Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw, Myanmar. The qualitative characters were recorded at different growing stages according to the International Union for the Protection of New Varieties of Plants (UPOV) Test Guide line (TG). The agglomerative cluster analysis was computed using Ward's hierarchical algorithm based of qualitative characters. According to the results, diverse qualitative traits were observed among the tested genotypes and genotypes varied different qualitative traits with different frequencies. Thus, comparisons of qualitative traits were made to know the extent of variation among maize varieties under investigation to estimate the genetic diversity. The tested maize genotypes could be grouped five clusters based on qualitative traits. Although some maize genotypes collected in an area are included in different groups because of the different characteristics they pose. Since qualitative characters have less environmental influences, these traits could be used for Distinctness, Uniformity and Stability (DUS) test and Value for Cultivation and Use (VCU) testing of plant varieties. Thus, the application of morphological markers according to UPOV descriptor could contribute to more efficient selection of parental pairs in the early generations of testing.

**Keywords** characters, cluster, maize genotypes, variation

## INTRODUCTION

Maize (*Zea mays* L.) is the world's third most important cereal, widely used in poultry and food industries next to wheat and rice. As it has higher yield potential than any other cereals, it is referred to as “miracle crop” or the “queen” of cereals. Maize is grown both as food for human beings and fodder for animals (Malhotra, 2017). In Myanmar, maize is the second most important cereal after rice. In 2017-2018, maize growing area is 504,000 ha and production is 1940,000 MT. As demand for maize has increased annually since 2009, the maize growing area has expanded year by year (DoP, 2018). Most of cultivars grown in Myanmar are introduced hybrid varieties and these varieties are registered according to the Seed Law and Regulation. Although the registered varieties are increasing year by year, the procedure on clarification as a new variety is weak. Awareness of genetic diversity among elite breeding materials or adapted cultivars has an important role in the improvement of crop plants.

Many tools are now available to study the relationships among the cultivars, including various types of molecular markers; however, morphological characterization is the first step in the description and classification of germplasm (Smith and Smith, 1989). There is an important role of morphological data in the management of genetic resources that are conserved in *ex-situ* gene-banks. The characterization and grouping of lines helps the breeders to avoid duplication in sampling populations and to aid in the identification of varieties and hybrids (Madhukeshwara and Sajjan, 2015). Although there are the ways to collect the morphological character by using International Board for Plant Genetic Resources (IBPGR) descriptor and International Union for the Protection of New Varieties of Plants (UPOV) Test Guide line (TG), collection of the data according to UPOV TG is better for characterization of the genotypes which are used to set up reference varieties in TG preparation, and Distinctness, Uniformity and Stability (DUS) testing. The inventory and agro-morphological characterization of these genetic resources (improved varieties and local's accessions) are essential to provide a sound database on the characteristics of the maize (Salami et al., 2015). Therefore, this experiment was conducted to characterize qualitative characters of maize and to identify similarity among maize genotypes.

## METHODOLOGY

This experiment was carried out during the wet season from June to October, 2019, at Maize and other Cereal Crops Research Section, Department of Agricultural Research (DAR), Yezin Nay Pyi Taw, Myanmar which was located at 19° 49' 33" N; 96° 16' 44" E; 102 m above sea level. In the present investigation, 38 hybrids (developed from DAR and imported by private companies) and 12 inbred lines and open pollinated varieties (OPVs) (developed from DAR, Yezin) were utilized. These genotypes are selected based on the most recommended for importation in 2018-19, as new varieties approved by 10<sup>th</sup>-14<sup>th</sup> National Seed Committee (NSC) and currently grown in Regions and Divisions.

All tested genotypes were evaluated in Randomized Completely Block Design with three replications. Each entry (genotype) was grown at a spacing of 75 cm × 25 cm, in a plot size of two rows with 4 m length. Two seeds were sown in each hill and thinning was done 14 days after sowing and single plant per hill was left. For fertilizer application, Urea, Triple Super Phosphate and Muriate of Potash were applied as basal at the rate of 123.5 kg ha<sup>-1</sup>, 123.5 kg ha<sup>-1</sup> and 61.75 kg ha<sup>-1</sup>, respectively. Then, the first and second sides dressing of 61.75 kg ha<sup>-1</sup> Urea, 30.86 kg ha<sup>-1</sup> Muriate of Potash were applied three weeks after sowing and five weeks after sowing, respectively. Inter-cultivation was done two times; just before the fertilizer applications which control weeds and also improve soil aeration. The qualitative parameters were recorded on each tested genotype in each replication on five representative plants according to UPOV test guidelines. The qualitative characters were recorded at different growing stages following UPOV TG. The agglomerative cluster analysis

was computed based on qualitative and morphological characters using Ward's hierarchical algorithm according to the procedure as described by Singh and Chaudhary (1977).

## RESULTS AND DISCUSSION

### Characterization of Maize Genotypes

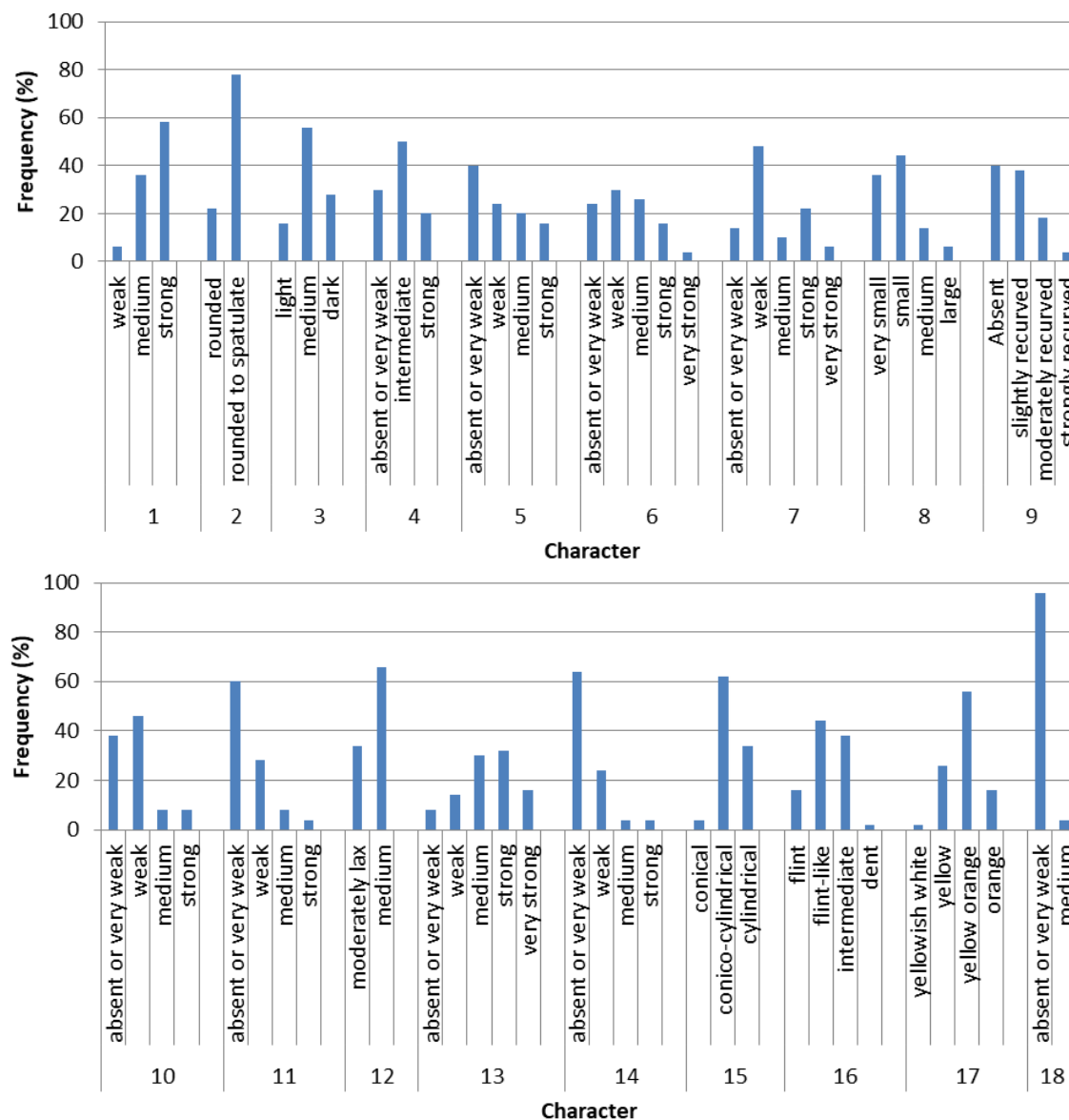
The qualitative traits of tested maize genotypes were classified according to UPOV test guidelines. Variations of 18 qualitative characters in 50 maize genotypes are shown in Fig. 1. Some distinctive characteristics of tested maize genotypes are presented in Plate 1. The anthocyanin coloration of first leaf sheet possessed five groups such as absence, weak, medium, strong and very strong color anthocyanin. There were 29 genotypes (58%) having strong anthocyanin, 18 genotypes (36%) having medium and 3 genotypes (6%) having weak anthocyanin. There was no genotype with absence of anthocyanin and very strong anthocyanin coloration. In shape of first leaf apex, the maximum percentage of genotypes exhibited rounded to speculate type (78.00%) and the minimum accessions showed round apex type (22%) of leaf apex shape. The intensity of green color in foliage was classified into three groups such as light, medium and dark green for tested maize genotypes. Of the total, 28 genotypes showed medium green color foliage (56%), 14 genotypes (28%) had dark green foliage and other 8 genotypes were observed (16%) light green foliage. The undulation of margin in first leaf blade in tested genotypes was classified as absence, intermediate and strong. Among the genotypes, 25 genotypes possessed intermediate undulation of margin of leaf blade, 15 genotypes were absent and the rest 10 genotypes had strong undulation leaf blade margin.

Five characters regarding with tassel were examined in this study: tassel anthocyanin coloration at the base of glume, anthocyanin coloration of glume excluding base, anther anthocyanin coloration, the angle between main axis and lateral branches and curvature of lateral branches of tassel. In this result, different genotypes possessed different tassel characters. The types of spikelets density in tassel was classified into three types. Among the 50 genotypes, most were medium density of spikelets (66%) and the rest was moderately lax (34%), and there was no moderately dense density of spikelets. In, anthocyanin coloration of silk of ear, no anthocyanin (19 genotypes = 38%), weak anthocyanin (23 genotypes = 46%), medium anthocyanin (8%) and strong anthocyanin (8%) colors were found among the 50 genotypes. Among the maize genotypes studied, 30 genotypes (60 %) were found no purple anthocyanin in brace root, 14 genotypes (28%) had weak anthocyanin, 4 genotypes (8%) had medium color and 2 genotypes (4%) were noticed as strong anthocyanin.

Leaf sheath and stem (internode) anthocyanin color were classified five groups as absent or very weak anthocyanin, weak, medium, strong and very strong. Leaf sheath anthocyanin was dispersed at all groups as very strong anthocyanin color (16%), strong anthocyanin color (32%), medium anthocyanin color (30%), weak anthocyanin color (14%) and very weak or absence of anthocyanin color (8%). In stem color, absence of anthocyanin (68%), weak anthocyanin (24%), medium (4%) and light strong anthocyanin (4%) were observed among the genotypes. There was no genotype with strong anthocyanin color in internode color. The shape of maize ear was characterized as conical, conical-cylindrical and cylindrical. Most of the tested genotypes had conical-cylindrical type of ear (62%), followed by cylindrical type of ear (34%) and the rest (4%) had conical type of ear.

In types of grain, UPOC TG showed nine types: flint, flint like, intermediate, dent-like, dent, sweet, pop, waxy and flour. In this study, four types of grain were observed among the genotypes. Most of the genotypes possessed flint like grain showing about 44% of total. Nineteen out of 50 (38%) showed intermediate type of grain, 16% had flint of grain, and the rest genotype about 2% had dent type of grain. There were 10 types of color of grain top such as white, yellowish white, yellow, yellowish orange, orange, red orange, red, purple, brownish and blue black color. In this study, only four types of color of grain top were observed among the tested genotypes with the frequencies of 2%

in yellowish white, 26% in yellow, 56% in yellowish yellow and 16% in orange. No more color of grain top was examined. Although five types of anthocyanin coloration of glumes of cob were classified, only two distinct colors with distinct frequencies like absent anthocyanin (96%) and medium anthocyanin (4%) were observed among the tested maize genotypes. According to the results, comparisons of qualitative traits were made to know the extent of variation among maize varieties under investigation to estimate the genetic diversity.

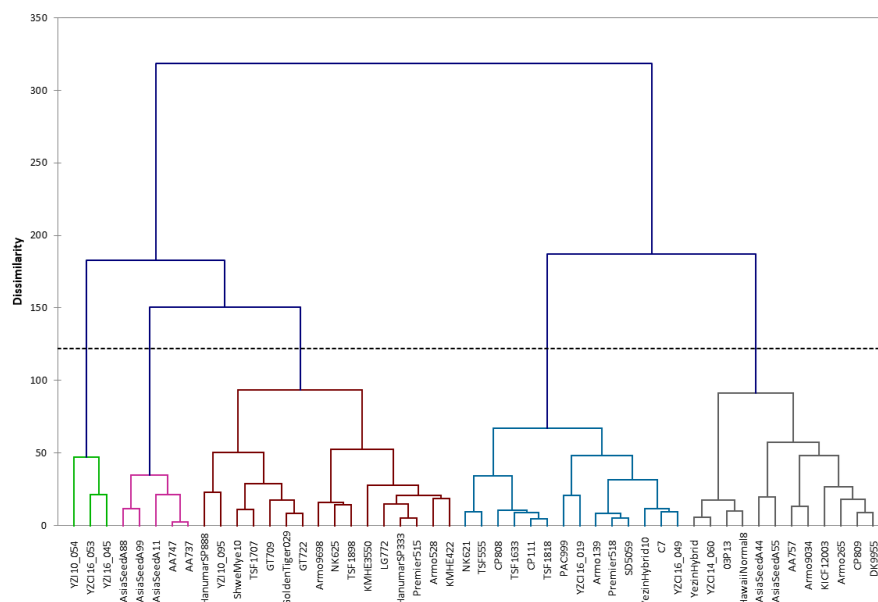


**Fig. 1 Variation of different qualitative characters in maize genotypes**

Note: 1-anthocyanin coloration of first leaf sheet, 2-apex shape of first leaf, 3-intensity of green in foliage, 4-undulation of leaf blade margin, 5-anthocyanin coloration at base of tassel glume. 6-anthocyanin coloration of tassel glumes, 7-anthocyanin coloration of anthers, 8-tassel angle between main axis and lateral branches, 9-curvature of lateral branches, 10-anthocyanin coloration of silks. 11-anthocyanin coloration of brace roots, 12-density of spikelets, 13-anthocyanin coloration of leaf sheath, 14-anthocyanin coloration of internodes, 15-ear shape, 16-type of grain, 17-grain color, and 18-anthocyanin coloration of glumes of cob

## Cluster analysis of maize genotypes

The cluster analysis, using WARD method based on the squared Euclidean distance criteria, was conducted for measuring genetic diversity and relatedness among the studied genotypes by using qualitative characters (Fig. 2). The studied maize hybrids were grouped into five clusters, showing the existence of considerable genetic diversity among 50 maize genotypes. Some of the hybrids of the same geographical region (source) were observed in different groups. Maximum number of genotypes were grouped in Cluster III (16 genotypes), followed by cluster IV (14 genotypes) and Cluster I (12 genotypes) whereas cluster V contained the least number of genotypes (only three genotypes) followed by cluster II (five genotypes) (Table 1). It can be concluded that the genotypes present in the same region were genetically distinct from each other. These results are in accordance with previous findings of Babic et al. (2016) who concluded that the grouping of maize lines based on the scale-measured characteristics was not in accordance with the information of their origin and even related line pairs were not grouped in the same cluster.



**Fig. 2 Dendrogram of maize genotypes based on morphological distances by a visual assessment of the group of plants and single measurement of individual plants or part of plants**

**Table 1 Cluster based on qualitative characters**

Cluster	No.	Name of genotypes
I	12	03P13, AA-757, Armo 265, Armo 9034, Asia Seed A44, Asia Seed A55, CP 809, DK 9955, Hawaii Norma 18, KICF12003, Yezin Hybrid, YZCI 14-060
II	5	AA 747, AA737, Asia Seed A11, Asia Seed A88, Asia Seed A99
III	16	Armo 528, Armo 9698, GoldenTiger 029, GT 709, GT 722, Hanumar SP333, Hanumar SP888, KMHE 3550, KMHE 422, LG 772, NK 625, Premier 515, Shwe Mye 10, TSF 1707, TSF 1898, YZI10-095
IV	14	Armo 139, C7, CP 111, CP 808, NK 621, PAC 999, Premier 518, SD 5059, TSF 1633, TSF 1818, TSF 555, Yezin Hybrid 10, YZCI 16-019, YZCI 16-049
V	3	YZCI16-053, YZI10-054, YZI16-045

*Cophenetic correlation coefficient = 0.577*

## CONCLUSION

The total 50 maize genotypes (including hybrids and inbreds) were examined their qualitative characters. The results showed that genotypes varied different qualitative traits with different frequencies. The fifty maize genotypes, with the help of cluster analysis, were successfully characterized and accurately grouped into five clusters with distinct promising features. It should be noted that some maize genotypes collected in an area are included in different groups because of the different characteristics they pose. Thus, the application of morphological markers according to UPOV descriptor could contribute to more efficient selection of parental pairs in the early generations of testing.

## APPENDIX

Some distinct qualitative traits of maize from this study were summarized below.



## ACKNOWLEDGEMENTS

The authors are grateful to the responsible persons from DOA, DAR and YAU for their kind permission and collaborating to conduct this study. We acknowledge all the staff of Maize and other Cereal Crops Research Section, DAR for helping us.

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## **Biofunctool® Approach Assessing Soil Quality under Conservation Agriculture and Conventional Tillage for Rain-fed Lowland Rice Systems in Cambodia**

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**Abstract** Rice productivity is often limited by soil fertility depletion, water availability and access. Conservation Agriculture (CA) cropping systems have been designed and tested with the main objectives of restoring soil fertility, increasing productivity and profitability. This study assessed changes in soil health under rain-fed lowland rice under (i) conventional tillage (CT), (ii) CA (CA7: 7 years under CA) and (iii) green manure management for one year (CGM1) and for two years CGM2). Biofunctool®, a multi-functional soil assessment approach based on a set of seven soil indicators, was used to evaluate changes in three main soil functions (C transformation, nutrient cycling, and soil structure). In addition, soil chemical analyses were conducted in the 0-5, 5-10, 10-20 and 20-40 cm soil layers to assess changes in nutrient contents. Our results emphasized positive impacts of CA on C transformation, soil structure and nutrient contents. Soil organic carbon and total N were significantly higher ( $p < 0.05$ ) under CA7 in the 0-5 cm layer with up to  $+7.5 \text{ g C kg}^{-1}$  and  $+0.74 \text{ g N kg}^{-1}$ , respectively. Higher values of labile C and soil respiration ( $p < 0.05$ ) were observed under CA in the 0-5 and 5-10 cm layers. More stable soil aggregates and improved VESS values ( $p < 0.05$ ) were also observed under CA. CA and CGM had 2 to 3 times more available phosphorus than CT in the 0-5 cm layer, and higher values were observed under CA from a depth of 0-20 cm. Higher Ca, Mg and K contents were recorded under CA and CGM in the 0 to 40-cm soil layer. A SOC stabilization trend was observed in soils under CA (0-5 and 5-10 cm layers) while a SOC

mineralization trend was observed under CT and CGM. These results emphasize the positive impacts of CA on maintaining and/or enhancing soil health and in contributing to SOC accumulation. A diachronic analysis is now needed to assess the long-term on-farm impacts of CA on soil health and crop performances.

**Keywords** soil organic carbon dynamic, sustainable intensification, climate change adaptation

## INTRODUCTION

Cambodia produced over eight million tons of paddy for a total cultivated area of ~ 3.3 million ha (MAFF, 2018). Rice exports jumped from 100,000 tons in 2010 to ~ 540,000 tons in 2016. Based on topographic position, rice agroecosystems range from upland rice, rainfed lowland or upper sandy terraces, flooded rice in the plains, receding rice, and deep-water rice. Soil fertility across the rice agroecosystems ranges from medium to low (Biswas *et al.*, 2017). Yields are often limited by low levels of soil nutrients, fluctuating water levels in the paddy field and related impacts on the form and availability of nutrients in the soil (Pheav *et al.*, 2005). Despite the increase in rice yields over the last two decades, rice yield and profitability of farming systems around the Tonle Sap lake are still low. In addition, increasing use of chemical fertilizers and pesticides are recorded both in flooded and irrigated conditions raising concerns about environmental and health issues, and food safety. The low level of diversification, the increasing use of pesticides (Flor *et al.*, 2018) and the combination of practices that deplete soil fertility (i.e., continuous ploughing, use of rotary tillers, low inputs of organic compounds, burning or removal of crop residue to feed cattle) call for the design and assessment of alternative rice-based cropping systems. There is an urgent need to promote diversified rice cropping systems to maintain and improve soil quality, increase farmers' incomes while simultaneously contributing to safer food production. Several studies have demonstrated the positive impacts of diversified conservation agriculture (CA) cropping systems which promote the accumulation of soil organic carbon (SOC) and improve soil fertility (Boddey *et al.*, 2010). Conservation agriculture (CA) is based on three technical principles with (i) minimum soil disturbance (i.e., no tillage), (ii) a permanent soil cover, and (iii) diversified cropping systems (FAO, 2014). Diversified CA cropping systems with high biomass-C inputs (Séguy *et al.*, 2006) insure a continuous supply of fresh organic compounds, thereby improving soil aggregation (Tivet *et al.*, 2013), increasing soil biodiversity (Lienhard *et al.*, 2013), and SOC content (de Moraes Sá *et al.*, 2015) while enhancing production and ecosystem services (Pittelkow *et al.*, 2015). Multifunctional soil assessments are needed to better understand the relationships between cropping system management and soil health. Thoumazeau *et al.* (2019 a, b) proposed an integrative, multifunctional approach, named Biofunctool®, that makes it possible to assess three main soil functions (i) carbon transformation, (ii) nutrient cycling, and (iii) soil structure, with a core set of in-field and low-tech indicators. Three indicators were used to assess the changes of the carbon transformation including the labile soil organic C fraction (permanganate oxidizable carbon: POXC) (Weil *et al.*, 2003), the basal soil respiration (SituResp®) (Thoumazeau *et al.*, 2017), and the soil biological activity using the bait lamina test (van Gestel *et al.*, 2003). Then, three indicators were used for the soil structure maintenance function by assessing soil aggregate water stability (AggSoil) at 0-5 and 5-10 cm depths (Herrick *et al.*, 2001), water infiltration (Beerkan) (Thoumazeau *et al.*, 2019b), and visual evaluation of soil structure (VESS) at 0-30 cm depth (Guimarães *et al.*, 2011). Finally, the nutrient cycling function was assessed by quantifying available N, P, Ca, Mg and K.

## OBJECTIVE

We hypothesised that rice-based CA cropping systems have direct and positive effects on soil health, increase the main soil functions through C transformation, soil structure and nutrient contents. The



overall objective of the study was to conduct an integrative and quantified assessment of the relationships between contrasted rice cropping systems (i.e., conventional plough-based tillage (CT), CA and green manure management) and soil health on the flood plains of Lake Tonle Sap using the Biofunctool® approach.

## METHODOLOGY

### Study site:

In 2011, an on-farm experimental design was implemented in the hydromorphic plains in Kropour Kert village, Banan district, Battambang province (latitude 13°00'32.37" N, longitude 103°04'27.31"E, 18 m elevation, no slope). The soil in the 0-20 cm layer comprised 511 g kg<sup>-1</sup> clay, 339 g kg<sup>-1</sup> silt, 150 g kg<sup>-1</sup> sand and 5.19 pH (H<sub>2</sub>O). The soil is classified as a Vertisol by the FAO and as clayey soil according to the USDA soil classification. Mean annual precipitation was 1,306 mm and the mean temperature was 27.5 °C.

### Cropping systems:

Soil functions and soil physical-chemical characteristics were assessed for four main cropping systems (CA7, CGM1, CGM2 and CT). It should be noted that the on-farm assessment was based on an unequal number of fields under the same management, with two fields under CA management for 7 years (CA7), two fields under green manure management for one year (CGM1), three fields under green manure management for two years (CGM2), and seven fields under conventional plough-based management (CT). CA was based on no-tillage with a cover crop following wet season rice. Two main species were used, *Stylosanthes guianensis* (cv. Ubon Nina) and *Centrosema pascuorum* (cv. Cavalcade). Green manure management comprised ploughing the cover crop biomass into the soil a few weeks before rice was sown. Conventional tillage (CT) included ploughing (6-disc plough) and harrowing, and the residues of the previous crop were incorporated into the soil by ploughing or rotary tiller.

### Soil quality assessment:

According to the integrative view of the soil quality, the indicators used to assess changes in soil quality should be the result of soil biota-physical-chemical property interactions (Thoumazeau et al., 2019). The Biofunctool® approach was chosen to provide this integrative view and to describe the impacts of contrasted practices on soil quality. This approach is based on three main soil functions (i) soil carbon transformation, (ii) nutrient cycling and (iii) soil structure.

### Soil sampling and soil physical-chemical analysis:

Soil samples were collected on January 25<sup>th</sup>, 2018 in four soil layers (0-5, 5-10, 10-20, and 20-40 cm) with three replicates per field. The Biofunctool® approach was applied to the 0-5 and 5-10 cm layers. In addition, the same two soil layers (0-5 and 5-10 cm) were sampled per subplot to assess water-stable aggregates. Total C and N concentrations were analysed using a dry combustion method with an elemental CHN analyser (Wright et al., 2008), available P (Bray II method) (Bray and Kurtz, 1945), and available K, Ca, Mg (AAS method) (Pyle et al., 1995). Available N was quantified on samples sieved at 2-mm using the Kjeldahl method (Craft et al., 1991).

### Data analysis:

Statistical analysis was performed using R software (Dessau and Pipper, 2008). Each Biofunctool® indicator was first studied separately using a linear-mixed effects model (lme4 package, (Bates et al., 2015). Treatment was defined as the fixed factor and replicates (plots and inner-replicates) as random factors. After checking the normality of the model residuals and the homoscedasticity of residual variance, ANOVAs were run using the car package (Fox and Weisberg, 2013). This was followed by post-hoc mean comparisons, using the Shapiro-Wilk test with Bonferroni adjustment (Hothorn et al.,

2008). After studying each indicator separately, the indicators were subjected to Principal Component Analysis (PCA) (FactoMineR package) (Lê et al., 2008).

## RESULTS

### Soil Chemical Analysis

Under CA7, higher SOC and N contents ( $p < 0.05$ ) were recorded in the 0-5 cm soil layer, representing an increase of up to  $+7.5 \text{ g C kg}^{-1}$  and  $+0.74 \text{ g N kg}^{-1}$ , respectively (Table 2). Higher N contents were observed in the 0-5 and 5-10 cm soil layers under CA7 than under CT and significant differences between treatments were observed in all soil layers. In addition, higher stratification of SOC and N contents were observed under CA7 and CGM2 compared with CGM1 and CT in the top soil layer (0-10 cm). SOC and N contents were more uniformly distributed under CT mainly due to the successive effect of ploughing which mixed the topsoil layers.

**Table 1 Total soil organic carbon and nitrogen contents**

Fields	Total OC ( $\text{g kg}^{-1}$ )				Total N ( $\text{g kg}^{-1}$ )			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	17.75 b	9.97 ns	7.19 ab	4.86 ns	1.76 b	1.09 b	0.81 ab	0.63 ab
CGM2	11.52 a	9.90 ns	7.35 b	5.05 ns	1.15 a	1.07 ab	0.86 b	0.67 b
CGM1	10.22 a	9.56 ns	5.57 a	4.11 ns	1.13 a	1.05 ab	0.78 ab	0.66 b
CT	10.75 a	9.22 ns	5.74 a	4.51 ns	1.02 a	0.91 a	0.66 a	0.53 ab

Note: CA7: 7 years CA (experiment, 2 fields,  $n = 6$ ), CGM2: 2 years of cover crops as green manure (3 fields,  $n = 9$ ), CGM1: 1 year of cover crops as green manure (2 fields,  $n = 6$ ), CT: Conventional tillage as the control (7 fields,  $n = 21$ ). Different letters indicate significant differences according to Tukey's test.

### Soil Quality: C Transformation, Soil Structure and Nutrient Cycling

#### Carbon transformation:

Higher values of POXC and SituResp were observed in the 0-5 cm soil layer ( $p < 0.05$ ) under CA7 than under CGM2, CGM1 and CT, representing higher labile-C inputs and soil biological activity of the mesofauna and microflora. No significant difference was observed for the bait lamina, however higher substrate degradation was observed under CA7.

**Table 2 Soil carbon transformation**

Fields	POXC		SituResp®		Lamina baits	
	(mg C. $\text{kg}^{-1}$ )		(Absorbance difference)		(Substrate degraded)	
	0-5	5-10	0-5	5-10	0-5	5-10
CA7	877.3 b	404.0 ns	0.71 b	0.25 b	0.53 ns	0.36 ns
CGM2	443.7 a	324.7 ns	0.33 ab	0.12 ab	0.38 ns	0.29 ns
CGM1	429.7 a	320.2 ns	0.29 a	0.13 ab	0.46 ns	0.40 ns
CT	428.0 a	290.2 ns	0.26 a	0.11 a	0.40 ns	0.33 ns

Note: CA7: 7 years CA (experiment, 2 fields,  $n = 6$ ), CGM2: 2 years of cover crops as green manure (3 fields,  $n = 9$ ), CGM1: 1 year of cover crops as green manure (2 fields,  $n = 6$ ), CT: Conventional tillage as the control (7 fields,  $n = 21$ ). Different letters indicate significant differences according to Tukey's test. POXC: permanganate oxidizable carbon (POXC); Situ Resp: Soil respiration

#### Soil structure stability:

A significant difference was observed in the 5-10 cm soil layer with higher values under CA7 emphasizing the higher soil aggregate stability (Table 4). A low water infiltration rate was observed in

all fields with no significant differences between fields. CA7 exhibited the better visual soil structure with a lower score.

**Table 3 Soil structure stability**

Fields	Aggregate (score)		Beerkan (ml.mn <sup>-1</sup> )	VESS (score)		
	0-5	5-10		0-5	5-10	10-20
CA7	5.20 ns	5.36 ns	4.22 ns	2.00 a	2.00 a	2.50 a
CGM2	5.59 ns	4.89 ns	3.78 ns	2.78 c	2.78 a	2.83 a
CGM1	5.69 ns	4.81 ns	4.17 ns	2.10 a	3.00 b	3.25 b
CT	5.41 ns	4.61 ns	3.34 ns	2.29 b	3.75 c	3.50 b

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

### Nutrient contents:

Under CA7, higher concentrations of available nitrogen were recorded in the 0-5 cm soil layer ( $p < 0.05$ ) and in the other soil layers except in the 20-40 cm layer (Table 5). Two to three times higher available phosphorus was measured in the 0-5 cm layer under CA7 and CGM1 than under CT and higher values were observed under CA7 in the three top soil layers. Higher Ca, Mg and K contents were recorded under CA7 and CGM2 in all soil layers (Table 5).

**Table 4 Available phosphorus and nitrogen**

Fields	P (mg.kg <sup>-1</sup> )				N (g.kg <sup>-1</sup> )			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	12.34 c	6.52 b	2.50 b	1.53 ns	1.28 b	0.74 ns	0.55 b	0.28 a
CGM2	5.75 a	4.03 a	1.85 ab	1.51 ns	0.78 a	0.70 ns	0.58 b	0.44 b
CGM1	9.51 b	5.66 b	1.73 ab	1.50 ns	0.64 a	0.59 ns	0.26 a	0.23 a
CT	4.51 a	3.60 a	1.45 a	1.25 ns	0.81 a	0.59 ns	0.36 a	0.32 a

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

**Table 5 Available potassium, calcium and magnesium**

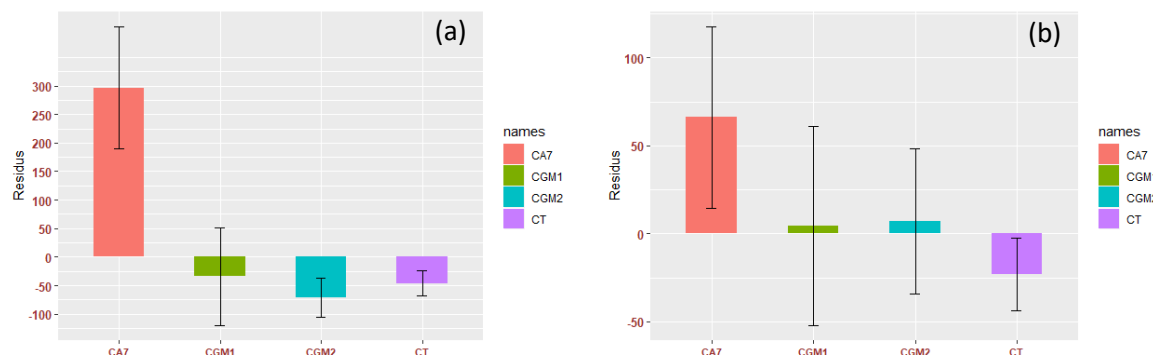
Fields	K (mg.kg <sup>-1</sup> )				Ca (mg.kg <sup>-1</sup> )				Mg (mg.kg <sup>-1</sup> )			
	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
CA7	27.27 c	11.70 ns	9.97 b	10.77 ns	926.6 c	840.7 b	812.8 b	747.5 c	118.8 c	106.2 b	103.3 b	98.7 ab
CGM2	22.93 bc	9.47 ns	10.37 b	10.93 ns	804.0 bc	794.6 b	809.0 b	714.4 bc	105.6 bc	106.9 b	132.9 c	124.3 b
CGM1	17.33 bc	8.60 ns	6.46 a	8.94 ns	648.1 ab	632.3 a	524.9 a	548.9 a	79.8 ab	78.5 a	75.4 a	77.1 a
CT	15.63 a	10.24 ns	8.60 ab	10.41 ns	687.1 a	692.5 a	609.0 a	630.8 ab	79.9 a	84.6 a	84.3 a	93.1 a

Note: CA7: 7 years CA (experiment, 2 fields, n = 6), CGM2: 2 years of cover crops as green manure (3 fields, n = 9), CGM1: 1 year of cover crops as green manure (2 fields, n = 6), CT: Conventional tillage as the control (7 fields, n = 21). Different letters indicate significant differences according to Tukey's test.

### SOC Stabilization vs. SOC Mineralization

Due to the unbalanced design, the trends and hypothesis presented hereafter need to be confirmed in a medium to long-term study. We nevertheless present our results here as the relationship between POXC and SituResp is a promising decision-making tool to assess SOC dynamics. A SOC stabilization

trend was only observed under CA7 (0-5 and 5-10 cm soil layers) (Fig. 1 a and b). For CT and CGM, a trend of SOC mineralization was observed with negative values of the residues of the linear relationship between POXC and SituResp. The SOC mineralization trend under CT could result from successive ploughing and lower inputs of biomass compared with CA. In addition, disruption of soil aggregates due to ploughing exposed SOC to microbial communities and to mineralization processes (Chen et al., 2009).

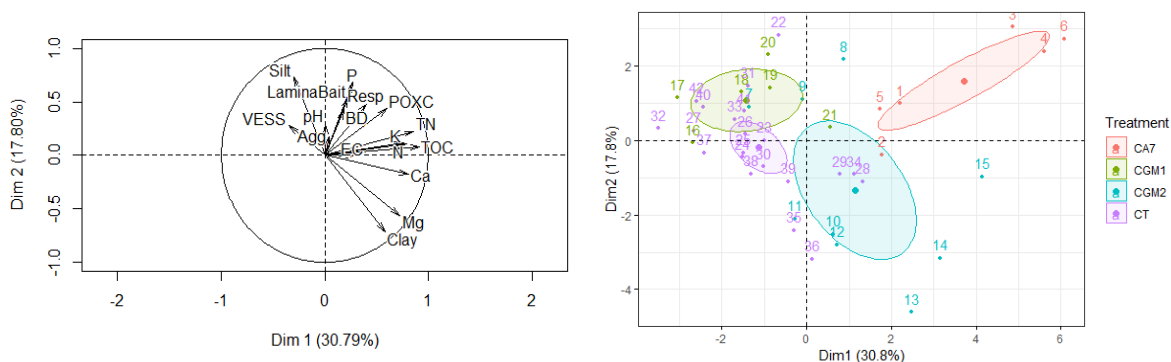


Note: CA7: 7 years CA (experiment), CGM2: 2 years of cover crops as green manure incorporating into the soil by tillage, CGM1: 1 year of cover crops as green manure incorporated into the soil by tillage, CT: Conventional tillage as the control. Regression was made between values of POXC and SituResp® (see Appendix 6). Residual mean values below zero represent a trend of mineralizable soil organic C, above zero values reflect a trend of short-term SOC stabilization. The vertical lines represent the standard error per treatment.

**Fig. 1 Mean values of regression residuals per treatment in the 0-5 cm (a) and 5-10 cm (b) soil layers**

### Principal Component Analysis

A principal component analysis (PCA) was conducted. Three main clusters were identified (1) CT and CGM1, (2) CGM2 and (3) CA7 (Fig. 2). The first axis separates CA7 from CGM1 and CT, while the second axis separates CA7 from CGM2. It should be noted that the main indicators of the Biofunctool® such as POXC, SituResp and lamina baits are correlated with long-term CA management (CA7). Other Biofunctool indicators of soil structure stability (i.e., VESS and soil aggregates) contributed less to both axes. As observed previously, CT and CGM1 are characterised by lower TOC, TN, Ca, Mg contents and SituResp compared with CA7 and CGM2.



Note: CA7: 7 years CA (experiment), CGM2: 2 years of cover crops as green manure, CGM1: 1 year of cover crops as green manure, CT: Conventional tillage

**Fig. 2 Principal component analysis of the impacts of cropping systems on soil functions and soil chemical properties in the 0-10 cm soil layer**

## DISCUSSION

This study assessed changes in soil quality under conventional plough-based and conservation agriculture management, using a multi-functional approach integrating a set of seven soil quality indicators related to three main soil functions (C transformation, nutrient cycling, and soil structure) (Thoumazeau et al., 2019). Soil physical-chemical analyses were also performed. Including all the indicators in a multivariate analysis made it possible to understand how soil functions in a clayey Vertisol in the flood plains of Tonle Sap lake are affected by contrasted agricultural practices under a tropical climate.

### Impacts of Contrasted Cropping Systems on Soil Chemical Properties

The main soil indicators were positively affected by long-term CA practices (CA7) with an increase in soil organic C and N contents, an increase in labile-C (POXC), an improvement of soil aggregation that can protect SOC from microbial oxidation compared with under CT. However, a SOC accumulation trend under CA cropping systems was only detected in the surface soil layer confirming the results of the study conducted by Hok et al. (2015). Soil organic C and N contents were stratified with depth under CA compared with CT as a consequence of crop residues being left on the surface of the soil, thereby regulating fluctuations in soil temperature and moisture, and limiting the SOC decomposition rate (Franzluebbers, 2008).

In accordance with the results of Hok et al. (2015) and Pheap, Lefevre et al. (2019), under CA, SOC and TN contents were higher in the topsoil layer. Zhang and He (2004) reported a gradual increase in SOC contents in the 0-15 cm layer in the first 30 years of rice cropping in South-East Asia. The highest accumulation of SOC and TN were associated with the higher biomass input and less disturbance (CA7) compared with other practices (CGM1, CGM2 and CT).

### Impact of Contrasted Cropping Systems on Three Soil Functions

#### Carbon transformation:

Part of the carbon transformation function, POXC, defined as a “soil fraction that is sensitive to cropping system management” (Pheap *et al.*, 2019) was indeed affected by the different practices with higher carbon contents measured in the 0-5 cm layer under CA7. Hok et al. (2018) reported an increase in POXC in the 0-10 cm layer under CA systems. In a similar experiment in Brazil, Sá et al. (2015) observed twice higher POXC under the no-till system down to a depth of 20 cm compared with under plough-based management. The increase in the top soil layer could be attributed to the difference in distribution of crop and cover crop residues between CA7 and other practices (Chatterjee and Lal, 2009) and due to a smaller amount of crop residues returned to the soil under CT than under CA7.

Concerning soil respiration, our results are in accordance with those of Pheap, Lefèvre et al. (2019), who reported that SituResp® values tended to be, or were significantly higher under CA, due to increased soil microbial activity linked to improved microorganism habitats and an increase in soil labile carbon. Used in combination, POXC and SituResp® play complementary roles by providing a framework for evaluating the relative dynamics of soil organic C stabilisation and mineralisation processes in agroecosystems (Hurisso et al., 2016). As observed by Pheap, Lefevre et al. (2019), CT tends to mineralise fresh organic carbon, while CA7 tends to stabilise C inputs.

#### Soil structure:

The absence of tillage combined with higher biomass inputs resulted in significant changes in soil physical properties, specifically improved soil aggregation (Lal and research, 1997). Soil physical properties improved rapidly under CA compared with under tillage-based systems. More stable aggregates in the top soil layers and better soil porosity are generally observed under CA (Busari et al.,

2015). As observed in our study, VESS was less sensitive to changes in the soil structure compared with water aggregate stability. This result is also in agreement with the results of other studies including those by Castioni et al. (2018) on a Brazilian Oxisol (55% clay) and by Thoumazeau et al. (2019b) for different land uses in Thailand.

#### **Nutrient cycling and contents:**

Ca, Mg and K contents were higher in the 0-10 cm soil layer under CA7 and CGM2 than under other practices. It is widely reported in the literature that CA with long-term use of cover crops recycle significant amounts of nutrients through the diversity of roots systems and aboveground biomass deposition. Continuous decomposition of plant residues enriches the main nutrient contents of the soil. Busari et al. (2015) reported that exchangeable Ca, Mg, and K were significantly higher in the surface soil under CA than under CT. Lower P, K, Ca and Mg contents were recorded in CT soils possibly due to the inversion of the top soil due to ploughing, which shifts less fertile subsoil to the surface with, in addition, possible leaching (Ali et al., 2006).

### **CONCLUSION**

A multifunctional soil assessment was conducted using the Biofunctool® approach to compare the impacts of contrasted rice cropping systems on soil health. Long-term CA cropping systems (CA7) and early green manure management for two consecutive years (CGM2) had positive impacts on the main soil functions compared with short-term green manure management (CGM1) and CT. These results showed that POXC, SituResp®, and VESS are sensitive in-field indicators of the impacts of annual rice cropping systems on soil health. By contrast, soil aggregate stability, beerkan, and lamina baits are less sensitive to changes. The relationship between POXC and SituResp® revealed a SOC accumulation trend under long-term CA management and is thus a promising in-field and low-cost indicator to assess the dynamics of soil organic C (stabilisation vs. mineralisation). The comparison conducted here, with an unbalanced number of fields among the different cropping systems, only highlights potential trends in changes in soil fertility. Additional studies are thus needed along with a diachronic analysis to assess the long-term on-farm impacts of CA on soil health, crop performances and on the provision of ecosystem services.

### **ACKNOWLEDGEMENTS**

This research was made possible through the support from the Department of Agricultural Land Resources Management (DALRM) from the General Directorate of Agriculture (GDA) that conducted field activities through the involvement of the team of the Conservation Agriculture Service Center (CASC) of Battambang province. We express our sincere gratitude to the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA), for funding the research activities. The field activities were made possible through the project Women in Agriculture Network Cambodia (WAgN, Royal University of Agriculture, University of Battambang and Pennsylvania State University) through support by the United States Agency for International Development Feed the Future Innovation Labs for Collaborative Research on Sustainable Intensification (Cooperative Agreement No. AID-OAA-L-14-00006, Kansas State University) and the Conservation Agriculture Network in South-East Asia (CANSEA). We also thank the Land Development Department (LDD) and Kasetsart University laboratory for handling and providing assistance for the soil analysis. We are thankful to Mr. Sambo Pheap, lecturer at the Faculty of Agronomy of the Royal University of Agriculture and Mr. Rotha Chork, Mr. Hang Socheat, Mr. Pengly Koun undergraduate students from RUA and the lab team for their assistance in collecting soil samples and conduct soil analysis.

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## **Assessment of Biogas Production Potential from Commercial Pig Farms in Cambodia**

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Received 28 December 2020    Accepted 30 June 2021    (\*Corresponding Author)

**Abstract** Commercial pig farms in Cambodia are increasing, representing 30% of the overall pig production in 2018. To run the farms, huge quantity of water is used on daily basis, and its large proportion ends up being wastewater that can cause air and water pollution, fly-related illnesses, and methane emissions. In response, anaerobic digestion (AD) is applied to convert waste into energy. Covered lagoons are an anaerobic biodigester that has long been used for commercial biogas plants in Cambodia due to acceptable investment cost and favorable climatic conditions for biogas production. However, lack of local technical data and technical assessment is a barrier to wider implementation of biogas systems in the country. Therefore, the study was conducted to explore the characteristics of commercial pig farms and wastewater use; to analyze the quality of biogas compositions from different covered lagoons; and to estimate biogas production, electricity generation, and CO<sub>2</sub> reduction equivalent from the collected data. The study was started from January to October 2020, selecting 9 farms with evaporative cooling systems for in-depth interviews, along with direct observations, biogas analysis, and power analysis. The findings indicate that all the studied farms were fattening farms operated under purchase contract with private companies. Pig production varied from 2,800 to 7,200 head per cycle, with two cycles per year. Moreover, daily wastewater was 0.033 m<sup>3</sup> d<sup>-1</sup> head<sup>-1</sup>, with dry matter (DM) accounting for 0.9%. Annual biogas production and electricity generation were 32.7 m<sup>3</sup> y<sup>-1</sup> head<sup>-1</sup> and 42.5 kWh y<sup>-1</sup> head<sup>-1</sup>, respectively. Biogas quality was 59.5% CH<sub>4</sub>, 31.5% CO<sub>2</sub>, 1.3% O<sub>2</sub>, and 2,256 ppm H<sub>2</sub>S. With biogas systems, individual farms could reduce CO<sub>2</sub> emission by 0.676 tCO<sub>2</sub>eq y<sup>-1</sup> head<sup>-1</sup>, which is economically and environmentally beneficial. However, a business model should be taken into account for successful implementation.

**Keywords** CO<sub>2</sub> reduction, covered lagoon, methane emission, pig manure

## INTRODUCTION

Pork is considered an important protein source for daily Cambodian diets. In this country, an average person eats 17.6 kg of meat per year, of which 9.29 kg is pork. Likewise, annual domestic demands for meat in 2018 was estimated at 285 thousand tons, and pork alone accounted for 52.8%. This high demand led to a 126% increase in local pig production between 2014 and 2017, from 2.44 million to approximately 3.18 million heads. A tendency toward large-scale production has emerged, as commercial pig farms rose by 30% in 2018 (MAFF, 2019). Commercial pig farms in Cambodia are farms with more than 100 fattening pigs, or more than 50 sows. There are around 500 farms in operation across 10 provinces, with 30-40% concentrated in Kampong Speu Province (NBP, 2019). Commercial pig farms in Cambodia are operated similarly with Thailand because they use evaporative cooling systems to maintain optimal temperatures inside the barns from 25 to 27 °C (Thanapongtharm, 2018). Such operation is vital for pig growth and disease prevention. For secure market and prices, farms turn to contract farming with private companies, such as C.P. Cambodia Co., Ltd., that provide both technical and financial support. Farm operation differs by pig type. In Cambodia, pig farms are classified into breeding farm and fattening farm. Among them, fattening farms are more popular. Fattening farms have three categories: small-sized (100 - 1,000 heads), medium-sized (1,001 - 5,000 heads), and large-sized (>5,000 heads) (MAFF, 2018).

Pig farms normally use large amounts of water for pig drinking, pig bathing, and barn cleaning. Daily water use rates vary by production type and pig weight. It is reported that average daily required water rates for breeding farms, fattening farming, and nursery farms are 92, 48, and 32 L d<sup>-1</sup> head<sup>-1</sup>, respectively. However, a large proportion (50-70%) ends up being wastewater (Nokyoo, 2016). Improper treatment of wastewater is associated with odor, flies, water pollution, and greenhouse gas emission. Some key elements used as pollutant indicators include chemical oxygen demand (COD), biological oxygen demand (BOD), total Kjeldahl nitrogen (TKN), and total suspended solid (TSS). A study by Tokhun (2010) indicates that untreated wastewater from large-scale pig farms in Thailand

contains 4,889 mg L<sup>-1</sup> COD, 3,555 mg L<sup>-1</sup> BOD, 481 mg L<sup>-1</sup> TKN, and 2,317 mg L<sup>-1</sup> TSS. These parameters are too high to be directly discharged into natural lakes. In the Cambodian wastewater standards for public water areas and sewers, COD, BOD, TSS, and nitrate (NO<sub>3</sub>) must be no more than 100, 80, 80, and 20 mg L<sup>-1</sup>, respectively (Council of Ministers, 1999). Thus, sound waste management is strongly required, as it is important for the sustainable operation of pig farms. One of the most effective wastewater treatment methods is the adoption of anaerobic digestion (AD). AD is known as a process under which organic matters, mainly in the form of fine particles, are fermented with the absence of air. The process consists of four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, with biogas produced as a final product and convertible into energy (Deublein and Steinhauser, 2011). Biogas is a gas mixture that contains 50-70% methane (CH<sub>4</sub>), 30-40% carbon dioxide (CO<sub>2</sub>), and other trace elements (EESI, 2017). CH<sub>4</sub> contained in biogas is the only source of energy such as heat and electricity. Nevertheless, it is harmful to the environment, if released into the atmosphere, because it is 28 times more powerful than CO<sub>2</sub> in terms of global temperature potential (GTP) for 100 years (IPCC, 2014). In contrast, converting biogas into electricity or upgrading it into bio-methane can reduce its harmfulness.

To promote manure management, the National Biodigester Program (NBP) was established in Cambodia in 2006 to turn cow manure into biogas for cooking and lighting. The program has built nearly 30 thousand biodigesters for smallholder farmers nationwide. In recent years, attention has been turned to large-scale biogas systems, which are covered lagoons. Covered lagoons are an AD technology, commonly used in commercial pig farms in Cambodia. It is reported that there are 44 covered lagoons in operation across the country (NBP, 2019). This number is still considered low in comparison to the potential pig farms and other biogas resources. This is due to lack of necessary documents and knowledge, or biogas skills. Therefore, in-depth studies on commercial pig farms are deemed vital for solving problems with wastewater and for economic profitability through energy generation.

## OBJECTIVE

The objectives of this study were (1) to explore the characteristics of commercial pig farms and wastewater use; (2) to analyze the quality of biogas from covered lagoons; and (3) to estimate methane production, electricity production, and CO<sub>2</sub> reduction equivalent.

## METHODOLOGY

The selection criteria for pig farms were farms that had thousands of pigs, used evaporative cooling systems, and were interested in setting up biogas systems. However, the scope of this research was based on a one-year study period only, from January to October 2020. To represent diverse pig farm characteristics, 9 commercial farms were selected from 6 different provinces: 5 from Kampong Speu and 1 each from Kampong Chhnang, Kampong Cham, Kampong Thom, Siem Reap, and Kratie. More farms were selected from Kampong Speu, as this province had the greatest farm number in the country. The study procedure was arranged by face-to-face interviews with farm owners on the site, direct observation, biogas analysis, and power analysis.

## Materials

Biogas quality is an important indicator to determine generator efficiency. A 5000 gas analyser, supplied by Geotech, UK, was used to analyze biogas quality based on the percentage of CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, and H<sub>2</sub>S in ppm with the maximum of 5,000 ppm.

Peak load is considered a vital indicator to determine the generator size for the farm. A Hioki

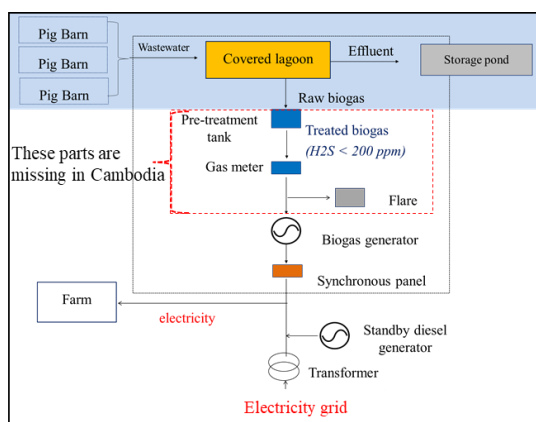
PW3365-20-01/5000 power logger was used to measure daily peak loads. It is capable of measuring three-phase voltage with high electrical current of up to 5,000 A.

### Sampling Methods and Data Analysis

In the study, a set of questionnaire was used to collect information on pig type, business type, pig number, barn number, cycle number, and duration within a cycle, as well as plans to increase farm size within the next few years. Moreover, information on sources of farm power supplies, existing biogas systems, biogas system type, generator type, and operation was also gathered. Annual electricity demand was estimated based on collection of electricity bills from the farms. All the information was verified by direct observation.

In the sampling process, biogas was directly measured before and after desulfurization—a process for removing hydrogen sulfide from the biogas systems. As desulfurizing systems did not function, biogas quality was only measured in the outlet pipe from covered lagoons. The measurement lasted 2 min, and average values were plotted from at least 3 different measurements. Before measuring new samples, the biogas analyzer was flushed. In the sampling process, peak loads were measured by attaching the power logger to the electricity panels of the farms for 1 hour. Night load was not recorded due to low electricity demand. Nevertheless, peak load recording was possible for a few farms only due to blackouts.

Total daily wastewater production from pig farms was estimated by combining daily water use for individual pigs, daily manure, and urine rate. In Thailand, daily wastewater left from cleaning, bathing, and drinking is estimated at 24 L d<sup>-1</sup> head<sup>-1</sup> for fattening farms (Kulpredarat, 2016). Because Thai pig farms share similarity with Cambodia, wastewater rate used in this study was assumed to be 30 L d<sup>-1</sup> head<sup>-1</sup>, about 20% more for conservative estimation. In this study, manure production used for individual pigs was 1.5 kg d<sup>-1</sup> head<sup>-1</sup> (Mek et al., 2018), and urine rate was assumed to be 2.5 L d<sup>-1</sup> head<sup>-1</sup>. These estimated values were roughly similar to the data in Thailand, where daily manure and urine production for typical fattening farms are 1.2-1.4 kg d<sup>-1</sup> head<sup>-1</sup> and 3.0-3.2 L d<sup>-1</sup> head<sup>-1</sup>, respectively (Nokyoo, 2016). Besides that, the evaporation rate was also included and assumed to be 0.5 m<sup>3</sup> d<sup>-1</sup> barn<sup>-1</sup>.



**Fig. 1 Diagram of full biogas systems in a typical pig farm, but in Cambodia, desulfurizing systems, gas meter, and flare are missing**

Daily biogas production was estimated by multiplying manure dry matter (DM) with biogas yield. It is reported that average pig manure contains 20% DM (DEFRA, 2011), and that biogas yield is 0.33 Nm<sup>3</sup> kg<sup>-1</sup> DM based on the experimental result by the Biogas Technology and Information Center (BTIC). Electricity production potential was calculated by multiplying biogas production with the conversion factor that varies from 1 to 1.7 depending on scale and average loading rate of the generator.

From biogas systems, CO<sub>2</sub> emission can be reduced in two ways: reduction by not emitting CH<sub>4</sub> directly into the atmosphere and reduction by using it to run biogas generators instead of grid electricity. In this regard, CO<sub>2</sub> reduction by avoidance of CH<sub>4</sub> release is equal to CH<sub>4</sub>-to-CO<sub>2</sub> equivalent x CH<sub>4</sub> density x Annual CH<sub>4</sub> production. Meanwhile, CO<sub>2</sub> reduction by avoidance of grid electricity is equal to electricity-to-CO<sub>2</sub> equivalent x annual electricity demand met by methane production.

Descriptive statistics and frequency distribution were applied to analyze the data using MS Excel. To determine relations between CH<sub>4</sub> and O<sub>2</sub>, or CH<sub>4</sub> and H<sub>2</sub>S, a simple linear regression was used at  $\alpha = 0.05$  probability level using R program 4.0.4, available for free online.

## RESULTS AND DISCUSSION

**Table 1 Characteristics of commercial pig farms in the studied areas (N = 9)**

Variable description	Frequency (N = 9)	Percentage (%)	Mean $\pm$ SE	Min	Max
Fattening farm					
Yes	9	100%			
No	0	-			
Contract farming					
Yes	9	100%			
No	0	-			
Production cycle (cycle/year)			2		
Barn Number			5.6 $\pm$ 0.6	4	9
< 5	3	33%			
$\geq 5$	6	67%			
Production size (head/cycle)			4,194 $\pm$ 470.4	2,800	7,200
< 1000	0	-			
1000 – 3000	2	22%			
3001 – 4999	5	56%			
$\geq 5000$	2	22%			
Plan to increase production					
Yes	4	44%			
No	5	56%			
Sources of Energy supply					
Electricity grid	5	56%			
Other Energy sources	4	44%			
Existing covered lagoons (m <sup>3</sup> )			4,085 $\pm$ 564	2,965	4,761
Yes	4	44%			
No	5	56%			
Biogas systems in operation					
Yes	3	33%			
No	6	67%			
Generator range (kVA)			186 $\pm$ 49.1	100	375
< 100	0	-			
100 – 200	2	67%			
> 200	1	33%			
Generator type					
New	0	-			
Modified	3	100%			
Pig number per barn (head/barn)			749 $\pm$ 11.8	740	800
working days (d/y)			330		
Daytime peak load (kW)			59.8 $\pm$ 9.4	32	97
Max peak load (kW/barn)			9.1 $\pm$ 0.4	7.8	10

## Pig Farm Characteristics and Wastewater Use

The studied farms were all fattening farms operated under official contract with private companies (Table 1). The farms had two cycles per year, 5.5 months each. In early production, the farms were given piglets and had to sell back pigs to the contracting companies at the end of the cycle, also known as all-in/all-out batch operations. The number of barns varied from 4 to 9, with an average of 5.6 barns per farm. Average production size was 4,194 heads per cycle, but varied from 2,800 to 7,200 heads per cycle. No farms had pigs less than 1,000 heads per cycle. Of all the farms, 7 were medium-sized farms (1,000 to 5,000 heads); 2 were large-sized farms (>5,000 heads).

In Table 1, when asked about plans to increase production within the next few years, about 44% of the farm owners said yes. The main electricity supplies came from the local electricity grid. Of all the interviewees, 5 depended solely on EDC, while 4 also used biogas as an alternative. The biogas systems in the farms were simple covered lagoons that had different sizes, but the average value was 4,085 m<sup>3</sup>. Only 3 farms had the systems in operation, while another one just stopped due to problems with the biogas generator. In addition, the farms that had biogas systems preferred to second-hand, modified diesel generators for economic reasons.

Farm electricity was mainly consumed by evaporative cooling systems and to some extent by pumping and lighting (Table 1). Average maximum daytime peak load the farms was 59.8 kW, but varied from 32 to 97 kW according to the production size. Maximum peak load per barn was also measured with full operation of evaporative cooling systems. It was 9.1 kW and varied from 7.8 to 10 kW. However, peak loads were low at night, in cold seasons, and in early production.

**Table 2 Estimated total wastewater production in the fattening farms (N = 9)**

Source	Unit	Average $\pm$ SE
Water	m <sup>3</sup> d <sup>-1</sup>	125.8 $\pm$ 14.1
Dung (fresh)	t d <sup>-1</sup>	6.3 $\pm$ 0.7
Urine	m <sup>3</sup> d <sup>-1</sup>	10.5 $\pm$ 1.2
Evaporation	m <sup>3</sup> d <sup>-1</sup>	2.8 $\pm$ 0.3
Total waste water	m <sup>3</sup> d <sup>-1</sup>	139.8 $\pm$ 15.7
	m <sup>3</sup> d <sup>-1</sup> head <sup>-1</sup>	0.033 $\pm$ 0.004
DM content	%	0.9%
Total DM	t d <sup>-1</sup>	1.3
Biogas	Nm <sup>3</sup> d <sup>-1</sup>	415
	Nm <sup>3</sup> y <sup>-1</sup>	137,033
	Nm <sup>3</sup> y <sup>-1</sup> head <sup>-1</sup>	32.7

Wastewater rate produced from pig bathing and barn cleaning was 125.8 m<sup>3</sup> day<sup>-1</sup> in each farm (Table 2). Fresh manure and urine production was 6.3 t d<sup>-1</sup> and 10.5 m<sup>3</sup> d<sup>-1</sup>, respectively. Evaporation rate was estimated at 2.8 m<sup>3</sup> d<sup>-1</sup>, so total wastewater produced by individual farms was 139.8 m<sup>3</sup> d<sup>-1</sup>, or 0.033 m<sup>3</sup> d<sup>-1</sup> head<sup>-1</sup>. DM accounted for 0.9%, so average daily biogas production in each farm was estimated to be 415 Nm<sup>3</sup> d<sup>-1</sup>, or equivalent to 137,033 Nm<sup>3</sup> y<sup>-1</sup>. Thus, biogas production rate per pig was 32.7 m<sup>3</sup> y<sup>-1</sup> head<sup>-1</sup>. However, this calculated value was just an average because swine growth stage affects biogas production yield (Amara et al., 2015).

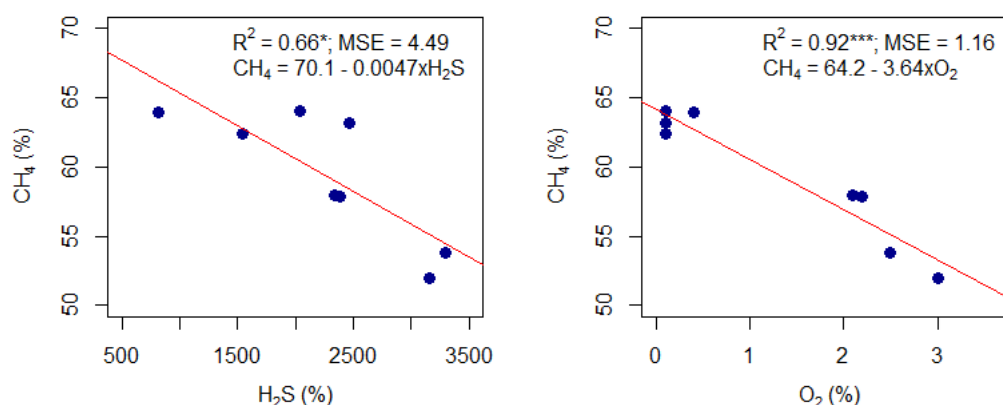
## Biogas Quality

In Table 3, CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, and H<sub>2</sub>S were 59.5%, 31.5%, 1.3%, and 2256 ppm, respectively. O<sub>2</sub> measured in this study might come from air that had entered the biogas system. Its presence suggests that there is also N<sub>2</sub> in the biogas, in a ratio of approximately 4:1. So 1.3% O<sub>2</sub> will mean 5.2% N<sub>2</sub>, possibly more. Biogas also contains water vapor, which may then lower CH<sub>4</sub> content. So, less air needs

to be mixed for the generator. High H<sub>2</sub>S content may reduce the generator lifespan through corrosion. Thus, desulfurizing systems are needed to lower its content before running any biogas generator. Nevertheless, the measured biogas quality was similar to a study by Sweeten, Fulhage, and Humenik (1981), who reported 50-60% methane, 40-50% CO<sub>2</sub>, and H<sub>2</sub>S <10,000 ppm. Dumont (2015) reported higher methane content (60-70%), lower CO<sub>2</sub> content (30-40%), and H<sub>2</sub>S (10-20,000 ppm) for biogas produced from organic waste.

**Table 3 Biogas quality measured on the pig farms that had biogas systems (N = 9)**

Biogas quality	Unit	Mean $\pm$ SE	Min	Max
CH <sub>4</sub>	%	59.5 $\pm$ 3.0	52.0	64.5
CO <sub>2</sub>	%	31.5 $\pm$ 2.8	26.9	40.0
O <sub>2</sub>	%	1.3 $\pm$ 0.7	0.1	3.0
H <sub>2</sub> S	ppm	2,256 $\pm$ 504	818	3,295



**Fig. 2 Relationships between CH<sub>4</sub> and H<sub>2</sub>S (left); CH<sub>4</sub> and O<sub>2</sub> (right); sample size (N = 9)**

In Fig. 2, CH<sub>4</sub> had a negative relationship with both H<sub>2</sub>S and O<sub>2</sub>, meaning that increased concentrations of H<sub>2</sub>S or O<sub>2</sub> may lower CH<sub>4</sub> content. H<sub>2</sub>S reduction is made possible by desulfurization through the use of desulfurizing systems, and O<sub>2</sub> reduction by preventing air from entering the system through proper sealing. However, Siripat (2019) and Deublein and Steinhauser (2011) reported that increased H<sub>2</sub>S content reduces biogas quantity, not CH<sub>4</sub> directly.

### Methane Production, Electricity Generation and CO<sub>2</sub> Reduction

In table 4, annual electricity production in each farm was estimated to be 178,142 kWh y<sup>-1</sup>, but varied by farm size. Pig weight varies by month, or by age, so does daily manure production. Daily biogas production also varies accordingly, but tends to increase constantly from early production to the end of the cycle. At the same time, electricity demands at the farms also showed variations, as the need for barn cooling varied with pig age and daily outside temperature. Thus, not all biogas could be used to produce electricity. In the study, the estimated average annual electricity demand for each farm was 166,667 kWh y<sup>-1</sup>, and electricity produced from biogas could meet only an average of 75%, or 127,700 kWh y<sup>-1</sup>. This estimation may vary according to farm production size.

Biogas systems also contribute to reduction in greenhouse gas emissions. In this study, the annual CO<sub>2</sub> reduction equivalent was estimated to be 2,832 tCO<sub>2</sub>eq y<sup>-1</sup> in each farm. This amount was huge, and further reduction could be achieved when more and more farms turn to using biogas systems. In short, about 0.676 tCO<sub>2</sub>eq y<sup>-1</sup> head<sup>-1</sup> could be reduced per pig. This CO<sub>2</sub> reduction equivalent was higher, when compared to Peerapong and Limmeechokchai (2017), whose estimatin was 0.469 tCO<sub>2</sub>eq

y<sup>-1</sup> head<sup>-1</sup> for a typical pig farm in Thailand. Thus, a business model should be considered for wider adoption of biogas systems for farm benefits and for the environment.

**Table 4 Estimation of electricity demand potential and CO<sub>2</sub> reduction (N = 9)**

Description	Unit	Value
Potential electricity production	kWh y <sup>-1</sup>	178,142
Estimated electricity for farm coverage	kWh y <sup>-1</sup>	124,700
Estimated farm demand	kWh y <sup>-1</sup>	166,667
Percentage of farm coverage from biogas	%	75
CO <sub>2</sub> reduction	Unit	
From CH <sub>4</sub> reduction equivalent	tCO <sub>2</sub> eq y <sup>-1</sup>	2,751
From grid electricity equivalent	tCO <sub>2</sub> eq y <sup>-1</sup>	82
Total		2,832
CO <sub>2</sub> reduction per head/year	tCO <sub>2</sub> eq y <sup>-1</sup> head <sup>-1</sup>	0.676

## CONCLUSION

Commercial pig farms in Cambodia were studied in terms of pig production, wastewater use for biogas systems, electricity demands and contribution to CO<sub>2</sub> reduction. It can be seen that these farms are typical, and some already used covered lagoons to produce biogas. The farm size is huge, and there is a high potential for further development of biogas systems that may benefits socially, economically, and environmentally. However, further studies shall be made on profitability of actual biogas investment as a model for farm investment and policy making.

## ACKNOWLEDGEMENTS

The study was made possible thanks to the project “Reduction of Greenhouse Gas Emission through Promotion of Commercial Biogas Plant in Cambodia” implemented by United Nations Industrial Development Organization (UNIDO) that not only provided funding, but also continuously assisted in strengthening the research team. Many thanks may also go to the farm owners that allowed for the interviews and site visits and to local authorities that helped facilitate the connections among us.

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## Evaluation of Some Nutritional Values and Antioxidant Activities of Dried Tea Leaves in Seasonally

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Received 30 December 2020    Accepted 30 July 2021    (\*Corresponding Author)

**Abstract** Tea Leaf is one of the traditional food in Myanmar. It is eaten as well as drunk with two main forms which are wet tea leaf (Myanmar name, Lahpet so) and dried tea leaf (Myanmar name, Lahpet chauk). Tea leaves can be picked starting from April until October. At Namhsan area, Northern Shan State in Myanmar, tea leaves are generally divided into six kinds depending on the plucked season. There are “Shwephi-Oo”, “Shwephi-Hnaung”, “Khakan-Oo”, “Khakan-Hnaung”, “Kha-Naing”, and “Kha-Hawt” or “Hnin Tet”. In this study, some nutritional value and antioxidant activities of six kinds of dried tea leaves in Namhsan region were evaluated by the aiming of which kind of dried tea leaves have the best quality for consumers. The analyses included examination of the dried tea leaves were their nutritional value (carbohydrate, protein, and amino acids), assessment of selected minerals, caffeine, catechin mixture, dietary fiber and total ash. Nutritional contents of tea leaves were examined by preliminary phytochemical screening method and the selected mineral contents (Ca, Zn, Fe, Mn) were analyzed by atomic absorption spectrophotometry (AAS). Total ash contents were determined by ignition method. Antioxidant activities of dried tea leaves were evaluated by the DPPH free radical scavenging assay. Caffeine and catechin mixture (flavonoid) were extracted by using chemical reagents such as dichloromethane and chloroform. From the observed results, dried tea leaves in Namhsan region were rich in mineral contents (2.33-5.48 mg/kg of Ca, 1.40-1.94 mg/kg of Zn, 1.06-1.77 mg/kg of Fe and 8.06-21.06 mg/kg of Mn), 0.525-3.413% protein, 8-11.5% crude fiber, 4.20-7.39% total ash, 0.075-0.200% caffeine, 0.4-2.0% catechin mixture and high antioxidant activities (2.3-5.9 µg/L, IC<sub>50</sub> value of DPPH scavenging assay) were found. Based on the studied nutritional values and antioxidant activity results, Shwephi-Oo dried tea leaf was confirmed that the best quality.

**Keywords** dried tea leave, nutritional value, antioxidant activity, phytochemical screening, free radical scavenging, AAS

## INTRODUCTION

Tea is grown in different states of Myanmar, mainly in Shan state. The best quality and major cultivation are in Namhsan region, Northern Shan State. The total tea plantation area is around 70,000 hectares with an annual production of 78 million Kg green leaves. Namhsan area is a mountainous

region with deep slope that forms a natural drainage system, vital for tea plantation (website 1). There are generally six kinds of tea seasonal names in use for the tea leaf. These are "Shwephi-Oo" if the tea leaf is picked 3<sup>rd</sup> week of March to 2<sup>nd</sup> week of April, "Shwephi-Hnaung" if it is picked 3<sup>rd</sup> week of April to 2<sup>nd</sup> week of the May, "Khakan-Oo" if it is picked 3<sup>th</sup> week of May to 2<sup>nd</sup> week of July, "Khakan-Hnaung" if it is picked mid-July to 2<sup>nd</sup> week of August, "Kha-Naing" if it is picked 3<sup>rd</sup> week of August to end-September, and, "Kha-Hawt" or "Hnin-Tet" if it is picked 1<sup>st</sup> October to end-November. The period of these seasons may be differed in locally.

Tea leaves contain thousands of the chemical compounds. When tea leaves are processed, the chemical compounds within them break down, form complexes with one another and form new compounds. The most important compounds in fresh tea leaves are polyphenols, amino acids, enzymes, pigments, carbohydrates, methylxanthines, minerals and many volatile flavor and aroma compounds. These components are responsible for producing teas with desirable appearance, aroma, and taste (Tony, 2019). All types of tea leaves (green tea, black tea, white tea, oolong tea, and pu-erh tea) contain unique antioxidants called flavonoids. The most potent of these, known as Epigallocatechin gallate (EGCG), may help against free radicals that can contribute to cancer, heart disease, and clogged arteries. All these teas also have caffeine and theanine, which affect the brain and seem to heighten mental alertness.

The more processed the tea leaves, usually the less polyphenol content. Polyphenols include flavonoids. When the tea leaves are oxidized or fermented, so they have lower concentrations of polyphenols than fresh green tea leaves; but their antioxidizing power is still high (Julie, 2009). Taste and aroma, as well as price and brand are the main factors impacting consumers' preferences with regard to tea of their choice; on the other hand consumers less frequently pay attention to the chemical composition and nutritional value of tea (Maria, 2017). Therefore, in this study, some nutritional values and antioxidant activity of dried tea leaves were examined by dividing the six kinds based on their plucked periods.

## **OBJECTIVE**

This study assessed the nutritional values and antioxidant activities of dried tea leaves in seasonally and confirmed the best quality dried tea leave based on the studied results.

## **MATERIALS AND METHODS**

### **Sample Collection**

Tea leaves were plucked in one of the tea farms from Zayangyi village in Namhsan region, Nothern Shan State, Myanmar. "Shwephi-Oo" kind of tea leaves were plucked in 2<sup>nd</sup> week of April 2018, "Shwephi-Hnaung" was plucked in 4<sup>th</sup> week of April 2018, "Khakan-Oo" was plucked in 4<sup>th</sup> week of May 2018, "Khakan-Hnaung" was plucked in 3<sup>rd</sup> week of July 2018, "Kha-Naing" was plucked in 3<sup>rd</sup> week of August 2018 and, "Kha-Hawt" was plucked in 1<sup>st</sup> week of October 2018. After plucking, all kinds of tea leaves were cleaned and dried at room temperature for two weeks. The dried tea leaves were stored in the air tight plastic bags before the analyses were performed.

### **Sample Preparation**

Aqueous extract of all kinds of dried tea leaves were prepared for the drinking purpose of dried tea leave infusion with boiling water.

### **Preliminary Phytochemical Analysis of Dried Tea Leaves**

Preliminary phytochemical investigations of aqueous extract of dried tea leaves were carried out to know what chemical constituents were involved in the dried tea leaves according to the standard procedure of qualitative test for preliminary phytochemical screening. The procedure and results are presented in Table 1 and 2, respectively.

**Table 1 Procedure for preliminary phytochemical screening**

Constituents	Procedure	Observation
Alkaloid	Dragendroff's test, Few mL of sample filtrate + 1-2mL of Dragendroff's reagent	Reddish brown precipitate
Carbohydrate	Barfoed's test, 1 mL of sample filtrate + 1 mL of Barfoed's reagent (heated for 2 min)	Red precipitate (monosaccharides)
Glycoside	Borntrager's test, 2 mL of sample filtrate hydrolysate + 3 mL chloroform (shaken well) + chloroform layer separate + 10 % ammonia solution	Pink colored solution
Protein and amino acids	Ninhydrin test, 2 mL of sample filtrate + 2 drops of Ninhydrin solution (10 mg Ninhydrin + 200 mL acetone)	Purple colored solution (Amino acids)
Flavonoid	Conc. H <sub>2</sub> SO <sub>4</sub> test, plant extract + conc. H <sub>2</sub> SO <sub>4</sub>	Orange color
Phenolic compounds	Ferric chloride test, Extract aqueous solution + few drops of 5 % ferric chloride solution	Dark green/bluish black color
Tannins	Braymer's test, 1 mL of sample filtrate + 3 mL distilled water + 3 drops of 10 % ferric chloride sol:	Blue-green color

### Determination of Some Nutritional and Mineral Contents of Dried Tea Leaves

Some nutritional (protein, total ash and crude fiber) contents of dried tea leaves were examined. The crude protein contents of the samples were determined by Macro-kjeldahl method (AOAC, 1990). Total ash was obtained by igniting tea sample in muffle furnace until it is free from carbon (Food Chemistry, 2012). The 5 g of each dried tea leave sample was taken in a tarred silica dish/crucible. It was heated at 100°C in an oven until moisture is expelled and incinerated at as low a temperature as possible. Then the dish was placed in furnace at  $525 \pm 20^\circ\text{C}$  for about 1 hour and leave until white ash is obtained. The dish was transferred in a desiccator for cooling and weighted the dish. The results were expressed as % total ash.

The crude fiber contents in the dried tea leave samples were examined by the method given in "The chemical analysis of Foods" (Joslyn, 1970).

The selected mineral contents (Ca, Zn, Fe, and Mn) were analyzed by atomic absorption spectrophotometer. 1 g of each dried tea sample was dissolved in 10 mL of 1 M nitric acid and boiled to complete the dissolution and filtrated. The precipitate was washed with 1 M nitric acid and filtered. The obtained filtrate was transferred to 25 mL volumetric flask and fill up to the level with double distilled water. Then the digested solution was analyzed with AAS spectrophotometer (SHIMADZU AA-7000). The results are described in Table 2.

### Determination of Caffeine, Catechin Mixture and Antioxidant Activities of Dried Tea Leaves

Caffeine and catechin mixture (flavonoid) were extracted by using chemical reagents such as dichloromethane or chloroform and ethyl acetate. 10 g of ground tea leaves were extracted with 300 mL of pure water at 80 °C for 40 min. Leaves were removed by filtration using a filter paper (Whatmann, No. 1). Aqueous tea infusion was initially partitioned with chloroform then second

partition was carried out with ethyl acetate (Row and Jin, 2006). Caffeine and catechin mixture were calculated by the equation 1 and 2 respectively (Ezgi, 2015).

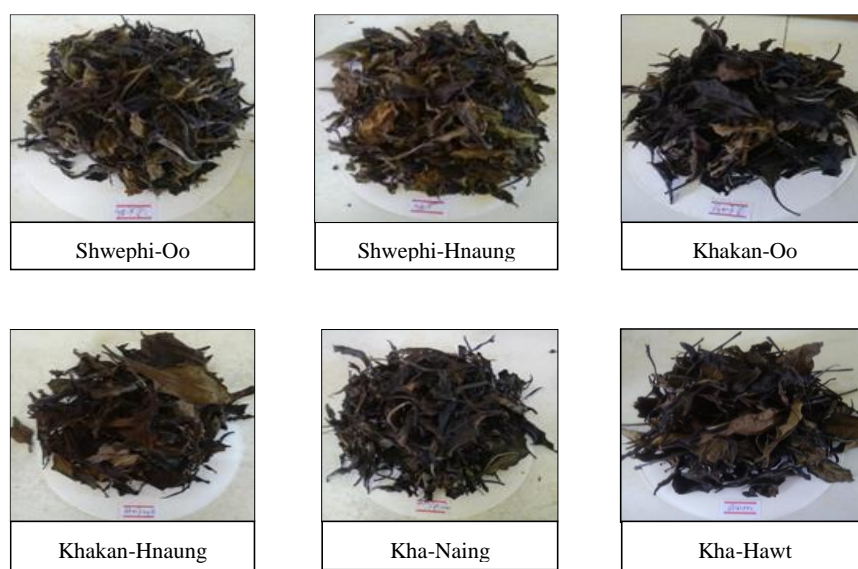
$$\% \text{ Caffeine (w/w)} = (\text{mass of caffeine extract} / \text{mass of tea sample}) \times 100 \quad (1)$$

$$\% \text{ Catechin (w/w)} = (\text{mass of catechin extract} / \text{mass of tea sample}) \times 100 \quad (2)$$

Antioxidant activities of dried tea leaves were evaluated by the DPPH free radical scavenging assay according to Lee *et al*, 2004. The results are described in Table 3. All experiments were performed in the laboratory of Department of Chemistry, Taunggyi University, Southern Shan State, Myanmar.

## RESULTS AND DISCUSSION

Fig. 1 shows the collected six kinds of air dried tea leaves from one of the tea farm in Zayangyi Village, Namhsan Region, Northern Shan State, Myanmar.



**Fig. 1 The six kinds of dried tea leaves**

**Table 2 Preliminary phytochemical screening of six kinds of dried tea leaves**

Sample (H <sub>2</sub> O extract)	Alkaloid	Carbohydrate	Glycoside	Protein and amino acids	Flavonoid	Phenolic compounds	Tannins
Shwephi-Oo	+	-	+	+	+	+	+
Shwephi-Hnaung	+	-	+	+	+	+	+
Khakan-Oo	+	-	-	+	+	+	+
Khakan-Hnaung	+	-	-	+	+	+	+
Kha-Naing	+	-	-	+	+	+	+
Kha-hawt	+	-	-	+	+	+	+

\*(+) = Present, (-) = absent

The qualitative tests of preliminary phytochemical screening for aqueous extract of six kinds of dried tea leaves showed the results which described in Table 2. According to these results, alkaloid, protein and amino acids, flavonoid, phenolic compounds and tannins were observed in all kinds of dried tea leaves. Glycoside was found in the two kinds, Shwephi-Oo and Shwephi-Hnaung. Carbohydrate was found to be absent in all kinds of dried tea leaves. By viewing these results, it is recommended that all kinds of dried tea leaves infusion with boiling water could significantly contribute to the health management and our daily need of secondary metabolites.

Nutrients can be divided into two categories: macronutrients, and micronutrients. Macronutrients are those nutrients that the body needs in large amounts. These provide the body with energy (calories). There are seven main classes of nutrients that the body needs. These are carbohydrates, proteins, fats, vitamins, minerals, fibre and water. It is important that everyone consumes these seven nutrients on a daily basis to help them build their bodies and maintain their health. Micronutrients are those nutrients that the body needs in smaller amounts. There are 7 essential plant nutrient elements defined as micronutrients [boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), chlorine (Cl)]. They constitute in total less than 1% of the dry weight of most plants (website 2). Table 3 shows the observed results of some nutritional and mineral contents of six kinds of dried tea leaves. From these results, it is found that the nutritional values are more or less differ from one another according to their plucked seasons. The protein (3.413%), crude fiber (11.5%) and zinc (1.9409 mg/kg) contents are the richest in “Kha-Naing” kind. The richest iron content (1.7661 mg/kg) is found in “Khakan-Oo” kind. The other parameters, total ash (7.39%), calcium (5.4764 mg/kg) and manganese (21.0559 mg/kg) are observed as higher significance in “Shwephi-Oo” kind. Despite the little difference in contents, on the basis of these observation, all kinds of tea leaves appeared that the presence of significance sources of nutritional and minerals for daily necessary human diet.

**Table 3 Some nutritional and mineral contents of six kinds of dried tea leaves**

Sample	Amount of parameters						
	Protein (%)	Crude fiber (%)	Total ash (%)	Ca (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Shwephi-Oo	0.525	8	7.39	5.4764	1.5873	21.0559	1.3953
Shwephi-Hnaung	0.525	8	6.60	5.4413	1.5079	15.1911	1.3514
Khakan-Oo	2.625	10.5	5.79	3.0048	1.8865	10.8227	1.7661
Khakan-Hnaung	1.138	10	5.39	4.4226	1.4070	8.0566	1.0664
Kha-Naing	3.413	11.5	6.60	3.3061	1.9409	11.6994	1.7569
Kha-hawt	2.144	8	4.20	2.3382	1.7858	11.1704	1.5577

Table 4 shows the amount of caffeine, catechin mixture and antioxidant activities of six kinds of dried tea leaves. The caffeine and catechin mixture contents were significantly higher in “Shwephi-Oo” kind. Numerous studies have indicated that catechins and other polyphenols in tea exhibit powerful antioxidant activities. They function as antioxidants in vitro by scavenging nitrogen species and reactive oxygen generated due to a variety of oxidative stress and by sequestering metal ions. The stable organic free radical, DPPH, has been considered as a useful reagent for determining free radical scavenging capacity of antioxidant materials (Tao, et al, 2019). The observed antioxidant results were compared with that of ascorbic acid as standard materials. The IC<sub>50</sub> values (the effective concentration of 50% inhibition) were showed that all kinds of tea leaves have significance antioxidant activities. Among them, “Shwephi-Oo” has highest caffeine, catechin and antioxidant activity. It is considerable that “Shwephi-Oo” is the foremost plucked season kind at the beginning of summer. It was free from rain and the leaves were fresh and best strength. Therefore, it is confirmed that the quality of tea leaves depend upon the plucked season as well as other geographical conditions.

**Table 4 The amounts of caffeine, catechin mixture and antioxidant activities of six kinds of dried tea leaves**

Sample	Amount of parameters		
	Caffeine (%)	Catechin mixture (%)	Antioxidant activity (DPPH scavenging assay, IC <sub>50</sub> value), (µg/L) ascorbic acid (Standard)
Shwephi-Oo	0.200	2.0	2.3
Shwephi-Hnaung	0.075	1.3	2.7
Khakan-Oo	0.100	0.4	4.8
Khakan-Hnaung	0.100	0.6	4.1
Kha-Naing	0.125	0.8	4.7
Kha-hawt	0.075	0.5	5.9

## CONCLUSION

From the experimental results, six kinds of dried tea leaves in Namhsan region have valuable nutritional values and high antioxidant activity. Among them, the selected mineral contents, total ash, catechin mixture, caffeine content and antioxidant activity were found higher rich in “Shwephi-Oo” than another five kinds of dried tea leave apart from protein, crude fiber, Zn and Fe contents. Therefore, “Shwephi-Oo” kind of dried tea leave is generally regarded as the best quality for consumers.

## ACKNOWLEDGEMENTS

The authors would like to express special gratitude to Pro-rector Dr Win Win Ei, Taunggyi University and our Ministry of Education for giving us the opportunity to present this research paper. I also thanks to Professor and Head, Dr Ah Mar Yi, Department of Chemistry, Taunggyi University, our co-workers and friends for their guidance, helpful and encouragements during this field. We also thank the reviewers of IJERD for their help in improving the quality of our research paper and their critical reviews.

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Recently, in developing countries, subsistence agriculture is being converted to export-oriented mono-culture, and the amounts of agricultural chemicals applied to the farmland are increasing every year. The applied chemicals in farmland cause serious environmental problems downstream such as eutrophication, unusual growth of aquatic plants, decrease in dissolved oxygen and accumulation of bottom mud in water resources. Also, there seem to be many cases in which people apply agricultural chemicals without understanding its impact to health and food safety. Therefore, it is necessary to promote and enhance understanding of sustainable rural development among local stakeholders including farmers.

Sustainable rural development aims to meet human needs while preserving the natural environment. As it should cover not only social and economic development but also natural environment conservation, no single organization can achieve sufficiently the aspirations of sustainable rural development. Collaboration among international, governmental and non-governmental organizations, together with the academe and scientific sector, is indispensable.

The knowledge and intelligence accumulated in universities and research institutions are also expected to make the programs facilitated by the international, governmental and non-governmental organizations more adequately implemented and meaningful to societal development. However, these cases especially those implemented locally have been scattered without having been summarized well or recorded in annals academic or scientific societies.

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