Title	Investigation on the Enzyme activity of TAUNG- THAMAN Lake soil Treated with water Hyacinth Fertilizer
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INVESTIGATION ON THE ENZYME ACTIVITY OF TAUNG –THAMAN LAKE SOIL TREATED WITH WATER HYACINTH FERTILIZER

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ABSTRACT

In this research paper, Taung-thaman Lake soil sample was collected from the place that is situated near Owe-bo village, Amarapura Township, Mandalay Region. Water hyacinth fertilizer was prepared with EM (Effective microorganism) solution. EM solution was prepared from wastes of the fruits and vegetables. Taung-thaman Lake soil sample was treated with water hyacinth fertilizer by different ratio. The pH values of soil samples before and after addition of water hyacinth fertilizer were measured by using pH meter. The moisture contents of soil samples before and after addition of water hyacinth fertilizer were determined by Oven method. The elemental contents of soil samples before and after addition of water hyacinth fertilizer were measured by EDXRF. Enzyme activity (glucosidase and protease activity) of soil samples before and after addition of water hyacinth fertilizer were determined by spectrophotometric method.

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INTRODUCTION

The world population is increasing day by day and year by year. There is an urgent need for increasing food production to feed the increasing population. Myanmar Naing Nagn, the agricultural country is trying to increase the yield of not only rice but also crops. Yield of many crops must be quantitatively high and qualitatively excellent. High yield of crops depend on good soil.

Soil is one of the factors for growing crops. Special attention should be paid to the chemical analysis of soil. Agricultural products may vary from year to year depending on the constituents of the soil. Soil quality can be changed by using natural fertilizers, manure and humus. Manure is

obtained by the decomposition of animal dung and urine. Humus comes from plant residues. (Emerson, P., 1925)

Soil enzymes are used as soil quality indicators. Soil enzymes mediate biochemical transformations involving organic residue decomposition and nutrient cycling in soil. Decomposition of plant or animal residues in soil releases essential nutrients required for plant growth. Soil is a dynamic living system where all biochemical activity proceeds through enzymatic processes.

Water hyacinth fertilizer, organic fertilizer plays vital role for better production and becomes alternative source of essential plant nutrients. Moreover the organic wastes were increased and become environmental problem. Large quantities of water hyacinth waste are available from Taung-thaman Lake. (Archana, S., 1971)

Enzymes are the vital activators in life processes, likewise in the soil they are known to play an essential role in maintaining soil health and its environment. Soil enzyme play key biochemical functions in the overall process of organic matter decomposition in soil system. The enzymatic activity in the soil is mainly of microbial origin.

All soils contain a group of enzymes that determine soil metabolic processes. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amount of organic matter content. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes.

Soil enzymes may include amylase, arylsulphtases, glucosidase, chitinase, dehydrogenase, phosphatase, protease and urease released from plants, animals, organic compounds and microorganisms. Soil nutrient such as organic matter are important drivers of soil microbial community composition. Soil is a living dynamic system containing many free enzymes.

Soil enzymes play critical role in catalyzing reactions leading to organic matter decomposition and serve as bioindicators of biochemical and microbial soil activity. In this research Taung-thaman Lake soil samples treated with water hyacinth fertilizer were used to measure enzyme (α-glucosidase and protease) activity.

Botanical Description of Water Hyacinth

Figure (1.1) Water Hyacinth

Family name	-	Pontederiaceae
Botanical name	-	*Eichhornia crassipes* (Mart). Solms
English name	-	Water Hyacinth
Local name	-	Beda

Fertilizer

A fertilizer is a material supplies nutrients to plants, the technical term "fertilizer" denotes any materials, organic or synthetic applied to the soil or sprayed on plants to add nutrients for plant growth.

Fertilizer, organic or inorganic mineral substances occurring in nature or manmade are used as plant nutrients to engender changes in the chemical properties of the soil for plant growth. Chemical salt, which contains plant nutrients were used as fertilizers only during the last 100 years.

Fertilizers are an increasingly important factor in a prosperous agriculture and fertilizers are used primarily to increase the supply of available plant nutrients in the soil and also to balance the plant-nutrient ratio. (Boyd, C. E. 1968)

Humus

Humus comes from plant residues. Humus gives the soil a dark color and retains nutrients and water. It cannot easily be decomposed further. The fine organic matter, and humus in particular, have the excellent

properties. It improve the soil structure and the resistance of the soil against the erosive action of rain and wind. It retains water and releases it slowly, so that water is available to the plants (water storage capacity) over a longer period. It retains nutrients and releases them to the plants slowly over a longer period. And then it contains the main nutrients: nitrogen (N), phosphorus (P) and potassium (K), which become available to the plants after decomposition.

The micro-organisms are mainly responsible for further breaking down part of the humus into carbon dioxide, water and nutrients for the plants. This process is called mineralization: nutrients are released and can be taken up directly by plant roots.

The rate of humus production and mineralization in the soil depend on a number of factors. In a hot climate the micro-organisms are more active and the organic materials will break down more rapid than in a cold climate. Also the acidity of the soil, the composition of the organic matter, the humidity and the availability of oxygen strongly influence the rate of decomposition.

Soil Quality Indicators

Soil enzymes increase the reaction rate at which plant residues decompose and release plant available nutrients. The substance acted upon by a soil enzyme is called the substrate. For example glucosidase (soil enzyme) cleaves glucose from glucoside (substrate) ,a compound common in plants.

Soil enzyme play an important role in organic matter decomposition and nutrient cycling. Enzymes are the direct mediators for biological catabolism of soil organic and mineral components.

Some soil enzymes are Amylase, Arylsulphtase, Glucosidase, Cellulase, Chitinase, Dehydrogenase, Phosphatase, Protease and Urease. It is very essential to understand the possible roles of soil enzymes in order to maintain soil health and its fertility management in ecosystems. These enzymes, usually found in the soil, may have significant effects on soil biology.

Glucosidase

Glucosidase is a common and predominant enzyme in soils. It is named according to the type of bond that it hydrolyses. This enzyme plays

an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various glucosidase present in plant debris decomposing in the ecosystem. Its final product is glucose, an important carbon energy source of life to microbes in the soil. Glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilize the soil organic matter, and can be used to detect management effect on soils.

Protease

Proteases in the soil play a significant role in N mineralization, an important process regulating the amount of plant available N and plant growth. This enzyme in the soil is generally associated with inorganic and organic colloids. The amount of this extra cellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem. There is a need to study the properties and factors affecting naturally occurring enzyme complexes such as those involving protease enzymes in the soil ecosystem as they may reveal some unknown role(s) in maintaining soil health and fertility.

MATERIALS AND METHODS

Sample Collection

Taung-thaman Lake soil sample was collected from the place that is situated near Owe-bo village, Amarapura Township, Mandalay Region. Water hyacinths were collected from Taung-thaman Lake, Amarapura Township, Mandalay Region. Water hyacinths were cut into small pieces and then allowed to dry at room temperature.

Preparation of EM Solution

Wastes of the fruits and vegetables from Tagontine bazzar and six litre of water were mixed in bucket to ferment for two months. After two months, EM (Effective Microorganism) solution was obtained.

Figure-(1) EM (Effective Microorganism) solution

Preparation of Water Hyacinth Fertilizer

The water hyacinth samples (200g) and 2 Liter of EM solution were mixed. This creates a condition which allows for fermentation to occur. It allows to ferment for 2 month. The prepared fertilizer produced bad fermented smell. If the smell is putrid smell, in a state of foul decay or decomposition, it should be dried. In this way, organic fertilizer was obtained.

Figure-(2) EM (Effective Microorganism) solution and Water Hyacinth

Determination of Yield Percent of Prepared Water Hyacinth Fertilizer

The prepared fertilizer were dried and the yield percent of prepared water hyacinth fertilizer was determined based upon the total weight of adding selected material.

Table (1) Taung-thaman Lake Soil Sample Treated with Water Hyacinth Fertilizer by Different Ratio

Sample	Taung-thaman Lake soil (g)	Water hyacinth fertilizer (g)	Percent (%)	Various Ratio
I	100	0	0	100:0
II	95	5	5	95:5
III	90	10	10	90:10
IV	85	15	15	85:15

Sample I - Taung-thaman Lake Soil

Sample II - Taung-thaman Lake Soil Treated with Water Hyacinth Fertilizer (5%)

Sample III - Taung-thaman Lake Soil Treated with Water Hyacinth Fertilizer (10%)

Sample IV - Taung-thaman Lake Soil Treated with Water Hyacinth Fertilizer (15%)

Elemental Analysis of Soil Samples Before and After Addition of Water Hyacinth Fertilizer

The elements that contain in soil samples before and after addition of water hyacinth fertilizer were analyzed by using EDXRF at Department of Physics, University of Mandalay.

Estimation of pH

About 2.5 g of sample was weighed accurately and placed into a bottle and 10 cm^3 of distilled water was added (the ratio of sample to water was 1:4) and shaken for half an hour. Then pH was measured by digital pH meter.

Determination of Moisture

Accurately weighed (20g) of sample powder was added into a petridish previously dried and cooled in a desicator. The dish containing the sample was placed in an oven and dried for 30 minutes at 101° ± 1°C.

The dish then removed from the oven and cooled in a desicator at room temperature and weighed. The procedure was replaced until the loss in weigh did not exceed 0.05% per minute during the drying period.

Calculation

$$\text{Moisture \%} = \frac{\text{loss in wt (g)}}{\text{wt of sample (g)}} \times 100$$

Construction of Calibration Curve for Standard Glucose Solution

Sample

Glucose (analar)

Solutions Prepared

(1) Preparation of 0.2 % Benzoic Acid Solution

(2) Preparation of Standard Glucose Solutions

(3) Alkaline Copper Reagent Solution (Somogyi Reagent)

(4) Arsenomolybdate Colour Reagent Solution (Nelson Reagent)

Procedure

Standard glucose solution (0.5 ml) was pipetted into a test tube containing 1 ml of alkaline copper reagent solution and the contents were mixed well. The test tube was heated on a vigorously boiling water bath for 10 min.

Next the test tube was cooled under running tap water for 1 min and 1 ml of arsenomolybdate colour reagent solution was added to the test tube. The solution mixture gave a deep blue colour after shaking vigorously. This solution was diluted to 10 ml with distilled water and mixed by inversion. Arsenomolybdate chromogenic solution was obtained. Similarly, 0.5 ml of standard glucose solutions II, III, IV and V were prepared according to the above procedure.

A blank solution was prepared by carrying out as described above except that 0.5 ml of distilled water was used instead of 0.5 ml of standard glucose solution.

The absorbances of five arsenomolybdate chromogenic solutions obtained from standard glucose solutions I, II, III, IV, and V were measured at 750 nm with a UV-visible spectrophotometer.

Determination of Glucosidase Activity of Soil Samples before and after Addition of Fertilizer

Sample

Each soil sample (I, II, III and IV)(2g) was dissolved in 10 ml of acetate buffer.

Solutions Prepared

(1) Preparation of 0.2 M Acetic Acid Solution

(2) Preparation of 0.2 M Sodium Acetate Solution

(3) Preparation of 0.1 M Acetate Buffer Solution (pH 5)

(4) Preparation of 2 % Starch Solution (pH 4.8)

(5) Preparation of Soil (Glucosidase) Solution

Soil samples (1 g) was dissolved in 10 ml of acetate buffer (pH 4.8) and the volume made up to 100 ml with the buffer in a volumetric flask.

Procedure

The soil enzyme solution, sample I (0.1 ml) was added to a test tube containing 0.1 ml of 2 % starch solution (pH 4.8) and the solution mixture was incubated at 37°C for 10 min. Similarly sample II, III and IV solution were prepared as mentioned above. After incubation for 10 min, 1 ml each of alkaline copper reagent solution was added to each test tube containing the solution mixture to stop the reaction and mixed well.

Then the test tubes were heated on a vigorously boiling water bath for 10 min and cooled under running tap water for 1 min. One ml each of arsenomalybdate colour reagent solution was added to each test tube and mixed well. The volume was made up to 10 ml in a test tube with distilled water and mixed thoroughly.

The remaining procedure was the same as that mentioned above except that 0.2 ml of standard glucose solution III and 0.2 ml of acetate buffer (pH 4.8) were used as blank solution instead of 0.1 ml of enzyme solution and 0.1 ml of 2 % starch solution (pH 4.8).

The absorbance of the solutions were measured at 750 nm with a UV-visible spectrophotometer and the blank solution was used as reference.

Determination of Protease Activity of Soil Samples before and after Addition of Fertilizer

Sample

Each soil sample (I, II, III and IV)(2g) was dissolved in 10 ml of potassium phosphate buffer, pH 7.5.

Solutions Prepared

(1) Preparation of 0.05 M Potassium Phosphate Buffer, pH7.5 Solution
(2) Preparation of 0.65% Casein Solution
(3) Preparation of 0.11M Trichloroacetic Acid Solution

Procedure

The soil enzyme solution, sample I (0.1 ml) was added to a test tube containing 0.1 ml of 0.65% casein solution and the solution mixture was incubated at 37°C for 10 min. Similarly sample II, III and IV solution were prepared as mentioned above. After incubation for 10 min, 1 ml each of trichloroacetic acid solution was added to each test tube containing the solution mixture to stop the reaction and mixed well.

The absorbance of the solutions were measured at 660 nm with a UV-visible spectrophotometer and the blank solution was used as reference.

RESULTS AND DISCUSSION

Yield Percent of Prepared Organic Fertilizer

The yield percent of prepared organic fertilizer was determined based on the total weight of material that was used. The result was shown in Table (2).

Table (2) - Yield Percent of Prepared Water Hyacinth Fertilizer

Total weight of adding water hyacinth (g)	Dried weight of prepared water hyacinth fertilizers (g)	Yield (%)
200	135.71	67.85

pH Values of Soil Samples

pH values of soil samples before and after addition of water hyacinth fertilizer were determined and the results are shown in Table(3).

Table (3) Results of pH values for Soil Samples Before and After Addition of Fertilizer

No.	Sample Solutions	pH
1.	I	7.3
2.	II	7.5
3.	III	7.6
4.	IV	7.6

There were no appreciable changes in pH values of soil samples before and after addition of water hyacinth fertilizer.

Elemental Present in Selected Samples

The elements that contain in selected samples were analyzed by using EDXRF at Department of Physics, University of Mandalay. These results are shown in Table (4).

Table (4) - Elemental Present in Selected Samples by EDXRF Analysis

No	Element	Unit	Soil	Water Hyancinth	Soil + Fertilize
1	Aluminiu	(%)	5.69100	0.24700	4.08200
2	Silicon	(%)	16.2200	0.67100	11.3200
3	Phosphoro	(%)	0.03853	0.21920	0.18520
4	Sulphur	(%)	0.00706	0.08100	0.04079
5	Chlorine	(%)	0.02169	6.24100	2.28400
6	Potassium	(%)	1.78400	2.60000	2.49100
7	Calcium	(%)	1.25000	1.84300	1.75400
8	Titanium	(%)	0.44800	0.02370	0.30520
9	Vanadium	(%)	0.01081	0.00270	0.00707
10	Chromium	(%)	0.00927	0.00430	0.00639
11	Manganes	(%)	0.05351	0.09920	0.04716
12	Iron	(%)	4.17000	0.34780	2.91200
13	Copper	(%)	0.00347	0.00070	0.00256
14	Zinc	(%)	0.00959	0.00460	0.00803
15	Bromine	(%)	0.00023	0.00400	0.00133
16	Rubidium	(%)	0.01658	0.00240	0.01245
17	Strontium	(%)	0.00898	0.00658	0.00906
18	Barium	(%)	0.05530	0.02360	0.03630

Moisture Content of Selected Samples

The moisture content of selected samples were determined and these results are shown in Table (5).

Table (5) - Percentage of Moisture Present in Soil Samples Before and After Addition of Fertilizer

No.	Samples	moisture content (%)
1.	I	6.514
2.	II	4.000
3.	III	3.400
4.	IV	3.500

The moisture content of soil sample before addition of fertilizer was greater than that of soil samples after addition of fertilizer.

Construction of Calibration Curve for Standard Glucose Solution

For quantitative analysis of a compound by visible spectroscopy, it is firstly necessary to know the wavelength of maximum absorption (λ_{max}). The wavelength of maximum absorption was found at 750 nm.

In the present research standard glucose solutions were made to react with Nelson Somogyi reagent and the absorbances of standard glucose solutions I, II, III, IV and V were determined by UV-visible spectrophotometer. The results are shown in table 6.

Table (6) Relationship between Absorbance and Concentration of Standard Glucose Solution

Std Glucose Solution	Conenration (x 10^{-6}M)	Absorbance
I	6.94	0.1530
II	9.72	0.4400
III	13.88	0.6930
IV	19.43	0.9900
V	27.75	1.2820

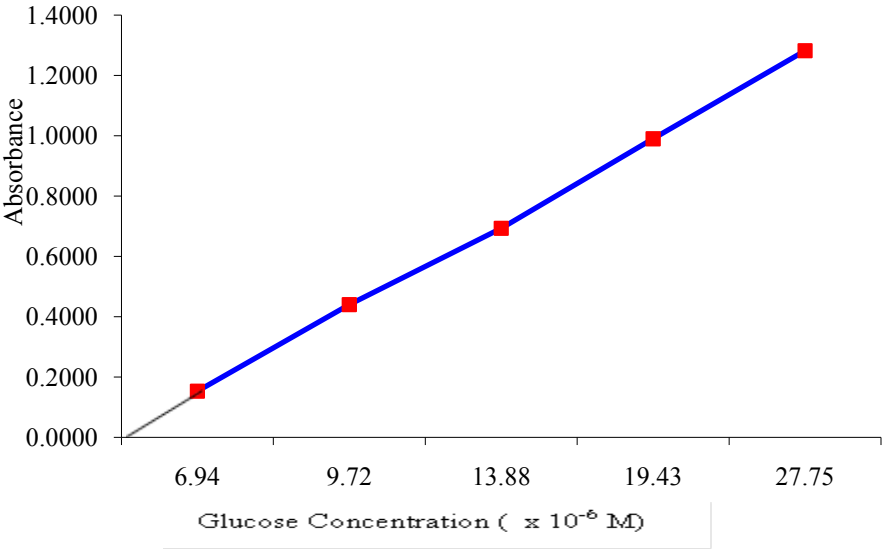

Figure (3) - Plot of Absorbance as a Function of Concentration of Glucose

Glucosidase Activity of the Sample Solution

Estimation of glucose formed by the reaction of starch and sample solutions (glucosidase enzyme) was carried out by Nelson-Somogyi method. Glucose concentrations can be determined directly by UV spectroscopy. Enzyme activity can be calculated by the equation.

$$\text{Enzyme activity} = \frac{\text{Glucose formed from enzyme action}(\mu\text{mole})}{\text{time (min)} \times \text{volume of enzyme used (mL)}}$$

Table (7) Glucosidase Enzyme Activity of Soil Samples

Sample Solutions	Absorbance at 750nm	Molarity of Glucose (x 10^{-6} M)	Amount of Glucose (x 10^{-6} mmole)	Enzyme Activity (x 10^{-5} µ mole /min/ml)
I	0.447	10.643	106.43	106.43
II	0.517	12.310	123.10	123.10
III	0.611	15.738	157.38	157.38
IV	0.736	17.524	175.24	175.24

Glucosidase enzyme activity (carbon cycling function) of soil sample solutions were increased with increase in fertilizer ratio.

Protease Activity of the Enzyme Solution

The absorbance of samples was measured by a spectrophotometer using a respective wavelength. The absorbance values of samples was converted to the amount of product (tyrosine) using standard curve. Standard curve was used from literature. Enzyme activity can be calculated by the equation.

$$\text{Enzyme activity} = \frac{\text{Glucose formed from enzyme action}(\mu\text{mole})}{\text{time (min)} \times \text{volume of enzyme used (mL)}}$$

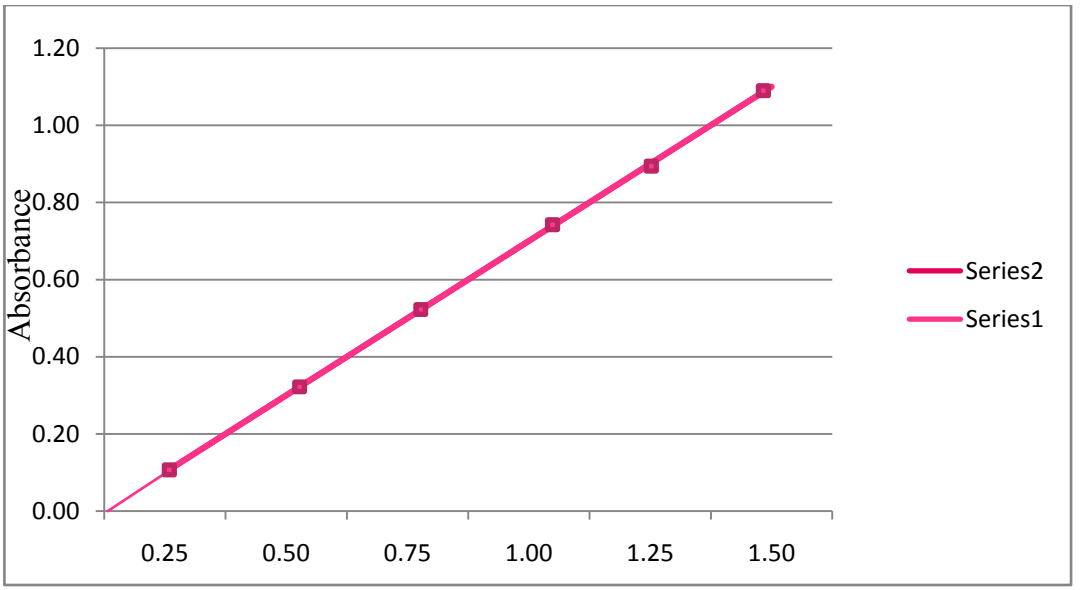

Figure (4) -Plot of Absorbance as a function of concentration of standard tyrosine solution

Table (8) Protease Enzyme Activity of Soil Samples

Sample Solutions	Absorbance at 750nm	Molarity of Tyrosine (x 10^{-4} M)	Amount of Tyrosine (µmole)	Enzyme Activity (x 10^{-2} µ mole /min/ml)
I	0.051	5.856	5.856	5.856
II	0.057	6.575	6.575	6.575
III	0.067	7.679	7.679	7.679
IV	0.074	8.508	8.508	8.508

Protease enzyme activity (nitrogen cycling function) of soil sample solutions were increased with increase in fertilizer ratio.

CONCLUSION

In this research paper, Taung-thaman Lake soil sample was collected from the place that is situated near Owe-bo village, Amarapura Township, Mandalay Region. Water hyacinth fertilizer was prepared with EM (Effective microorganism) solution. EM solution was prepared from wastes of the fruits and vegetables.

Taung-thaman Lake soil sample was treated with water hyacinth fertilizer by different ratio. Sample I was taken Taung-thaman Lake soil before the addition of fertilizer. Samples II, III and IV were taken Taung-thaman Lake soil samples treated with water hyacinth fertilizer (5%, 10% and 15%) ,10 days later the addition of fertilizer.

The elemental contents of soil samples before and after addition of water hyacinth fertilizer were measured by EDXRF. Water hyacinth contains high amount (integer value) of chlorine, potassium and calcium and moderate amount (one decimal) of aluminum, silicon, phosphorous and manganese.

Soil contains high amount (integer value) of aluminum, silicon, potassium, calcium and iron moderate amount (one decimal) of titanium and (two decimal) phosphorus, chlorine, vanadium, manganese , rubidium , zirconium and barium.

Soil treated with fertilizer contains high amount (integer value) of aluminum, silicon, chlorine, potassium, calcium and iron moderate amount (one decimal) of phosphorus and titanium and (two decimal) sulfur , manganese, rubidium, zirconium and barium .

The pH values of soil samples before and after addition of water hyacinth fertilizer were measured by using pH meter. There were no appreciable changes in pH values for soil samples before and after addition of water hyacinth fertilizer.

The moisture contents of soil samples before and after addition of water hyacinth fertilizer were determined by Oven method. The moisture content of soil sample before addition of fertilizer was greater than that of soil samples after addition of fertilizer.

Enzyme activity of soil samples were determined by spectrophotometric method. Glucosidsase activity (carbon cycling function) was measured by using starch (substrate) and acetate buffer system.

Glucosidsase activities were increased with increase in fertilizer ratio. This enzyme plays an important role in soil because it is involved in catalyzing the hydrolysis and biodegradation of various glucosidase present in plant waste decomposing in the ecosystem.

Protease activity (nitrogen cycling function) of soil samples was measured by using casein (substrate) and phosphate buffer system. Protease activities were increased with increase in fertilizer ratio. Soil enzyme activities are the direct expression of the soil community to metabolic requirements and available nutrients. It was suggested that further enzyme activities (urease, phosphatase, amidase , dehydrogenase , etc:) and other substrate for this fertilizer should be studied.

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REFERENCES

Archana, S. 1971. Eradication and utilization of water hyacinth. A review. Current Sci. 4:51-55.

Boyd, C. E. 1968 Evaluation of some common aquatic weeds as possible feed-stuffs. Hyacinth Contr.J.7:26-27.

Emerson, P., (1925), "Soil Characteristics", Mc Graw-Hill Book Co, Inc., London.

Kamal, I. A. and E. C. S. Little. 1970. The potential utilization of water hyacinth for horticulture in the Sundan. PANS 16:488-496.

Knipling, F. C. S. H. West and W. T. Haller. 1970. Growth characteristics, yield potential, and nutrient content of water hyacinths. Soil and Crop Sci. Soc. Of Fla. Proc. 30:51-63.

Miner, J. R. J. W. Wooten, and J. D. Dodd. 1971. Water hyacinths to further treat anaerobic lagoon efflunent.

In Live stock waste management and pollution abatemint. Intern. Sympossium on Livestock Waste. Amer. Soc. Agr. Eng. Proc. 271:170-173.

Rogers, H. H. and D. E. Davis. 1972. Nutrient removal by water hyacinth. Weed Sci.20:423-428.