EVALUTION OF VERMIWASH FROM LOCALLY AVAILABLE ORGANIC MATERIALS

By

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DECLARATION

The work described in this thesis was carried out by me at the University of Peradeniya, Faculty of Agriculture and Department of Soil Science under the supervision of Dr. K.A. Nandasena and Dr. Tilak Hewawasam. A report on this has not been submitted to any other University for another degree.

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ABSTRACT

Vermiculture biotechnology is a modern concept of harnessing an ecosystem for effective utilization of organic residuals with the help of earthworms. Earthworms are one of the important members of soil fauna, which is traditionally been known as beneficial in agriculture. The role of earthworms in farming was known to farmers since long, but their role in waste treatment is a new area. Earthworms can convert organic wastes in to valuable products such as; vermicompost, vermicast, vermiwash, vermibiopesticides, vitamins, enzymes and antibiotics.

The present research was conducted to evaluate the composition of vermiwash produced from the locally available organic materials such as rice straw and cow dung. There were two treatments such as vermiwash composting unit and control composting unit. Rice straw cow dung mixture was inoculated with earthworms and used as vermiwash composting unit. Control composting unit was prepared without earthworms. This experiment was done in replicates (three vermiwash composting units and three control composting units). All units were moistened every day using 100ml of distilled water. Extract (from vermiwash composting units and control composting units) was collected monthly over three months and subjected to chemical analysis.

According to the statistical analysis, there was a significant difference (Pr>t, 0.05) between two treatments (vermiwash composting samples and control composting samples). Organic Carbon, Phosphorus, Potassium, Sulphur, Calcium and Magnesium were showed significantly higher values in vermiwash composting samples than control composting samples. There was no significant difference for nitrogen and copper in both treatments. Treatment effect was varied for some nutrients (Zinc, Manganese) for some months. Variation of nutrients values with time were observed for Organic Carbon, Nitrogen, Phosphorus, Sulphur and Manganese.

From the results, it can be concluded that the vermiwash produced from the locally available organic materials could be used as an environmentally friendly foliar organic fertilizer in agriculture.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Introduction

Relatively little fertilizer is currently used in the developing countries because most of the farmers can not find the cash to buy it especially since the recent increase in fertilizer prices. Fertilizer usage in developing countries is growing slowly and may be expected to continue to do so, aided by increasing local manufacture of green manuring, bulky organic manuring, composting, and vermicomposting. (Webster and Wilson, 1980).

Composting is a natural process that occurs in nature in which organic matter is decomposed by microorganisms forming humus like substances. The finished product obtained from composting may have fertilizer value. Consequently compost can be used for improving the fertility of marginal and arable lands. The process itself is not new. Many farmers have found compost to make excellent materials for litter or bedding (Merkel, 1981). Vermiculture biotechnology is a modern concept of harnessing an ecosystem for effective utilization of organic residues with the help of earthworms (Pandy, 1999).

The role of earthworms in farming was known to farmers since long, but their role in waste treatment and producing vermicompost and vermiwash are new areas to develop an alternative and economic method for the treatment of sewage generated by small community through vermiculture (Pandy, 1996). Vermiculture biotechnology harnesses non toxic organic residues both solid as well as liquid as substrates to produce vermi casting, vermicompost and vermiwash are resource for sustainable agriculture and wasteland development.

Vermicompost is a method of making compost with the use of earthworms, which generally live in soil, eat biomass and excrete it in digested form. It is estimated that 1800 worms which is an ideal population for one square meter can feed on 80 tonnes of humus per year (Dahama, 1996).

Vermicasting is the end result of the feed taken in by earthworms. These earthworm castings contain a considerable quantity of humus of more than 20% and other way quality depends on the 'Waste' taken in by the worms and the Nitrogen, Phosphorous and Potassium (NPK)

content of the feed. The humus portion of casting is responsible for stabilization of organic matter.

Vermiwash is the liquid fertilizer collected after passage of water through a column of worms acted soil. Vermiwash is a one, which can be prepared at low cost and also a good foliar spray. It can be sprayed on plants as a foliar spray or may be diluted in 10% cows urine as organic pesticides.

The present study was planned with the overall objectives of evaluating the composition of vermiwash prepared using locally available organic materials.

1.2 Objective

Objective of the study;

1. To evaluate the nutritive values of vermiwash produced from locally available organic materials.

CHAPTER 2
LITERATURE REVIEW

2.1 Plant nutrition

Arable plants require nutrients, water, sunlight, and optimum atmospheric and soil environment. Nutrients and water are taken by the plants from soil via their root system (Somani, 1996). Plants can absorb and use nutrients only if they are in simple forms, usually ionic (Simpson, 1986).

2.2 Nutrient sources

Crop and animal residues, fertilizer, compost and domestic and industrial wastes are the principal sources of plant nutrients. In Sri Lanka farmers mainly use chemical fertilizers and organic manure such as compost and cow dung. Recently, however, farmers turn to use different organic products such as vermiwash and vermicompost for their crops.

2.2.1 Composting

Composting is an ancient practice where by farmer a have converted organic wastes on to resources that provide nutrients to crops and enhance soil tilth, fertility and productivity. Through composting, organic wastes are decomposed, nutrients are available to plants (Gupta, 1999). It has been in practice for many countries by farmers who have stacked animal manure in to piles or gardeners who have placed garbage, leaves, grass cutting, etc in to pits (Merkel, 1981).

The aerobic decomposition takes place in piles and in bins kept sufficiently moist to support the decay. Home production of compost is more widespread and has increased in recent years due to restrictions on the burning of leaves and other domestic wastes in residential areas. This is a blessing in disguise composting ensures the conservation of some nutrients that are lost when residues are burned (Brady, 1990).

The finished product from composting may have fertilizer value. Many farmers have found compost to make excellent materials for litter or bedding. It is moisture absorbent, odorless,

and eliminate the need to purchase bedding from an outside source. Consequently compost can be used for improving the fertility of marginal and arable land.

2.2.2 Essentials of composting

As already mentioned the basic requirements for compost making are the compostable waste materials, air and moisture (Agarwal, 1985).

2.3 Soil fauna

The soil fauna is based on the principle that soil fauna can regulate decomposition process. The activities of a wide range of organisms affect physical properties of the soil they inhabit (Reddy, 1995). In soil fertility related work, soil fauna can be classified in to ecological groups to help in understanding their role in organic matter formation and decomposition. Mainly on two dominent groups, Termites and Earthworms (Woomer and Swift, 1994).

2.3.1 Effects of soil fauna

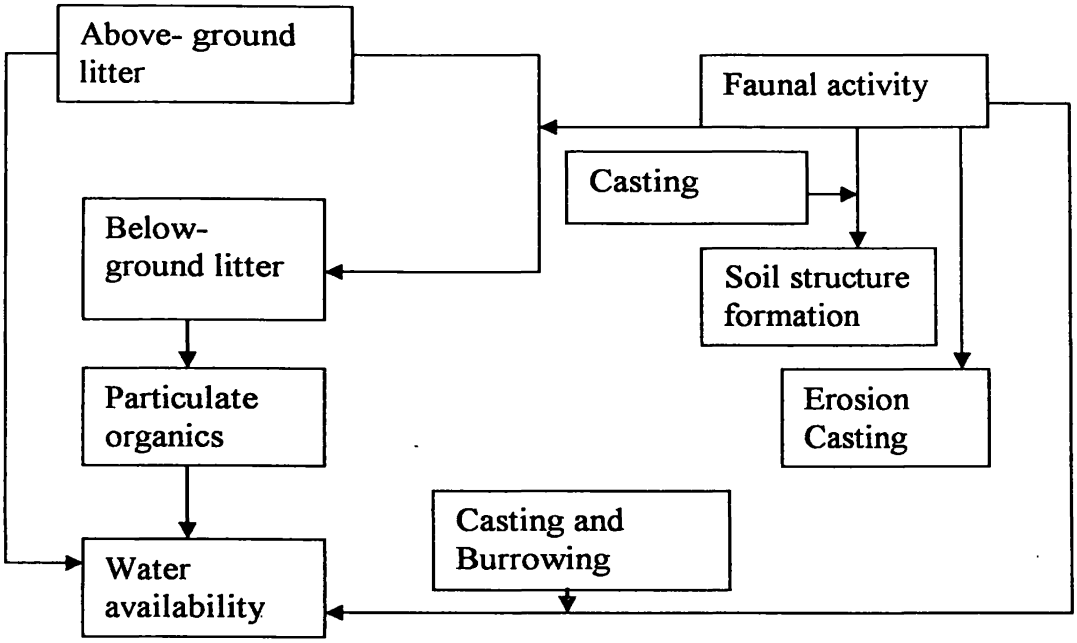

Fig 2.1 Effects of soil faunal activities on soil processes (From Woomer and Swift, 1994).

2.4 Earthworms

Earthworms can be defined as terrestrial invertebrates that belong to the order Oligochaeta, class Chaetopod, phylum Annelida. Their characteristic features are that they are externally segmented with a corresponding internal segmentation, and usually have setae on all segments. They are hermaphrodite, and produce cocoons contain eggs, which hatch in to immature worms that are similar to the adults except in size and development their genital organs. Earthworms range in size from a fraction of cm, to exceptional individuals, which may measure 2.75cm in length and 3cm in diameter (Edwards and Lofty, 1977).

2.4.1 General activity of earthworms

Earthworms occur in diverse habitats. Organic materials like manure, compost, litter, humus effluents and kitchen drainage are highly attractive for some species. Earthworms are omnivores but they mostly derive nutrition from dead organic matter, which generally does not occur abundantly in the soil. As a result they are adapted to swallow large quantities of soil for extracting sufficient nourishment from it. The soil inhabiting protozoan, nematodes,

rotifers, bacteria, fungi, etc have been recorded from the content of their gut. They are capable of with standing considerable starvation with water loss of up to 70% of their body weight (Jairajapuri, 1993).

The earthworms consume the soil organic matter and convert it to humus with a short period of time. With 24 hours they can pass soil equivalent to their own weight through the alimentary cannel. The activity of most earthworms is interrupted during dry periods or under high temperatures. To overcome the adverse period they usually move in to the deeper soil layers and may undergo 'diapause' or transform in to quiescent stage (Jairajapuri, 1993).

2.4.2 Ecological categories of earthworms

Three main ecological categories (strategy based) have been proposed to classify earthworms: Epigeic, Anecic, and Endogeic.

2.4.2.1 Epigeic

They are litter dwellers, creating to burrow systems in the soil. So their effects are limited to the upper few centimeters of the soil litter interface. They are litter transformers. By community this litters, they modify its physical and chemical status. Epigeics are unable to survive in disturbed ecosystems, unless there is a significant litter components (Fragoso *et al.*, 1997). They include saprophagous arthropods and small pigmented earthworms (Woomer and Swift, 1994).

2.4.2.2 Anecic

They are soil dwellers, creating permanent burroes in the soil. They are large in size, dorsally pigmented and feed on litter, and animal excreta, and originally rich in soil. Since Anecic depend on the presence of litter for their survival, their role in soil function in intensifying agricultural systems is likely to be small unless a significant litter system is present (Fragoso *et al.*, 1997).

2.4.2.3 Endogeic

They are soil dwellers and soil feeders. They have developed complex interactions with soil microorganisms to obtain nutrients from low quality foods. Also predominantly make horizontal burrows (Fragoso *et al.,* 1997).

Epigeic can be largely used in the composting processes and Anecics can be used in both composting and soil improvement process. Endogeics can be used in soil forming processes (Ismail, 1996).

2.4.3 Benefits of earthworms

2.4.3.1 Role in organic matter cycle

Plant organic materials that reach to soil subjected to decomposition of both microorganisms and animals. The micro flora may decompose very soft materials but tougher plant materials like stems, root materials, do not breakdown without being disintegrated by some means. Few common earthworm species seem to be move responsible for large proportion of fragmentation of litter. They consume large amount of litter and the amount they turn over seems to be more. They pass a mixture of organic and inorganic matter through their guts when feeding or burrowing. Humification is the final stage of organic matter decomposition and this is basically the breaking down of large particles of organic matter to a complex amorphous colloids contain phenolic materials. It is said that the final stage of humification is due to intestinal micro flora in the earthworm guts (Edwards and Lofty, 1977).

2.4.3.2 Effect on nitrogen mineralization

Earthworms make available mineralized nitrogen for plant growth. They consume large amount of plant organic matter that contains considerable quantities of nitrogen, and much of it is returned to soil in their excretions. Earthworms greatly increase soil fertility and this could be a one reason for that (Edwards and Lofty, 1977).

2.4.3.3 Effect on microorganisms and decomposition

The excreta of earthworm contain more fungi, actinomycetes, and butric acid forming bacteria and cellulose decomposing bacteria. It has been reported that microorganisms in soil are increased as mush as five times by present of earthworms. Also it has been reported that growth of certain fungi stopped whenever an earthworm was introduced. Earthworm excreta (cast, etc) are rich in simple nitrogenous compounds, which may stimulate other microbial decomposition processes as well (Edwards and Lofty, 1977).

2.4.3.4 Effect on soil structure

Ingestion of soil particles breakdown of organic matter intimate mixing of these fractions, ejection of this materials as surface or subsurface cast, burrowing through the soil and bringing sub soil to the surface are the activities of earthworms that have most influence on soil structure. During these processes they throughly mix the soil form water stable aggregates, aerated the soil improve it is water holding capacity.

Soil with earthworms drain from four to ten times faster than soils without earthworms. Clearly they greatly increase the aeration and structure of soil. Also they influence the drainage of water from soil and moisture holding capacity of soil, which are important factors for growing crops.

2.4.3.5 Effect on soil amelioration

Adding earthworm cast to soil can improve greatly it is structure and fertility. Vermi casts usually have a higher pH, and more total and exchangeable magnesium, base capacity and moisture equivalent.

2.4.3.6 Effect on counteracting leaching

By the downward movement of water, many soluble mineral nutrients are lost to plant roots. This leaching is, in some measure, counteracted by the action of earthworms, which will especially if the nutrients are in organic form, consume them and returned them to the upper levels of the soil layer, where they are deposited in the form of casings (Minnich, 1977).

2.5 Earthworms in agriculture

In agriculture practices, earthworms play a key role as they improve the soil texture, enrich the soil, enrich the nutrients in the crops, act as powerful biopesticides and protect the useful micro flora of the soil (Pandy, 1999). They eat soil and rock particles as grinding medium for waste organic residues. Soil excretedly the earthworms has a natural acidity/alkalinity (pH) and contains balanced plant nutrients available form. These are held by the process of ion exchange, making them available to roots when required, but preventing their leaching to the ground water thus protecting their losses and consequent ground water pollution. They stopped up decomposition of crop residues and convert them in to balanced plant nutrients, water and carbon dioxide. These are produced and effectively utilized in a slow release manner (Pandy, 1999).

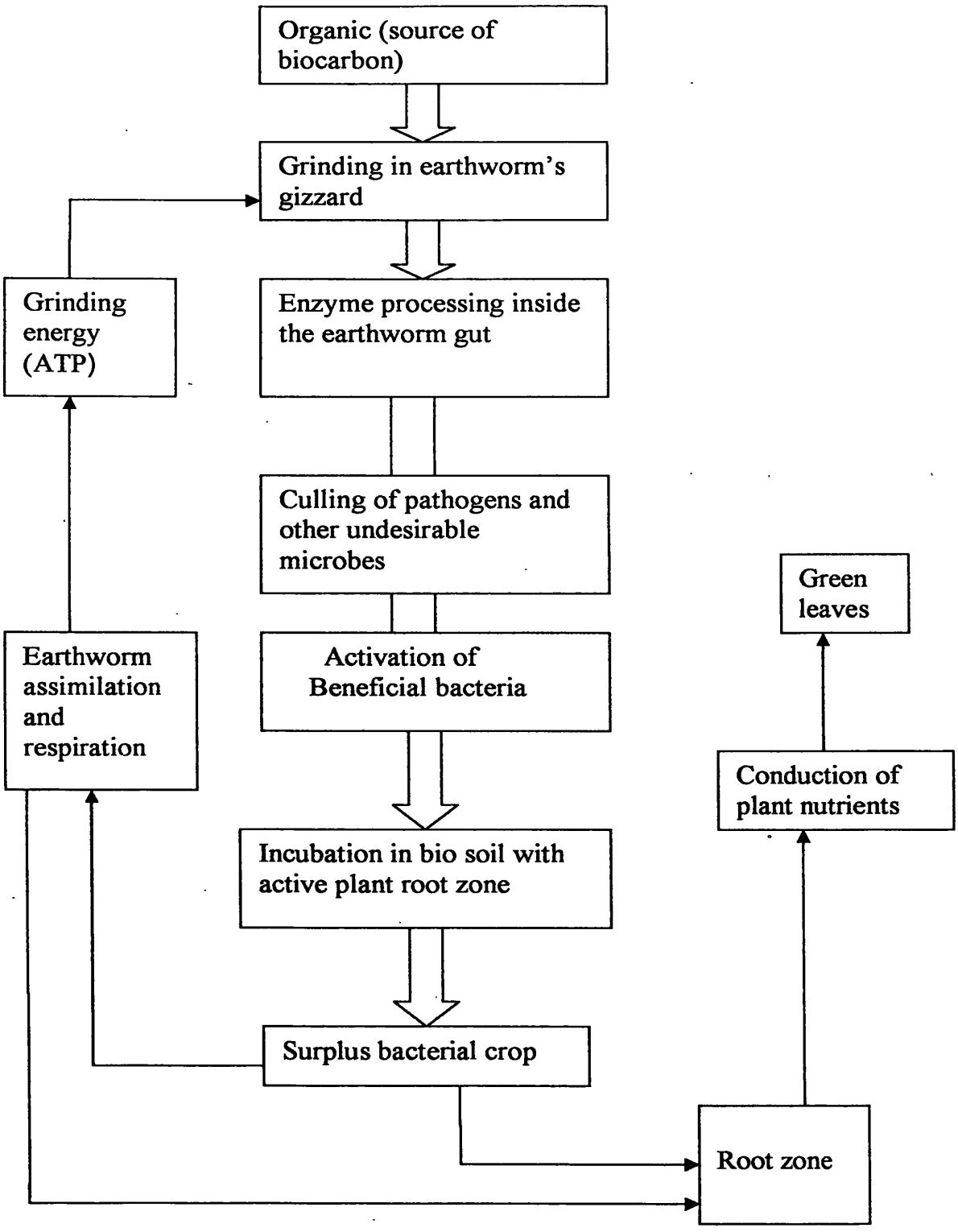

Fig 2.2 Earthworms farm bacteria and plants (From Pandy, 1999).

The living soil produced by the earthworms has an enhanced ability to absorb the atmospheric moisture. This soil is structurally stable. The beneficial microorganisms, which use crop residues to fix atmospheric nitrogen, stabilize the soil phosphorous, make potassium, calcium, magnesium, sulphur and essential microelements available produce vitamins, antibiotics and plant growth hormones. The earthworms as their food consume excess microorganisms. They eliminate soil pathogens and ineffective microorganisms by selective predation (Pandy, 1999).

The excrement or castings of earthworms, which consist largely have digested soil and particles of organic matter, is more chemically neutral than the surrounding soil. So by consuming soil, processing it and excreting the remainder as castings. Sufficient numbers of earthworms help keep a field closer to the neutral pH range. Soil that is excessively acidic or alkaline can inhibit the growth of plant and microbes.

Earthworms ensure proper utilization of organic residues such as sugarcane trash, banana stems, paddy and wheat straw, coir waste and weeds. Earthworms use these residues at the rate of 10 tons per hectare. Their fertilizer value is as good as other composts, which would need 200 tons of raw organic residues to produce the same amount. With the use of vermicasting, the cost of application of chemical fertilizers and pesticides is saved, labour in tilling the ground is not required and crops require less water for irrigation (Pandy, 1999).

2.6 Vermitechnology

It is the technology to use surface and sub surface local verities of earthworms in composting and soil management (Ismail, 1996).

Vermitechnology is the method of converting of waste to useful products through the action of earthworms. It comprises three main processes.

1. Vermiculture - Rearing of earthworms.
2. Vermicomposting – Biodegradation of waste biomass in an earthwormic way.
3. Vermiconservation – Mass maintenance of sustainability of wasteland through earthworms.

Utilizable product and benefits of vermitechnology are waste biomass management, animal protein production, organic abatement, wasteland conservation and land reclamation, production of worm worked manure, soil fertility and enhancement in plant production (Senapati, 1993).

2.6.1 Importance of vermitechnology

The availability of nutrients for sustained crop production has become a serious constraint in agriculture with the increased cost and shortage of fertilizers. Vermitechnology, which is concentrated on earthworms, could be made use of to meet needs for plant nutrients, recycling of biodegradable organic wastes and in solving problems of deteriorating soil conditions.

Environmental improvement is an accepted national goal. Because of low population and availability of inexpensive energy and enough raw materials, recycling of used materials was not considered necessary in the past. More ever, the amount of waste produced was within optimal limit and taken care of by nature. Under the present condition of acute energy crises and environmental degradation due to steep rise in population, it is very essential to develop suitable technology for recovery of energy from non conventional sources like organic wastes, which were once through to be of nouse. The concept of resource recycling particularly relevant to agricultural production. The problem of organic recycling in soil improvement and crop production may be tacked by,

1. Improvement in the process of composting by reduction in the processing period and enrichment in the quality and

2. Utilization of available organic residues an inorganic waste in the natural plant production cycle.

Waste biomass from domestic, agriculture, urban, and industrial sources are main causes of organic pollution in developing countries in which major portion of refuse, more than 60% contain decomposable materials. Appropriate disposal of waste is most essential and beneficial from ecological and economical point of view. Decomposers like earthworms are

also rate regulators and biocatalyst at organism level. They stimulate composting both in enhancing manuring value and decreasing time (Senapati, 1993).

2.6.2 Development of vermitechnology

2.6.2.1 At global level

The concept of vermitechnology was started from the middle of the 20th century. The first vermicomposting plant was setup in Holland. Since then vermicomposting has been earnestly undertaken in USA, Italy, Japan, and now being initiated in France, Israel, etc. Along with sufficient stories, there are also instances like the Philippines where vermicomposting industries have collapsed because of lack of social acceptance and extention education. Vermiconservation of wastelands is of recent origin and there is a greater scope for its development all over the world (Senapati, 1993).

2.6.2.2. At Sri Lankan level

In Sri Lanka, earthworm species were identified some extent by Stephenson in 1925. In the recent past the demand for vermitechnology has been increasing. However the technology is popular in the large plantations such as Bogawantalawa, Anilkanda, Stassons and Need wood, as well as a few small tea holders in Deraniyagala area. Institutions such as the Tea Research Institute, Coconut Research Institute, Open University, University of Ruhuna, and Post Graduate Institute of Science are conducting experimentations on vermitechnology. In Addition some non government organizations such as "Gamiseva Sevana" Galaha and "Friends of Sri Lanka", Deraniyagala are activity promoting the technology among rural sector in Sri Lanka.

2.7 Vermitech methods

2.7.1 Vermiculture

Vermiculture is feasible in suitable contains or boxes made up lightweight materials like plastics, wood, tin etc. Size of the container may vary according to the need. It is said that specially designed wooden box to be more convenient and useful. The bottom of the box with provided with few holes. Plastic window screen is placed on the inside bottom with ajute cloth lining on top of the screened sides before the culture medium is added. Top of the box

is covered with a jute cloth frame. A mixture of 1/3 soil and 2/3 organic matter is considered to be more useful in culture (Julka and Paliwal, 1993).

Also they can be reared with small containers filled with compost, cow dung and kitchen refuses. Sufficient moisture and adequate organic residues are considered ideal for their growth. Three months after culturing, the worms may be taken from cultures and when needed, can be introduced in the desired fields, gardens, etc. If used inorchards the worms may be released in the pits in which the trees or plants are growing (Jairajapuri, 1993).

2.7.2 Vermicast

The earthworms feed on decaying organic matter in the soil and after it is assimilation in the alimentary cannel excrete the soil as "Cast", which is rich in nutrients. With in 24 hours they can pass soil almost equivalent to their own weight through the alimentary cannel. Thus the soil being constantly and continuously turned over and again by these worms and the amount brought to the surface is quite considerable. Cast contains various amino acids, minerals and microorganisms, which humify the organic matter in the surrounding soil and act as bio fertilizer for plants (Jairajapuri, 1993).

Soil with vermicast in comparison to soil without these has 5 times more nitrogen, 7 times more phosphorous, 11 times more potassium, 2 times more magnesium and calcium each. All these along with other trace elements and soil nutrients soluble in water are readily available to the root system (Gupta, 1999). The casts also form suitable base for free living beneficial microbes, whose activities are essential for releasing of nutrients to higher plants. In tropical countries, the prevailing environmental condition and type of soil bring about leaching of nutrients at a rapid rate.

The existing microbial populations fail to remain due to lack of energy requirements for their activity. Under such conditions, regular applications of vermicast to field improve the physico chemical and biological properties of soil (Kale, 1993). In addition, it has been reported that the vermicast contains many growth promoting substances secreted by earthworms such as auxins, cytokinins and vitamins B_{12} responsible for the lush growth of plants.

Table 2.1 Comparative characteristics of Earthworm casts and soils (From Brady, 1990).

Characteristic	Earthworm casts	Soils
Silt and clay (%)	38.8	22.2
Bulk density (Mg/m^3)	1.11	1.28
Structural stability	849	65
Cation exchange capacity (c mol / Kg)	13.8	3.5
Exchangeable calcium (c mol / Kg)	8.9	2.0
Exchangeable potassium (c mol /Kg)	0.6	0.2
Soluble Phosphorous (ppm)	17.8	6.1
Total N (ppm)	0.33	0.12

2.7.3 Vermicompost

It is a method of making compost with the use of earthworms, which generally live in soil, eat biomass and excrete it in digested form (Gupta, 1999). This can be done either in pits or concrete tanks or well rings or in wooden or plastic crates appropriate to a given situation. Vermicomposting is setup by first placing a basal layer of vermibed comprising of broken bricks or pebbles (3-4 cm) followed by layer of coarse sand to a total thickness of 6-7 cm to ensure proper drainage. This is followed by a 15cm layer of loamy soil. In to this soil are inoculated about 100 locally collected earthworms (About 50 surface and 50 sub surface varieties). Small lumps of cattle dung (fresh or dry) are then scattered over soil and covered with a 10cm layer of hay.

Water is sprayed till the entire setup is moist but not wet. The unit is kept covered with broad leaves like coconut or palmyrah. Old jute bags can be used for covering. Watering the unit is continued and the unit is monitored for 30 days. Organic refuse is added from the 31st day as a spread on the bed after removing the fronds. The spread should not exceed 5 cm in thickness at each applications, the refuse is turnover without disturbing the bed. The day

enough refuse has been added in to the unit, just kept watering and turning over and 45 days later the compost is ready for harvest.

As the organic refuse change to a soft, spongy, sweet smelling, dark brown compost, adding water is stopped (42nd day). The compost can be harvested and they should be placed in the form of a cone on solid ground in bright sunlight. After sieving at through a 2mm or 2.5mm sieve, if necessary, can be packed in polythene bags to retain moisture.

2.7.3.1 As organic manure

Vermicompost as organic manure at field level, in pot mixes and in nursery beds has been tested to study the effect on various crops. It was found that the load on the organic manure and chemical fertilizer application is almost reduced by 25 to 50 percent on application of vermicompost. The efficiently of bio fertilizers improved on application to crop along with vermicompost. Vermicompost has stimulatory effect on seedling establishment and vegetative propagation of plants. This is related to the earthworm exudates and metabolites of the microbes associated with vermicompost. Vermicomposting with urban organic solid waste, part from curbing the organic pollution caused by putrefying organic waste helps in minimizing the use of chemicals in agriculture. Many of the farmers practicing organic farming are of the opinion that the repeated use of vermicomposting to field have brought down the incidence of diseases in crops (Pandy, 1999).

2.7.3.2 Range of nutrients

Table 2.2 Range of nutrients in vermicompost (From Pandy, 1999).

Nutrient	Range
Organic Carbon (C)	9.15 – 17.98
Total Nitrogen (N)	0.50 – 1.50
Available Phosphorous (P)	0.10 – 0.30
Available Potassium (K)	0.15 – 0.56
Available Sodium (Na)	0.06 – 0.30
Calcium (Ca) and Magnesium (Mg) (MEQ / 100g)	22.67 – 47.60
Copper (ppm)	2.00 – 9.50
Iron (ppm)	2.00 – 9.30
Zinc (ppm)	5.70 – 11.50
Available Sulphur (S)	128.00 – 548.00

2.7.3.3 Physico – chemical analysis

Table 2.3 Physico – chemical analysis of vermicompost (Pandy, 1999).

Characteristics	Raw waste	Vermicompost
pH (1:2.5)	8.20	7.85
Electric Conductivity (EC) (1:5)	12.4	33.30
Total Carbon (%)	4.5	5.25
Organic matter (%)	7.76	9.05
Total Nitrogen (ppm)	2300	2700
Available Potassium (ppm)	2880	6480

2.7.4 Vermiwash

Vermiwash is an extract of compost, the wash of earthworms present in the medium. Vermiwash is the liquid fertilizer collected after the passage of water through column of warm acted soil. To produce vermiwash, vermiwash unit is needed (Ismail, 1996).

Some of the farmers have vermiwash units in their garden. In vermiwash, the worms capabilities of decomposing organic matter, and turning nutrients in to a form available to plants, are being utilized. The earthworms eat decaying plants and soil, digest the material, and excrete it. This excretion is a liquid, which consist of nutrients such as calcium, potassium, nitrogen and phosphorous. Also vermiwash has been reported to be enrich with enzymes, hormones and vitamins. Vermiwash is a collection of this liquid and plants are able to absorb it. A vermiwash unit can easily be made in a barrel, bucket or mud pot, and consists of large of pebbles, coarse sand, fine sand, soil, cow dung, dry leaves and compost. Worms are added to the soil layer. The units sprinkled with water every day and then, to maintain the decaying process. The vermiwash is collected from a tap on the bottom of the bucket (Ismail, 1993).

2.7 Effect on vermitechnology on crop yields

Some of the effects of earthworms on soil take much long time to produce detectable effects on plant growth. But there are more evidences that vermitechnology has increased crop yields.

Application of vermicompost and earthworm increases the yields of paddy crops ranging up to 95% in grains and 128% in straw and roots (Senapati *et al.*, 1993). Large number of earthworms added to soil doubled the dry matter yield of spring wheat and increased clover yield ten times although pea yields were decreased. Addition of live worms to a garden soil increased yield of oats by 71% (Edwards and Lofty, 1977).

After inoculation of earthworms to a maize cultivation, significant difference in above ground by matter production was observed. In this case respective increase of 40%, 152%, and 130% were noted for leaf weight, cob biomass and the number of cobs. More over earthworm treatments decreased the root: shoot ratio by 20-40% thus indicating improved conditions for

nutrient supply (Derourd *et al.*, 1997). It has been shown earthworms introduced to the soil in which fruit trees were grown, more roots grew in the earthworm inoculated soils than in those without worms (Edwards and Lofty, 1977).

2.8 Benefits of vermiculture

1. Vermiculture biotechnology is unique because it can utilize organic residues that cannot be utilized by any bioconversion technology. Moreover, utilization is complete.

2. Vermiculture offers high solids retention time, even up to 6-12 months. This is achieved without any increase in hydraulic retention time and bioreactor size.

3. Vermiculture harnesses a mixed culture of beneficial soil bacteria as the most diverse and productive bioprocessing agents. Vermiculture is self regulated.

4. Bacteria are the prime bioconversion agents in vermiculture. These are selected by earthworms depending upon the changing substrate and demand on end product. Earthworms also carry out continuous upgradation of bacterial culture while they are doing their job.

5. Vermicastings enable farmers to harness all the known and unknown beneficial microorganisms in the field. Vermicasting is a sustainable biofertilizer.

CHAPTER 3
MATERIALS AND METHODS

3.1 Location

The experiments were carried out at the University of Peradeniya under normal climatic conditions during the period of October 2003 to February 2004. The characteristics of the location are wet zone of the southwest and central hill country, average 2500mm of rainfall mostly throughout the year, temperature is 25 Celsius.

3.2 Materials

An empty plastic containers (3 feet height and 2 feet diameter) with one open side were used as composting units. Draining taps were fitted to each container to collect composting extract (Figures 3.1 and 3.2). Black colour was applied outside of the every container. Earthworms were collected from Mawella farm at University of Peradeniya.

3.3 Experimental design

There were two treatments with three replicates, one treatment was the vermiwash composting unit (with earthworms) and the other was control composting unit (without earthworms) (Table 3.1).

Table 3.1 Design of composting units.

Number	Composting Unit	Type
1	Vermiwash composting unit	With earthworms
2	Vermiwash composting unit	With earthworms
3	Vermiwash composting unit	With earthworms
4	Control composting unit	Without earthworms
5	Control composting unit	Without earthworms
6	Control composting unit	Without earthworms

3.3.1 Establishment of composting units

A 4cm layer of pebbles was placed at the bottom of the container. Then a 2cm layer of coarse sand was placed. These two layers facilitate the down ward movement of composting extract.

Cow dung and surface soil were mixed as ratio of 3:1 (6 kg of cow dung and 2kg of surface soil) and placed on the sand layer. Only three containers were inoculated with about 200 numbers of earthworms. Ricestraw (about 1 kg) was placed on the top of the soil+cowdung layer of the each container.

The units were moistened every day using 100ml of distilled water. Water percolated through the compost and burrows made by earthworms and get collected at the base. Extract was collected monthly over three months by openning the tap and subjected to chemical analysis.

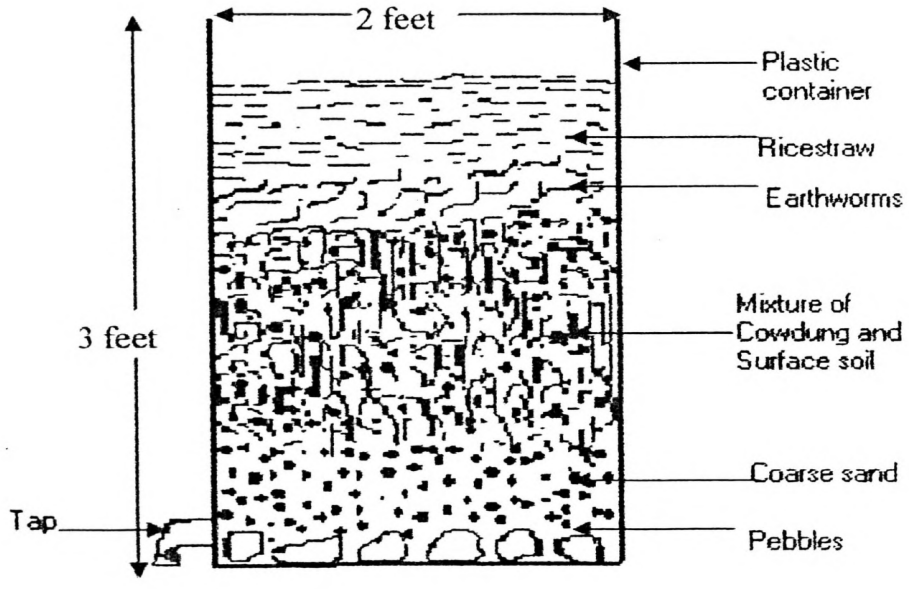

Fig 3.1 Vermiwash composting unit (with earthworms)

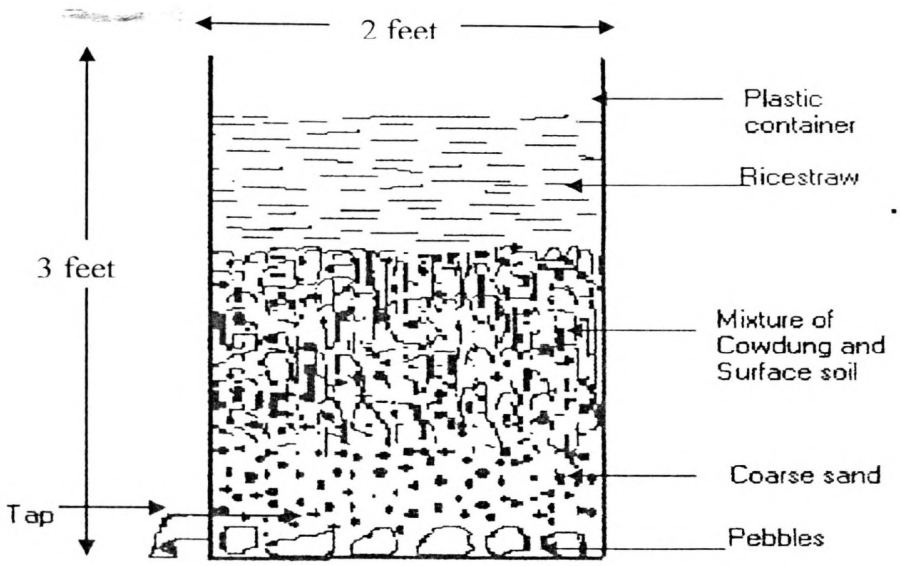

Fig 3.2 Control composting unit (without earthworms)

3.3.2 Analysis of nutrient composition

All compost extract were analyzed for pH, Electric Conductivity (EC), Organic carbon (C), Total Nitrogen (N), Phosphorus (P), Potassium (K), Sulphur (S), Magnesium (Mg), Calcium (Ca) and micro nutrients such as Copper (Cu), Zinc (Zn) and Manganese (Mn).

3.3.2.1 pH

pH was measured using pH meter.

3.3.2.2 Electric Conductivity (EC)

EC was measured using EC meter.

3.3.2.3 Organic carbon (C)

Organic carbon was determined according to the Walky and Black method (Walky and Black, 1934) (see Appendix 1).

3.3.2.4 Total Nitrogen (N)

Total Nitrogen was measured according to the Kjeldahl method (Bremmer and Mulvany, 1992) (see Appendix 2).

3.3.2.5 Sulphur (S)

Sulphur was measured according to the Turbiditimetric method after acid digestion of the samples (see Appendix 3).

3.3.2.6 Phosphorus (P)

Phosphorous was determined using Spectrophotometer after acid digestion of the samples (see Appendix 4).

3.3.2.7 Potassium (K)

Potassium was determined using Flamme photometer after acid digestion of the samples.

3.3.2.8 Calcium (Ca) and Magnesium (Mg)

Calcium and Magnesium were determined using Atomic Absorption Spectrometry (AAS) method.

3.3.2.9 Micronutrients

Manganese (Mn), Molybdenum (Mo), Copper (Cu) and Zinc (Zn) were measured using Atomic Absorption Spectrometry (AAS) method.

3.4 Data Analysis

Data were analyzed using Statistical Analysis System (SAS) software package. T test procedure was carried out to observe the significant difference between treatments and regression procedure was carried out to observe the nutrients variation with time.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Quantity of composting extract

In general, there was not a significant variation in quantity of composting extract for 3 months. Amounts of extract produced during the experimental period are presented in table 4.1.

Table 4.1 Quantity of composting extract produced during the three month of the experiment.

Sample No	Treatment	Type	Volume in December (ml)	Volume in January (ml)	Volume in February (ml)
1	Vermiwash composting unit	With earthworms	1400	1400	1400
2	Vermiwash composting unit	With earthworms	1300	1400	1300
3	Vermiwash composting unit	With earthworms	1300	1300	1300
4	Control composting unit	Without earthworms	1400	1300	1300
5	Control composting unit	Without earthworms	1300	1400	1300
6	Control composting unit	Without earthworms	1300	1300	1400

4.2 Variation of chemical parameters

4.2.1 pH

There was a significant difference (Pr>t, <0.05) of pH between two treatments (vermiwash composting samples and control composting samples) for months of December and January. But there was no significant difference, of pH between two treatments for February.

Initially the materials are slightly acidic. As composting proceeds acid forming bacteria cause the compost to be acidic, lowering the pH. With the time microbes in the compost then begin to metabolize the inorganic nitrogen to ammonium nitrogen, causing the pH to rise.

Table 4.2 Treatment comparison for pH

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	6.78	7.72	7.89	0.15	0.43	0.42
Control composting samples	6.38	7.42	7.72	0.28	0.04	0.41

4.2.2 Electric Conductivity (EC)

There was a significant difference (Pr>t, <0.05) of EC (Electrical Conductivity) between two treatments for month of December, January and February.

According to the significant value, there was a different of EC between vermiwash composting samples and control composting samples. Lower EC values were observed in vermiwash composting samples than control composting samples for month of December. But EC values of vermiwash composting samples were increased with time. Higher EC values were observed in vermiwash composting samples for January and February.

The increase of EC in vermiwash composting samples could be attributed to the release of high amount of ions (plant nutrients) from the decomposing litter with earthworms. This enrichment of nutrients clearly indicates usefulness of earthworms in vermicomposting.

Table 4.3 Treatment comparison of Electric Conductivity (EC)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	4.87	17.03	17.98	0.59	0.62	0.48
Control composting samples	6.15	13.53	13.83	0.28	0.56	0.52

4.3 Variation of macronutrients

4.3.1 Organic carbon (C)

There was a significant difference of organic carbon between two treatments for considered time period. According to the statistical analysis, there was a significantly high amount of organic carbon in vermiwash composting samples than control composting samples.

Variation of organic carbon was a significantly different with time. That means organic carbon of two treatments showed an increasing trend with time. The increased amount of organic carbon may be due to the accelerated decomposition of litter.

Table 4.4 Treatment comparison for organic carbon (C)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	1.16	1.62	1.96	0.26	0.30	0.31
Control composting samples	0.29	0.84	0.98	0.23	0.20	0.40

4.2.3.2 Total nitrogen (N)

There was no significant difference in total nitrogen between two treatments for given three months. Therefore, total nitrogen values were not significantly difference in both vermiwash composting samples and control composting samples.

Because as composting begins, the microorganisms require carbon as source of energy for growth and nitrogen for protein synthesis. As composting proceeds, the carbon to nitrogen ratio (C/N ratio) continuously decrease with time, Since nitrogen remains relatively constant and the carbon is release as carbon dioxide gas.

Table 4.5 Treatment comparison of total nitrogen (N)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	1.88	1.96	1.98	0.03	0.02	0.04
Control composting samples	0.51	0.98	0.68	0.20	0.42	0.60

4.3.3 Phosphorous (P)

There was a significant difference for phosphorous between two treatments for given months. There was a difference in phosphorous values between vermiwash composting samples and control composting samples. Higher values of phosphorous were observed in vermiwash composting samples than control composting samples for considered time period. Also variation of phosphorus was a significantly different with time.

Table 4.6 Treatment comparison of phosphorous (P)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.29	0.26	0.29	0.04	0.04	0.04
Control composting samples	0.21	0.20	0.21	0.04	0.02	0.03

4.3.4 Potassium (K)

There was a significant difference for potassium between two treatments for December and February. But there was no significant difference for potassium between two treatments for January. Variation of potassium was not significantly different. That means potassium of two treatments not showed a variation with time.

Table 4.7 Treatment comparison of Potassium (K)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	2.38	2.52	2.55	0.40	0.39	0.29
Control composting samples	1.98	2.00	2.07	0.16	0.46	0.24

4.3.5 Sulphur (S)

There was a significant difference for Sulphur between two treatments for considered time period. There was a different of values of sulphur between vermiwash composting samples and control composting samples. Higher values of sulphur were observed in vermiwash composting samples.

Variation of sulphur was significantly different with time. Therefore sulphur of two treatments showed a variation with time.

Table 4.8 Treatment comparison for Sulphur (S)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.22	0.23	0.24	0.02	0.03	0.02
Control composting samples	0.16	0.18	0.17	0.02	0.02	0.02

4.3.6 Calcium (Ca)

There was a significant difference of calcium between two treatments. According to the significant values there was a different amount of calcium between vermiwash composting samples and control composting samples. Higher values of calcium were observed in vermiwash composting samples. Also calcium of two treatments not showed a variation with time.

Table 4.9 Treatment comparison of Calcium (Ca)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.28	0.24	0.25	0.05	0.08	0.08
Control composting samples	0.14	0.10	0.10	0.10	0.08	0.08

4.3.7 Magnesium (Mg)

There was a significant difference of magnesium between two treatments for considered time period. According to the significant differences, there was a different amount of magnesium between vermiwash composting samples and control composting samples. Higher values of magnesium were observed in vermiwash composting samples for considered time period. Magnesium of two treatments not showed a variation with time.

Table 4.10 Treatment comparison of magnesium (Mg)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.52	0.63	0.64	0.12	0.12	0.08
Control composting samples	0.28	0.12	0.19	0.16	0.13	0.22

According to the overall results of macronutrients, phosphorous, potassium, sulphur, calcium and magnesium were showed significantly higher values in vermiwash composting samples. Because substantial proportions of nutrients contained in organic matter are in complex forms unavailable to the plants. They include in nitrogen, phosphorous, sulphur and other nutrients in the persistant organic materials. Other

organic matter, particularly from recent crop residues or manure, can be composed quickly by bacteria and earthworms and thus mineralized and nutrients become available to the plants as ammonium, nitrate, phosphate, sulphate and other ions.

4.4 Variation of micronutrient content

4.4.1 Manganese (Mn)

There was not significant difference for manganese between two treatments for month of December and January. But there was a significant difference of manganese between two treatments for February.

According to the significant differences, there was no difference of manganese values for both treatments for month of December and January. But significantly higher values of Manganese were observed in vermiwash composting samples. Variation of amounts of manganese was a significantly different with time.

Table 4.11 Treatment comparison for manganese (Mn)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.16	0.15	0.14	0.11	0.10	0.04
Control composting samples	0.02	0.03	0.02	0.02	0.02	0.02

4.4.2 Zinc (Zn)

There was a significant difference of zinc between two treatments for December. there was not a significant difference of zinc for January and February. For Decem significantly higher variation of values was observed between two treatments. But for January and February, zinc values were not significantly variation for both two treatments. Zinc of two treatments not a showed variation with time.

Table 4.12 Treatment comparison of zinc (Zn)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.01	0.03	0.01	0.00	0.02	0.01
Control composting samples	0.00	0.00	0.00	0.00	0.00	0.00

4.4.3 Copper (Cu)

There was not a significant difference of copper between two treatments for considered time. Therefore values of copper was not a significantly difference in both treatments. Also copper of two treatments not showed a variation with time.

Table 4.13 Treatment comparison of copper (Cu)

Treatment	Mean value			Std Dev		
	Dec	Jan	Feb	Dec	Jan	Feb
Vermiwash composting samples	0.02	0.06	0.04	0.01	0.07	0.01
Control composting samples	0.00	0.01	0.01	0.00	0.01	0.01

Treatment effect was varied for some nutrients such as zinc and manganese for some months. Also above mentioned nutrients varied monthly according to the decomposition rate of organic residuals.

CHAPTER 5
CONCLUSION

The vermiwash produced from the locally available organic materials is a nutrient rich organic solution, which can be used as a foliar fertilizer. This vermiwash can be produced in small scale within short time period with low input. From the results, it can be concluded that the vermiwash produced from the locally available organic materials could be used as an environmentally friendly foliar organic fertilizer in agriculture.

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APPENDIX 1

Measurement of Organic Carbon

Procedure

10 ml of 1/6N strength of Potassium dichromate ($K_2Cr_2O_7$) and 20ml of Conc.H_2SO_4 were added to 2ml of composting extract. After shaking one minute, it was allowed to stand on a sheet of asbestos for one hour. After one hour, 200ml of distilled water, 10ml Phosphoric acid and 1ml of Diphenylamine indicator solution were added to above solution. The solution was titrated against Ferrous Ammonium Sulphate solution drop by drop, until the color flashed to dark green. Again 0.5ml of Potassium dichromate ($K_2Cr_2O_7$) was added and titrated again until the color flashed to dark green. A blank titration was done simultaneously (Walky and Black, 1934).

Calculation

$2\ Cr_2O_7^{-2} + 3C + 16H^+ = 4Cr^{+3} + 3CO_2 + 8H_2O$

Volume of Potassium dichromate used = 10.5ml
Molarity of dichromate = 1/6M
Burette reading (with soil) = V1
Burette reading (blank) = V2

$2\ Cr_2O_7^{-2} + 6Fe^{2+} + 14H^+ = 2Cr^{+3} + 6Fe^{3+} + 7H_2O$

Moles of dichromate used = 1/6 * 10.5/1000 = X moles
Moles of Ferrous ammonium sulphate = 1/6 * 10.5/1000 *6
Molarity of Ferrous ammonium sulphate = 1/6 *10.5/1000 * 6/V2 * 1000 = M1
Moles of dichromate reacted with ferrous = M1 * v1 * 1/6 = Y moles
Moles of dichromate reacted with carbon = X – Y = Z moles
Grams of carbon reacted with dichromate = 3/2 * Z * 12
Organic carbon percentage (%) = 3/2 * Z * 12/2 *100

APPENDIX 2

Measurement of Total Nitrogen

Procedure

10ml of composting extract, one gram of catalyst mixture and 5 ml of conc. Sulphuric acid were added in to a digestion tube. The sample was digested using a digestion unit until the solution becomes light blue in color. After cooling to room temperature, it was transferred in to a 500ml conical flask using about 150ml distilled water. 20ml of 4% boric acid was placed in a receiving flask and two drops of mixed indicator were added and receiving flask kept beneath the condenser. Then 30ml of 10N NaOH (Sodium Hydroxide) was poured in to the conical flask containing sample and small quantity of Davadas alloy was also added to the sample. Subsequently flask was connected to distillation apparatus. Digest was distilled until the distillate in the receiving flask increased up to about 100ml. It was titrated the 0.01 N HCl (Hydrochloric) until color changes from green to pink. (Bremmer and Mulvany, 1992).

Calculation

$$NH_4^+ + OH^- \longrightarrow NH_3 + H_2O$$

$$NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$$

$$H_2BO_3^- + NH_4^+ + HCl \longrightarrow H_3BO_3 + NH_4Cl$$

Volume of 0.01 M HCl required = X ml

X ml of 0.01 HCl reacts with = 0.14* Xmg N

Percentage of total N in sample = 0.14 * X * 100%/1000

APPENDIX 3

Acid digestion

20ml of composting extract and 3ml of conc. Nitric acid (HNO_3) were added to a digestion tube.

Sample was digested about 15 minutes. After 2ml of Perchloric acid ($HCLO_4$) was added, and again sample was digested about 15 minutes.

Whole digested sample was wash down to a 10ml volumetric flask with distilled water and filtered using No. 44 filter paper.

APPENDIX 4

Measurement of Sulphur (S)

Procedure

Digested composting extract was transferred in to 50ml beaker.

10ml of 6.25% Ammonium solution, 5ml of Gum Acacia and 0.5g of Barium Chloride were added to beaker.

Beaker (with solution) was leaved to stand for about 10 minutes.

Absorbance was measured at 440nm wavelength using Spectrophotometer.

APPENDIX 5

Measurement of Phosphorous (P)

Procedure

Exactly 1ml of digested composting extract was added in to a 50ml volumetric flask and also 10ml of distilled water was added to flask.

8ml of reagent mixture was added and volumerized up to 50ml

It was leaved to stand for about 30 minutes. After absorbance was measured at 880nm wavelength using Spectrophotometer.

APPENDIX 6

Organic carbon measured in December

The TTEST Procedure

Variable	Class	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev
OC	1	6	0.8823	1.1567	1.431	0.1632	0.2614
OC	2	6	0.0446	0.2867	0.5288	0.144	0.2307
OC	Diff (1-2)		0.5528	0.87	1.1872	0.1723	0.2465

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
OC	Pooled	Equal	10	6.11	0.0001
OC	Satterthwaite	Unequal	9.85	6.11	0.0001

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
OC	Folded F	5	5	1.28	0.7905

APPENDIX 7

Organic Carbon measured in January

The T TEST Procedure

Variable	Class	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev
OC	1	6	1.3038	1.62	1.9362	0.1881	0.3013
OC	2	6	0.6351	0.8417	1.0482	0.1229	0.1968
OC	Diff (1-2)		0.4509	0.7783	1.1057	0.1778	0.2545

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
OC	Pooled	Equal	10	5.30	0.0003
OC	Satterthwaite	Unequal	8.61	5.30	0.0006

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
OC	Folded F	5	5	2.34	0.3714

APPENDIX 8

Organic Carbon measured in February

The T TEST Procedure

Variable	Class	N	Lower CL Mean	Mean	Upper CL Mean	Lower CL Std Dev	Std Dev
OC	1	6	1.637	1.9633	2.2897	0.1941	0.311
OC	2	6	0.568	0.9833	1.3987	0.2471	0.3958
OC	Diff (1-2)		0.5221	0.98	1.4379	0.2487	0.3559

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
OC	Pooled	Equal	10	4.77	0.0008
OC	Satterthwaite	Unequal	9.47	4.77	0.0009

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
OC	Folded F	5	5	1.62	0.6094

APPENDIX 9

The GLM Procedure (Regression procedure)

Dependent Variable: pH

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.09320016	0.09320016	4.03	0.0567
z	1	0.02023436	0.02023436	0.87	0.3595
time*z	1	0.04500496	0.04500496	1.94	0.1765

The GLM Procedure

Dependent Variable: Electric Conductivity (EC)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	516.1408333	516.1408333	89.73	<.0001
z	1	14.0958730	14.0958730	2.45	0.1273
time*z	1	44.2816667	44.2816667	7.70	0.0091

The GLM Procedure

Dependent Variable: Organic Carbon (C)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	1.95213333	1.95213333	23.07	<.0001
z	1	0.75461944	0.75461944	8.92	0.0054
time*z	1	0.01815000	0.01815000	0.21	0.6464

APPENDIX 10

The GLM Procedure

Dependent Variable: Nitrogen (N)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.03307500	0.03307500	0.29	0.5919
z	1	2.11200357	2.11200357	18.73	0.0001
time*z	1	0.00666667	0.00666667	0.06	0.8094

The GLM Procedure

Dependent Variable: Phosphorous (P)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.00000675	0.00000675	0.00	0.9686
z	1	0.03232268	0.03232268	7.52	0.0099
time*z	1	0.03088838	0.03088838	7.19	0.0115

The GLM Procedure

Dependent Variable: Sulphur (S)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.00234631	0.00234631	2.24	0.1446
z	1	0.00605257	0.00605257	5.78	0.0224
time*z	1	0.00057264	0.00057264	0.55	0.4652

The GLM Procedure

Dependent Variable: Potassium (K)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.99680064	0.99680064	0.06	0.8027
z	1	4.76041086	4.76041086	0.30	0.5857
time*z	1	0.03731960	0.03731960	0.00	0.9614

APPENDIX 11

The GLM Procedure

Dependent Variable: Calcium (Ca)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.00120741	0.00120741	0.00	0.9485
z	1	0.12125147	0.12125147	0.43	0.5186
time*z	1	0.06706716	0.06706716	0.24	0.6306

The GLM Procedure

Dependent Variable: Magnesium (Mg)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.00536321	0.00536321	0.13	0.7195
z	1	0.00053726	0.00053726	0.01	0.9094
time*z	1	0.13586577	0.13586577	3.33	0.0785

The GLM Procedure

Dependent Variable: Copper (Cu)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.00199530	0.00199530	0.06	0.8152
z	1	0.03014951	0.03014951	0.84	0.3675
time*z	1	0.03119845	0.03119845	0.87	0.3594

The GLM Procedure

Dependent Variable: Zinc (Zn)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.13859488	0.13859488	3.99	0.0583
z	1	0.11160084	0.11160084	3.21	0.0869
time*z	1	0.09028669	0.09028669	2.60	0.1212

APPENDIX 12

The GLM Procedure

Dependent Variable: Manganese (Mn)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
time	1	0.18396654	0.18396654	6.87	0.0156
z	1	0.22481632	0.22481632	8.39	0.0084
time*z	1	0.14660910	0.14660910	5.47	0.0288

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