PRODUCTION OF BIOFERTILIZER FROM VERMICOMPOSTING OF LANDFILL LEACHATE USING EARTHWORMS

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Dedicated to my family, and my friends.

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ABSTRACT

Nowadays, the accumulation of waste in landfill site is largely due to increasing of urbanization and industrialization in Malaysia. The non-proper management of wastes have worsened the condition whereby the amount of leachate produced from the waste led to critical environment issue. Thus, the objective of the study was to determine the effects of vermicomposting of landfill leachate using earthworms on biofertilizer production in 31 days. Liquid leachate that majorly consist of organic matter can yield highly nutritive biofertilizer through vermicomposting process. The process used earthworms to convert organic matter in leachate into plant-nutrients that enhance the growth of the plants and plants productivity. The amount of nitrate (N), phosphorus (P) and potassium (K) in vermicompost was found to increase while pH and number of earthworms declined at the end of the vermicomposting period. The vermibin containing 90 earthworms (approximately 52g) in a mixture of 150 ml of leachate and 800 g of soil obtained the highest concentration of NPK in vermicompost compared to those containing 39 earthworms and 15 earthworms. This indicates that the concentration of NPK increased with the high amount of earthworms. The number of earthworms decreased with decrease in pH in all vermibins after 31 days of vermicomposting period. V1 experienced increase 53–fold in N, 194-fold in P and 210–fold in K with 6.13 in pH after vermicomposting period due to earthworm activity during the process, thus improving the number of leaves (32 leaves). The data shows that vermicomposting is a suitable technology for the decomposition of landfill leachate into nutritive biofertilizer.

ABSTRAK

Masakini, perlonggokkan sampah di tapak pelupusan sampah majority adalah disebabkan oleh kemajuan pembangunan dan perindustrian di Malaysia. Pengurusan yang tidak terurus telah menyebabkan keadaan bertambah buruk di mana sejumlah air sisa yang pekat terhasil daripada tapak pelupusan telah menyebabkan isu pencemaran yang kritikal. Oleh itu, objektif kajian ini adlaah untuk mengenalpasti kesan vermicompost daripada air sisa sampah menggunakan cacing untuk menghasilkan biofertilizer. Cecair sampah yang mengandungi kebanyakannya adalah daripada bahan organic, di mana boleh menghasilkan biobaja yang berkhasiat melalui proses vermikompos. Proses ini yang menggunakan cacing untuk menukarkan bahan organik kepada nutrient pokok yang dapat meningkatkan pertumbuhan dan produktiviti pokok. Selepas proses vermikompos, kandungan nitrat (N), fosforus (P) dan potassium (K) didapati meningkat sementara pH dan bilangan cacing menurun. Vermikompos yang mengandungi 90 cacing yang dicampur 150 ml cecair sampah dan 798 g tanah menunjukan kandungan NPK yg paling tinggi berbanding vermikompos yg megandungi 39 dan 15 cacing. Ini menunjukan kandungan NPK yang tinggi dipengaruhi oleh bilangan cacing yang banyak. Sebaliknya, bacaan pH dan bilangan cacing menurun selepas 31 proses vermikompos. Disebabkan aktiviti cacing ketika proses, V1 menunjukan peningkatan NPK sebanyak 53-194-210 kali ganda, lalu meningkatkan bilangan daun. Hasil penyelidikan menunjukan vermikompos teknologi sesuai untuk penguraian organic di dalam cecair sampah kepada biobaja yang berkhasiat.

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%	Percentage
cm	Centimetre
g	Gram
k	Kilogram
L	Liter
m	Metre
m $/L$	Milligram per litre

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
BOD	Biochemical Oxygen Demands
CAGR	Compound Annual Growth Rate
Cd	Cadmium
COD	Chemical Oxygen Demands
Cr	Chromium
DO	Dissolved oxygen
Fe	Iron
HCl	Hydrochloric acid
IPI	Industrial Production Index
K	Potassium
Mg	Magnesium
MSW	Municipal Solid Waste
N	Nitrates
$NaNO_3$	Sodium Nitrates
NH_3	Ammonia
P	Phosphorus
P_2O_5	Phosphate
VOA	Volatile Organic Acid

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Malaysia is one of the developing countries sharing the same problem as other developed countries such as India and China when it comes to the municipal solid waste management. Rapid industrialization, increase in population and changes in consumption pattern, directly and indirectly, result in the generation of a large amount of municipal and industrial wastes, ranging from biodegradable to synthetic (Singh, et al., 2011). Malaysia has been producing wastes of more than 30, 000 tons per day. The amount of wastes generated continue to increase due to the rising population and development of the country. However, less than 12% of the waste is being recycled (Khan, 2015). Malaysia has been building sanitary landfills for solid waste disposal, but the accumulation of waste keeps on increasing along the year. This indicated the great potential of highly concentrated leachate production (Lee et al., 1994). Aljaradin, (2012) claimed that the migration of gas and leachate from landfill body into the surrounding environment present a serious environmental concern. Small amounts of leachate may contain highly toxic chemical, along with undesirable tastes and odours that can pollute large amounts of groundwater, thus making it unsuitable for domestic water supply (Lee, et al., 1994).

Therefore, vermicomposting is seen as a potential way to convert the biodegradable inorganic wastes into nutrient-rich end product by using earthworms (Mehta, & Karnwal, 2013). Thus, the objective of the research is to produce biofertilizer by vermicomposting of landfill leachate. The key potential of using earthworms is the ability to increase soil porosity by improving the exchange of oxygen-carbon dioxide with the atmosphere by creating permanent holes or tunnels in the organic matter (Duiker, & Stehouwer, 2008). Moreover, vermicomposting may enhance the mineralization of nutrients, thus boosting up the crop productivity. It also promotes soil aggregation and stabilizes the soil structure (Khan, & Ishaq, 2011).

1.2 Motivation

According to Global Biofertilizer Market size, biofertilizer production has been expected to reach US Dollar (USD) 1.88 billion by 2020 at a Compound Annual Growth Rate (CAGR) of 14.0% from 2015-2020 (Rohan, 2016). In addition, Asia-Pacific has conquered the third-largest after North America and Europe in biofertilizer industry. The main factor that aroused the demands for biofertilizer is the increase of awareness level regarding health and wellness gained from organic products and health hazards caused by the use of chemical fertilizers. Savci, (2012) claimed that chemical fertilizers consist elements such as $NaNO_3$ and NH_4NO_3, which caused deterioration of soil fertility and soil degradation reactions. Bio-fertilizers have several advantages over chemical fertilizer; they are non-pollutant, inexpensive, and utilize renewable resources (Saeed et al., 2015). Thus, biofertilizer is the best solution to replace the use of chemical fertilizers on agriculture practices. According to Bjerre (2012), the introduction of green revolution technologies in the modern agriculture is proven to help in the quality of crop products and safeguarding the soil fertility. Nonetheless, it is to accomplish Malaysia goals in nurturing biotechnology to convert biomass wastes as resources into high-value products and against the uses of chemical-based fertilizer on soil which give harm to the soil by creating soil erosion and greenhouse gas emission (UN Habitat, 2009; Mun, 2015).

1.3 Problem Statement

The increasing number of landfill have been recorded since 2008. This is due to the accumulation of wastes in landfill site. There are several factors that caused to the waste accumulation; low recycling, poor management as well as the increase generation of solid waste (Khan, 2015). Despite the massive amount of waste produced, utilization of disposal site space are still inefficient. Unknowingly, MSW landfill leachate that contains a variety of potentially highly hazardous chemicals can possibly bring significant health threat to those who consume waters from the river, water stream, and lakes (Lee, et al., 1994). Unfortunately, the excessive use of chemical fertilizer due to obtaining high yield of fruits and vegetables causes serious environmental problems especially on water resources, hence deteriorating the crop production quantity and quality of the product (Savci, 2012). These problems also lead to root rot disease and the emergence of new diseases to plants such as anthracnose, and stunted disease that affects the production of tomatoes plant ultimately (Satibi, 2014). These occurrences result in economic losses.

Even though vermicomposting industry in Malaysia has existed for several years, there is still lack of information. The lack of guidelines subsequently causes the low quality of vermicomposts production and marketing of immature vermicomposts. Therefore, this research aims to provide information on the best ratio for mixture soil, leachate and earthworms with the conditions suitable for vermicomposting as well for the growth of selective plants.

1.4 Objectives

The main focus of this research is to accomplish the following objectives:

1) To study the effects of vermicomposting of leachate using earthworms on biofertilizer production.
2) To determine the growth of tomato plants as effected by the application of vermicompost as a biofertilizer.

1.5 Scopes of Study

Following scopes have been identified, in order to achieve the objectives of the study:

1) Characterization of biofertilizer from vermicomposting of leachate in term of pH, Nitrate(N), Phosphate(P), and Potassium(K)
2) Study on the effect of different ratios of landfill leachate, soil and earthworms (L: S: E) (% wt./wt./vol) on the NPK production (15: 79.8: 5.2; 15: 82.0: 3.0; 15: 84.2: 0.8).
3) Phytotoxicity test on tomatoes plants such as plant height, and number of leaves.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Malaysia consist of Peninsular Malaysia and the states of Sabah and Sarawak located on the northern portion of the island of Borneo. Malaysia is a developing country where the year of 2020 is the year that Malaysia aimed to achieve the industrialized country status. In 2016, Department of Statistics Malaysia reported total population of Malaysia had increased for the past two years by 1.5 per cent annual growth rate from 30.7 million to 31.7 million people. From Figure 2.1, Selangor recorded the highest percentage of population of 19.9% followed by Sabah (12.0 %) and Johor (11.5%). This indicated that Selangor is the most population with 5.46 million and followed by Johor and Sabah with 3.21 million each (Department of Statistics Malaysia, 2016).

Figure 2.1: The population of Malaysia in 2016

Source: (Department of Statistics Malaysia, 2015)

The growth in population is ineradicably tied together with the increase of industrial sector (Whelpton, 2014) where Malaysia's industrial development has increased by 3.2% as reported by Malaysia's Index of Industrial Production (IPI). Thus, we can say that the industrial has been developed along with the growth of urbanization. Positive view from these growths, however, had led to many environmental problems. Consequently, large quantity of MSW generation, particularly in Peninsular Malaysia poses enormous challenges for the health of living being. Numerous landfill is built in for disposal purpose with the increment of waste generation rate and this is directly attributed to greenhouse gas pollution and ground water pollution. Landfilling industrial waste is considered as hazardous wastes as they are primarily chemical-based which extremely harmful to human, animal and plant life as these wastes leaches into soil and groundwater. Not only that, due to increase in population, a lot of organic wastes are produced year by year and this can be viewed in Figure 2.2. Thus, environmental protection expenditure also increased to 0.3 percent in 2014 and total of RM2.244billion was spent for environmental protection mainly in waste management(Department of Statistics Malaysia, 2015b).

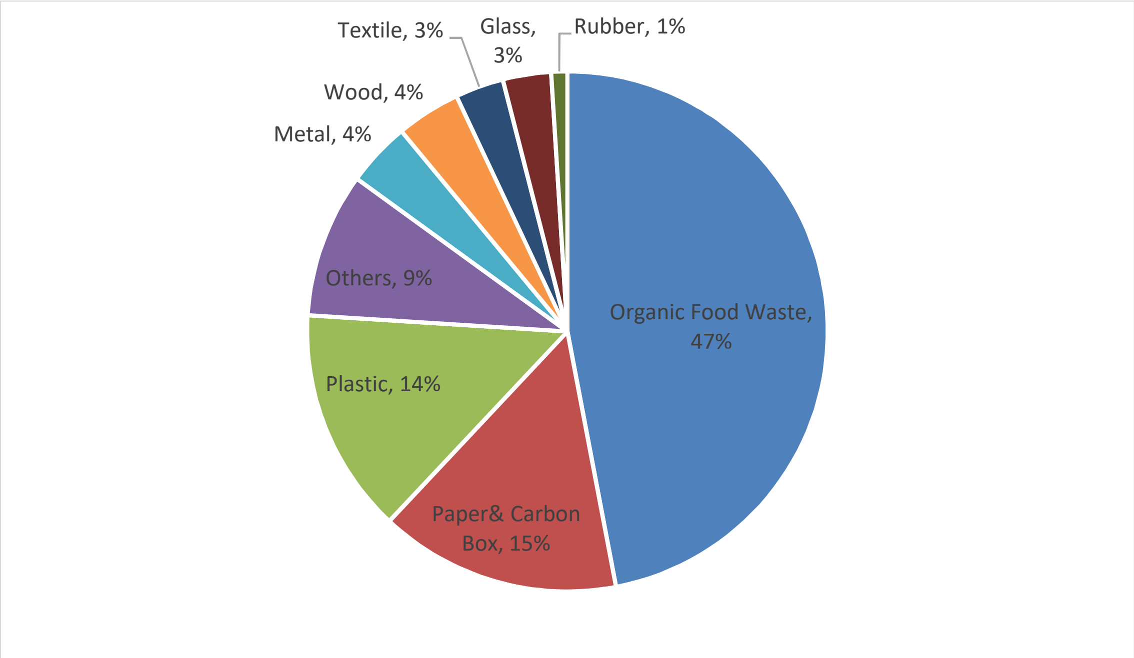

Figure 2.2: Composition of wastes disposal.

Source: (Mentari Alam EKO (Malaysia) Sdn Bhd, n.d.)

Throughout all the considered factors (population, industrialization, waste generation), the main concerned is the leachate generation from the accumulation of waste landfilled. It can give a significant cause to the contamination of soil and groundwater, potential exist of pathogen and the release odor (Raghab et al., 2013). Vermicomposting is the potential alternative method to the contamination of soil by diverting the organic matter in leachate using earthworms under controlled conditions of temperature, moisture content and pH to enhance soil fertilization. This process viewed as potential use for land application as biofertilizer and soil fertility. According to Zularisam et al., (2010), through the vermicomposting process, the organic portion of solid waste could be utilized in a profitable way. Vermicomposting has shown to be successful for processing sewage sludge (Gupta & Garg, 2008), paper mill sludge (Yadav & Madan, 2013), animal manures (Lazcano, et al., 2008) and rice husk (Lim, et al., 2012).

2.2 Leachate

Leachate generates through the process of the excess of rainwater percolating through the waste layers in a landfill (Taylor, et al., 2011). It has varied color based on their age, the amount of exposure to the sun as well as the composition of waste. Leachate composition consist of organic and inorganic chemicals, heavy metals, microbes as well as pathogens. Leachate can be divided into three categories, which are hydrolysis of solid waste and biological degradation, solubilisation of soluble salts contained in waste, and coagulation of particular matter. The quality of leachate produced influenced by these categories. The stabilization of waste has three major phases on biological waste decomposition processes over the time. This is where the leachate originates. In this chapter, all three major phases are describing the mechanism of mass transfer of waste to leachate.

2.2.1 Phase one: Aerobic Degradation phase

The first phase is when the degradable of organic matter begins as soon as the waste laid down at the landfills site. All typical catabolites of aerobic processes occur. In addition, the present bacteria and microbes within waste used up all the oxygen, usually the upper layer of MSW, where the oxygen is trapped in fresh waste and supplied by rainwater and diffusion(Heyer, & Stegmann, 1998). The duration of the aerobic process takes place in short time and usually not more than a month. This is because the composition of waste in MSW is majority organic waste. Cellulose is part of organic waste and degraded into glucose by extracellular enzymes. Later, it is being converted into water and carbon dioxide by bacteria within the waste. However, the period for the aerobic process can take time depending on the quantities of carbon dioxide production.

2.2.2 Phase two: Anaerobic Degradation phase

The formation of anaerobic phase is due to the demands of oxygen are more than the oxygen available. Three main different phases were occurred during anaerobic degradation phase; acidogenesis and acetogenesis phase, intermediate methanogenic phase and stabilized methanogenic phase. Thus, brief explanation of these phase were reported by Heyer & Stegmann, (1998)

2.2.2.1 Acidogenesis and Acetogenesis phase

Acidogenesis is a biological reaction involving breaking down of the hydrolyzed organic materials (fermented cellulose, etc.) to produce simpler and more soluble components by acidogenic bacteria. For example, production of volatile fatty acids, carbon dioxide, hydrogen sulfide (‘egg-rotten’ smell notable), ammonia and another by-product.

Acetogenic bacteria digest acetogenesis to produce carbon dioxide, hydrogen, and acetic acid. Acetogenesis main product is acetate through carbohydrate fermentation process.

Both processes caused the low pH value of leachate, increase the concentrations of volatile acids and low solubility of heavy metals present in leachate. Heavy metals are said to be dissolved during acid-fermentation due to low solubility.

2.2.2.2 Intermediate methanogenic phase

The second phase is known as methane generating where methanogenic bacteria started to grow slowly due to excess of organic volatile acids which are toxic to them. At the same time, the concentration of gas methane increases carbon dioxide, hydrogen, and volatile fatty acids decrease. Thus, the conversion of volatile fatty acids leads to the increase of pH values to alkalinity. As a result, the solubility of heavy metals, calcium, manganese, and iron into the soil decreased. Inversely, the concentration of ammonia nitrogen keeps increasing as the forming leachate is not removed regularly.

2.2.2.3 Stabilized methanogenic phase

At third phase, the characterization of the composition of leachate nearly to neutral pH values. Methanogenic bacteria assists in the methanogenic fermentation process in degradation of anaerobic as well maximize pH to the range 6 to 8. During this stage, biogas such as methane gas present is higher than in the second phase at 50%. Stabilization in leachate referred to the decreases of organic components, relatively low BOD, and ratio of BOD/COD values.

2.2.3 Final Aerobic phase

After methanogenic phase end, the air penetrates again into waste and the cycle continues until leachate been treated and discharge to water.

2.3 Impact of untreated landfill leachate towards environment.

Landfill leachate contain suspended matter from MSW might be adsorb in water and absorb into groundwater, causing a threat to a living being. According to (Clark, n.d.), water provides three significant roles in landfilling; as a medium for movement of contaminants, as a medium essential for bacterial activity in organic waste and for the chemical breakdown, and as a medium providing dilution. Zainol et al., (2012) studied that the production of leachate from waste disposal at landfill caused the percolation of the precipitate. Leachate has varied color depending on their age, amount of organic substances in the leachate as well as pH (Bhalla, et al., 2012). In addition, the amount exposure to the sun influenced the pH of the leachate. The main leachate composition consists of organic and inorganic chemicals, heavy metals, microbes, and pathogens. Thus, mobilizing earthworms helps increased the level of extractable N, P, K, zinc (Zn), forum (Fe) and manganese (Mn) by the availability of metals and their forms in composted material (Ghoname & Shafeek, 2005). In addition, the composition of heavy metals such as Mn, Zn, Ni, Cu and Pb gave the plant a nutritive content, especially fruits. (A.Ghanome & Shafeek., 2005; Adhikari & Khanal, 2015; Azizi et al., 2015; Zainol et al., 2012).

2.3.1 Impact of the emission of odour smell

The emission of odor to air due to the combination of gases released by bacteria chemical processes occur in a landfill site. These bacteria responsible for breaking down the waste from solid to liquid or from solid to a vapor. Referring to Figure 2.2, the composition of organic waste approximately 47 %. The organic waste tends to produce gas which potential to become odour. Gases such as ammonia and sulfides are the sources of odor in landfill gas. Pungent smell and rotten egg smell likely come from ammonia and sulfides respectively even in low concentration. The emission of odour brings unpleasant for people surrounding but harmless to health.

2.4 Treatment of landfill leachate

Zainol et al., (2012) studied that the production of leachate from waste disposal at landfill caused the percolation of the precipitate. It is analyzed with a high concentration of heavy metals such as Fe, Mn, Ni and Zn (Adhikari, & Khanal, 2015). Leachate also contain large amount of organic matter including dissolve matter, ammonia-nitrogen, alkalinity, acidity, inorganic salts, solids, phenol and other toxicant (Zainol et al., 2012).

2.4.1 pH

Leachate collected directly from lorry from Mercu Resolution Landfill site showed the pH reading at 8.41. pH more than 7.5 indicates the age of leachate (>10 years) and the stability of organic matter in landfill leachates (Baig, et al., 1937). However, the age of Sg. Ikan landfill is 5 years which pH should be 7. This showed that it was in the process of moving from fermentation phase to more natural values (Mohd Zin, et al., 2012). To overcome problems regarding leachate management, vermicomposting of landfill leachate is one of the solutions(Wu et al., 2014). The suitable pH for vermicomposting is between range 5 to 7(Zularisam, et al., 2010).

2.4.2 Heavy metals

Heavy metals such as copper (Cu), manganite (Mn), chromium (Cr), iron (Fe), zinc (Zn), nickel (Ni) and cadmium (Cd) are determined in leachate sample. In Adhikari & Khanal, (2015) research stated that majority of heavy metals found in leachate are Cu, Cr, Zn, Ni, Pb, and Cd. Meanwhile, the concentration of heavy metals in leachate depends on the age of leachate. Young leachate showed low in heavy metals Cu, Mn, Cr, Fe, Zn, Ni, and Cd. In Azizi et al., (2015) researched reported that vermicomposting process decreases the concentration of heavy metals in leachate. Furthermore, based on A.Ghoname & Shafeek., (2005), mobilizing earthworms helps increased the level of extractable N, P, K, Zn, Fe and Mn by the availability of metals. Plus, the composition of heavy metals present in leachate (Mn, Zn, Ni, Cu and Pb) gave the plant a nutritive content especially fruits. (A.Ghanome & Shafeek., 2005; Adhikari & Khanal, 2015; Azizi et al., 2015; Zainol et al., 2012).

2.5 Vermicomposting of leachate

Vermicomposting is a method of breaking down leachate using the potential earthworms as vermicomposter. In another hand, vermicomposting able to increase the fertility of soil by reducing the level of heavy metals and free from pathogens (Domínguez, et al., 2000; Sinha, et al., 2010). Sinha et al., (2010) studied that vermicompost becomes 'highly nutritive' rich in NPK and other nutrients as the worms release them during the vermicomposting process. These soil then suitable for fertilizer to growth the plants.

According to Sharma et al., (2005), the interaction between earthworms and microflora such as vermicomposting leachate can improve and maintain soil fertility. In addition, accumulation of heavy metals in leachate by earthworms not only converted them into nutrient for soil by accelerate nitrogen level but also decreased pathogen level in leachate (Kim, et al., 2012).

The suitable earthworms for vermicomposting are *epigeic* species. Epigeic earthworms affect the decomposition of organic matter directly through gut-associated processes; via ingestion by pharynx, digestion at gizzard part and casting through wasted from anus earthworms. An example of *epigeic* species that can be obtained in Asia are *Eudrilus eugeniae, Perionyx excavates, Eisenia Andrei* and *Lumbricus rubellus* (Sharma et al., 2005) and *Eisenia foetida* .

2.6 Optimum conditions for earthworms

Optimum conditions for earthworms are necessary for this research. Sinha et al., (2009) stated optimum conditions such as pH, temperature, moisture content and feedable to increase the reproduction of single worm by 2^8 (256 worms) for every 6 months. The purposed on increasing worms in vermicomposting thus led to a nutritive biofertilizer by increased ingest the heavy metals contains in leachate (Sinha,Herat,Bharambe,et al., 2010).

2.6.1 pH

The effect of pH to earthworms influenced the activity of the enzyme in the worms' gut. Some studied recommended pH for vermiculturing the earthworms in the range between 6 - 8 (Rostami, 2011; Kim, et al., 2012). Rostami, (2011) stated that worms in the vermicomposting process likely sensitive to pH and more favor on neutral pH. Suitable pH can help earthworms to grow rapidly and help to decompose organic matter in leachate efficiently(Sharma, et al., 2005).

2.6.2 Moisture content

Humidity is one of considered condition that affects the performance of earthworms in the vermicomposting process. It is one of the ways for earthworms to keep their skin wet and moist for respiration purposes. In Klok, (2007) researched specifically on *L.rubellus* earthworms species stated the humidity set for vermicomposting is at 61%.

However, moisture content still acceptable around 65-75% for the vermicomposting process as it depends on for different worms' species (Rostami, 2011).

2.6.3 Temperature

The optimum temperature for worms is in the range of 20°C -30°C. Based on many researchers reported that activities earthworm at this temperature is more active and able to vermicomposting efficiently (Adhikary, 2012; Marangon et al., 2014; Sinha et al., 2010; Sinha et al., 2014; Zularisam et al., 2010). Maximum temperature risen up to 40°C indicate the earthworms' activity in vermicomposting (Adhikari, & Khanal, 2015). However, optimum temperature depends on the difference species of earthworms as shown in Table 2.1.

Table 2.1: Optimum Temperature range for difference species of earthworms.

No.	Temperature range (°C)	Species	References
1	27-33	Eudrilus eugeniae	(Rajesh Banu, et al., 2001)
2	25	Eisenia fetida; Eudrilus eugeniae	(Pattnaik, & Reddy, 2010)
3	20-35	Eisenia fetida	(Zularisam, et al., 2010)
4	15-35	Eudrilus eugeniae; Eisenia fetida; Mega-scolex mega scolex	(Nitin Prakash Pandit, et al., 2012)
5	27	Eisenia fetida; Lampito mauriti	(Biosci, et al., 2014)
6	28-32	Perionyx excavates; Lampito mauriti; Drawida nelalensis	(Varma, et al., 2016)
7	26-35	Pheretima california	(Nitin Prakash Pandit, et al., 2012)

2.7 Mechanism of Worms in Vermicomposting Process

According to S, Jessy, & Ibrahim, (2014), earthworms itself is an effective tubular reactor which prevents the inactivation of the enzyme at high temperature through temperature regulatory mechanisms. The temperature regulatory mechanism helps to maintain the suitable temperature inside the earthworms' gut for bioprocess purposed.

Earthworms are classified as invertebrates where there are no bones and composed of many segments in a straight line. They can extend in sequences from mouth to anus (Kiyasudeen, et al., 2014). The entrance of feed is through the mouth and pharynx helps sucked in the incoming food particle to temporary storage. In temporary storage, food particles are mixed together and later moves to gizzard or digestive process part. Digestive muscle helps the coagulation of food and dirt and form a mixture. The glands released enzymes to assist chemical breakdown process of the organic material. After a finished chemical breakdown at gizzard, the mixture then sent to the intestine where various of nutrient are released by the aid of bacteria inside the gut. Bacteria ate the mixture of organic matter and released nutrients such as carbohydrates, proteins, minerals, and vitamins. These nutrients are supplied for worms and undigested organic matter together with soil's particles are thrown up through earthworm's anus. The waste is known as worm cast or vermicast. Vermicast is enriched with nutrients, revitalized and acid neutralized.

2.8 Pathogen Level Reduction during Vermicomposting

Vermicomposting is a mesophilic process. Thus, the temperature must below 35°C. Otherwise, earthworms could be inactive, flee, and die and disrupt the vermicomposting process. Vermicomposting involves mesophilic process while composting conduct thermophilic process. The thermophilic process is the process that used high-temperature range 50-70°C. High temperature practices during composting might eliminate many human pathogens such as E. *coli, Salmonella* spp., human viruses and helminths. However, the operation takes time in increasing the temperature to 50°C.

According to Edwards et al., (2011) the elimination of pathogens rate from contaminated leachate is twice as fast in the presence of earthworms as in its absence. An example of common pathogens presence in leachate is E. *coli, Salmonella* sp. and other Enterobacteriaceae. Apparently, pathogens destroyed as much as 98% after 17 days of vermicomposting, subsequent to the temperature risen by earthworms during vermicomposting. In addition, reduction of pathogens in vermicompost with nearly below the Class A limits after 6 to 10 weeks of the vermicomposting process were achieved and these is a strong evidence to use the vermicomposting process as an alternatives way for soil stabilization.

Pathogens destruction are caused by the competitive interaction with endemic bacteria which stimulate through the activity of earthworm on wastes. This activity also killed endoparasitic worms, bacteria, and viruses in organic matter. Multiplication of worms produced every 2 months vermicomposting was enough to increase the inactivation of viruses in organic matter. Kiyasudeen et al., (2014) reviewed the worm gut act as a reactor in vermicomposting where the organic matter in leachate is ingested as they are passed through the gut. These processes enhance the biological activity by increasing the surface area of ingested organic material. Thus, vermicast produced is highly nutritive with rich in a microbial activity that helps for plant growth. Moreover, for the last 50 years, many researchers had proven experimentally on the ability of earthworms in the reduction of pathogens level in vermicast. In conclusion, earthworms play crucible part on rapid reduction of pathogens in the vermicomposting process.

2.9 Accumulation of Heavy Metals by Earthworms during Vermicomposting Process

Leachate, particularly from a variety of sources such as industrial, household, construction and demolition, etc. (The World Bank Group, 2000), contain not only significant quantities of nutrients that may move into surface waters but also heavy metals and metalloids (Edwards, 2011). Both nonessential and essential metals excess can disrupt with soil microbial activities and plant growth. Consumption of tainted crops direct or indirectly affect to the wild animal and human health. According to Kinney et al., (2008), organic residues, including pharmaceuticals, which originate from biosolids and manure can be transferred to earthworms though the worms gut. Vermicomposting leachate poses a number of important and specific ecotoxicology that enhance crop growth such as N, P, K; nonetheless, the toxicity and a lot of heavy metals in leachate should be considered too.

According to Edwards, (2011), vermicomposting process produce effective plant growth media, containing large quantities of nutrient-rich, readily biodegradable organic matter that restrict metals availability via complexation, sorption, and precipitation reaction. Vermicomposter such as endogeic, epigeic, and anecic of earthworm species are capable of accumulating a number of nonessential and essential metals, thus enhancing the nutrient in vermicompost.

2.10 Biofertilizer

Biofertilizer is a natural product carrying living microorganisms derived from the root or cultivated soil (Kannahi, & Ramya, 2015). It also produced better seed germination and general plant growth (Khairuddin Abdul Rahim, 2012). Currently, companies that practicing production of biofertilizer are Felda, Felcra and Sime Darby where they used their plantations biomass wastes. Most research uses sewage sludge, vegetable waste, household waste, animal manure and biomass wastes, however in this research, landfill leachate used as substrates for biofertilizer production. This initiative was taken due to the production of leachate that rises along with the rise of accumulation of waste in a landfill site.

Leachate mainly from organic wastes where the degradation from it by earthworms' activity can optimize the growth of the biofertilizer microorganism. Thus, attribute to the large production of plant nutrients amendment in soil. Nevertheless, biofertilizer from vermicompost contains humic acid which helps regulate many processes of plant development included micro and macro nutrient adsorption. Abolmaaty, (2016) claimed earthworms promote better uptake of nutrient from the soil due to their activity in degrading organic matter.

Many types of research claimed that vermicompost potentially to control infection on the plant(Gutiérrez-Miceli, et al., 2008). This is due to plant pathogen controlled by earthworms during the vermicomposting process. Organic matter in leachate is effectively surpassed plant disease as they enhance microbial population and diversity in soil. In addition, non-thermophilic vermicompost acts as effective biocontrol agents aiding in the suppression of disease caused by oil-borne phytopathogenic fungi(Pathma, & Sakthivel, 2012). Vermicompost has been proven by lots of research and safe for environment used since they are eco-friendly and bio-pesticide(Pathma, & Sakthivel, 2012; Abolmaaty, 2016). Unless, chemical fertilizers where they give a high yield of crop instantly but the contents of chemicals composition do give harm to the environment and human health.

2.11 Quality Criteria for Vermicompost

Many countries have practice vermicomposting operation in large scale with different of wastes used; such as China, India, North Carolina and Australia where they demonstrate the viability of this renewable energy in these form and illustrate positive view on the expedition of vermicomposting roles in the recycling of organic waste. Furthermore, many scientific researchers claimed that vermicomposts fertilizer promoting the activity of beneficial microorganisms in the soil, transcend in plant resistance in diseases and pests, increasing the nutrient supplying of the soil to the root plants and reduce nutrient losses.

This section is to discuss the quality criteria for vermicompost in the various aspect that is important to achieve a standard of vermicompost fertilizer for better planting. Better standard of vermicompost indicates the maturity of vermicomposts in term of stability and pathogen seed sanitation. Potential quality criteria for vermicompost is the type of raw material used; type of earthworms' species; vermicomposting period and the density of earthworms left after a certain period. Each of these criteria influences the quality, maturity, and stability of vermicompost.

Vermicomposts should be kept under 75 to 90% of moisture content, to ensure that the material is not too dry which cause a reduction in plant resistance and rewetting. Low resistances are due to the changes in the composition of the microbial community in vermicompost and disturb the food web of soil. This extent causes the other beneficial qualities that dependent on this structure.

Vermicomposting of leachate in this research aims to produce biofertilizer that promotes plant growth instead depend on chemical fertilizer and evolve this new biotechnology in agriculture industrial. Thus, pH of the mixed of soil and leachate will be observed during the experiment. pH is an indicator for vermicompost acidity or vermicompost alkalinity which influence the availability of nutrient inside that improves fertility and plant growth aspect. Soil with pH range 5.5 to 8.0 is suitable for promoting plant growth and depending on the type of organic waste is used as substrates in vermicompost. Vermicomposts from sewage sludge had pH of 5.40 to 4.50 (Zularisam, et al., 2010; Sinha, et al., 2010); vermicomposts from sheep manure had pH of 7.6 to

8.1(Velasco-Velasco, et al., 2011) and vermicomposts from food waste with cow manure had pH of 8 (Nair, et al., 2006). Summarization of several vermicompost of different raw materials is shown in Table 2.2.

Table 2.2: Summarization for Quality Criteria of Vermicompost

No.	Type of raw material used	Type of earthworms' species	Amount of earthworms used per vermibin, g	Vermicomposting period	Final pH of vermicompost	Reference
1	Sewage sludge	*Eisenia fetida*	1000	21 days	4.50-5.40	(Zularisam, et al., 2010)
2	Sheep manure	*Eisenia fetida*	2500	2 months	8.60	(Gutiérrez-Miceli, et al., 2007)
3	Food waste and cow manure	60% *Eisenia fetida* and 40% *L.rubellus*	200	21 days	6.00-8.00	(Nair, et al., 2006)
4	20% Paper-mill sludge and 80% cattle manure	Clitellated and non-clitellated specimens of *E.andrei*	1500	70 days	8.1	(Elvira, et al., 1998)

2.12 The Benefits of Macronutrients and Micronutrients in Vermicompost

Vermicomposting process showing evidence that the process effectively eliminates human pathogen and sanitization of contaminated soil. Changes in chemical characteristics during vermicompost can be an indicator of the degree of stabilization. The most essential for the plant is macronutrient such as N-P-K. This primary nutrient is mostly needed by every plant in sufficient amount; however, over fertilizer of N-P-K led to environment problem as leach into the soil. Each of N-P-K has their own function for promoting plant development.

Nitrogen is necessary for protein production. During the vermicomposting process, nitrogen was released by earthworm during formation of various metabolic products such as growth stimulating hormones to get attached to available nitrogen (Sharma, et al., 2011). Nitrogen in the form of nitrates can be found in vermicompost in high or low concentration depend on the type of organic wastes used and pH. Sharma, et al., (2011) reported that at high pH, nitrogen is a loss as volatile ammonia, while neutral pH stabilizes the content of nitrate during vermicomposting. In vermicompost, the increased concentration of nitrates is due to microbial activity. Nitrates present in vermicompost are in ready forms for plant uptakes (Zaller, 2007). Nitrates content in fertilizer varies with the type of plant. According to Worthington, (2001), lower nitrates content are suitable for vegetables.

Phosphorus function as a nutrient for seedling development (Gellings, & Parmenter, 2004). According to Romero, et al., (2013), raw leachate contain a high amount of concentration phosphorus compared to digested leachate due to the amount of microorganism present. Therefore, with a greater amount of phosphorus probably can enhance the germination of seed. Phosphorus is classified as primary nutrients that are present in soil and the increase of phosphorus in vermicompost which is been trigger by the earthworms' activity during the process. Earthworms have degraded the organic matter in leachate into more soluble form from an insoluble form with the help of P-solubilizing microorganisms (phosphatases) that present in earthworm's gut (Pattnaik, & Reddy, 2010). Thus, making more available and exchangeable phosphorus for plants nutrient uptake. This nutrient assists in stabilizing soil fertility and plant growth.

Potassium is one of the major nutrient uptake by plants. The function of potassium is to improve the rigidity of the stalks and increasing disease resistance (Nicholson, 2016). Potassium level in fertilizer and soil also affects the concentrations and content change of chlorophyll of plants. This help the plants to overcome drought stress. Nevertheless, the potassium level required by plants depend on type of plants as claimed by Özyigit, & Bilgen, (2013). Deficiency of potassium can have caused reduction in chlorophyll concentration where the translocation of photosynthesis have been restrict from leaves to fruits during plant development. This caused poor development of leaf due to decrease in sucrose and starch contents of stems and flora buds.

Seven major micronutrients in fertilizer are Fe, Mn, Zn, Cu, B, Mo and Cl (Edwards, 2011). Vermicompost consist of macronutrients and micronutrients where both nutrients are known as primary and secondary nutrients respectively. Secondary nutrients are nutrient that averagely absorb by plants; however, the deficiency of these nutrient can be resulting to poor productivity. Macro and micro nutrients both showed an increased after vermicomposting process. Earthworms activities increase the mineralization process, thus enhancing the micronutrients (Cu, Zn, Mg and Fe) during vermicomposting period. This also increase the availability of these nutrients to be used for plant growth.

2.13 The Effects of Vermicompost on Plant Growth

Vermicompost showed a marketable increase in leaf area, dry weight of plant and improving fruit yield compared to soil without fertilizer. It is claimed that vermicompost contains growth promoting hormones which regulate for plant development such as increases germination, growth, flowering and fruiting. This is resulting due to earthworm gut associated microbes that enrich vermicompost with light-sensitive plant growth hormones. The presence of these hormones influenced the plant growth as making them stable and firm in soils more longer. Vermicompost not only improve and enhance the growth of plant but also reduced the occurrence of physiological disorder such as albinism and fruit malformation.

Albinism is characterized by partial or complete loss of chlorophyll pigments and incomplete differentiation of chloroplast membranes (Kumari, et al., 2009). Such incompetent has weakened photosynthesis process and can damage the plants. The plant can die at young stage without reaching maturity as the consequence of albinism. However, such disease can be reduced by using vermicompost a fertilizer where the incident of albinism can be reducing as much as 16.1% to 4.5% a claimed by Etingoff, (2015). This indicates vermicompost contains reducing nutrient-related disorder. Thus, significant increase in the number of fruits yield with better quality.

Fruit malfunction is the modification of vegetative arts. It gives the disease appereance, where all the axillary buds start growing simultaneously and sometimes the whole shoot become swollen (Sinha, & Majumder, n.d.). Fruit malformation has caused several effects at early fruit development such as misshapen or the fruits loss their shape symmetry, reduction insize and low pollen production. According to Ariza, et al., (2011), differences insize between well-shape and irregular fruits shape due to low reproductive capacibility means low capacity to secrete auxins. On the other hand, such disease trigger in early fruit ripening. However, fruit malformation has reduced about 11.5% to 4.0% when vermicompost is applied in pot. Thus, the improvement in plant growth might be due to the fact that earthworms enhance the physiochemical characteristics and nutrients of the processed leachate waste in soil. Vermicompost has supplying these nutrients in soluble and exchangeable form (nitrates, potassium and phosphate) in a balanced and sustained way which help in increasing antagonistic disease suppressing beneficial bacteria (S.Kiyasudeen, et al., 2016).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This research aimed to investigate the effect of varies amount of earthworms used on soil quality and their effect on the growth and productivity of tomato plants. This observation is to study on how the amount of earthworms on the degradation of organic matter in leachate. The soil content was analyzed with parameters such as the concentration of nitrates (N), phosphate (P), potassium (K), ammonia-nitrogen (NH_3) and pH. These parameters are important elements for plant growth where they are micronutrient that been absorbed by the plant. The vermicomposting process was conducted from 14th February 2017 to 14th March 2016.

Process flow for overall research design is shown in Figure 3.1 indicated the optimization of vermicomposting process. The NPK and ammonia reading for each vermibin are compared with control and standard vermicomposting using different substrates such as paper, kitchen waste and sludge after the vermicompost. In order to determine the maturity of vermicompost, color of leaf and length of shoot were taken as a parameter to produce nutritive biofertilizer.

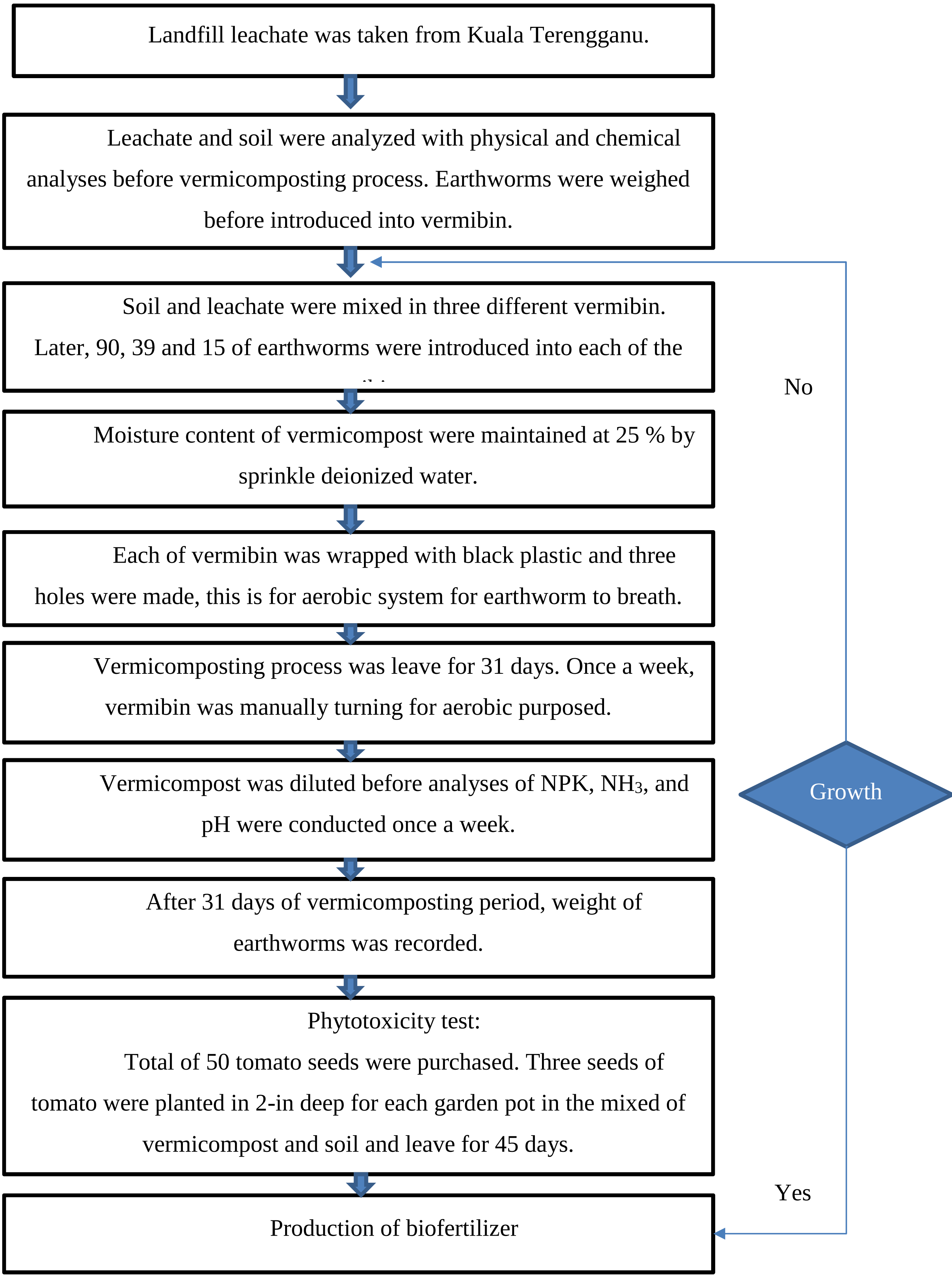

Figure 3.1: Process for overall research design.

3.2 Collection of Earthworms

Local worms were selected as vermicomposter for this study. Over 200 earthworms (approximately 243g) were purchased from a local seller in Gambang, Pahang, Malaysia. Three vermibins containing 90, 39, and 15 worms (approximately 52g, 30g. 8.g) respectively were used in this study (Domínguez et al., 2000; Kim et al., 2012; Rostami, 2011).

3.3 Preparation of Landfill Leachate

Landfill leachate was obtained from leachate pond at Sg. Ikan Landfill site Terengganu, Malaysia. Ten liters of leachate were tightly closed in a plastic bottle and kept in a refrigerator at 4°C temperature condition. The volume of leachate given for each vermibin was 150ml for 10cm depth of soil. Leachate was initially characterized for Nitrate-Phosphorus-Potassium (N-P-K), ammonium contents, and its pH before being introduced in vermibin.

3.2 Experimental Design

The experiments were conducted in plastic vermibin of size (m x m and m deep), each of capacity 150 ml of leachate, with holes at the bottom. The composting mixture consisted of different number of earthworms mixed with leachate and soil in the ratio; V1= 15:79.8:5.2 (% wt/wt/vol); V2= 15:82:3 (% wt/wt/vol) and V3=15:84.2:0.8 (% wt/wt/vol). Earthworms were introduced after soil and leachate were mixed in the vermibin. The duration of the experiment was 31 days. The moisture content in each vermibin was maintained to 25-50% for three days by sprinkling adequate quantity of water on vermibin. Each vermibin was covered with a black plastic to avoid vermicompost from the sun and left at room temperature. Vermicompost was manually turned over every week to aerate the waste and maintain good porosity in the vermibin. All three vermibin were replicated two times and soil-leachate without earthworms was used as control.

Table 3.1: Composition of different soil and earthworms in Vermibeds

Vermibeds	**Total volume of leachate,%**	**Total amount of soil, %**	**Amount of earthworms added, %**
V1	15	79.8	5.2
V2	15	82.0	3.0
V3	15	84.2	0.8
V4	15	79.8	5.2
V5	15	82.0	3.0
V6	15	84.2	0.8
Control	15	85.0	0

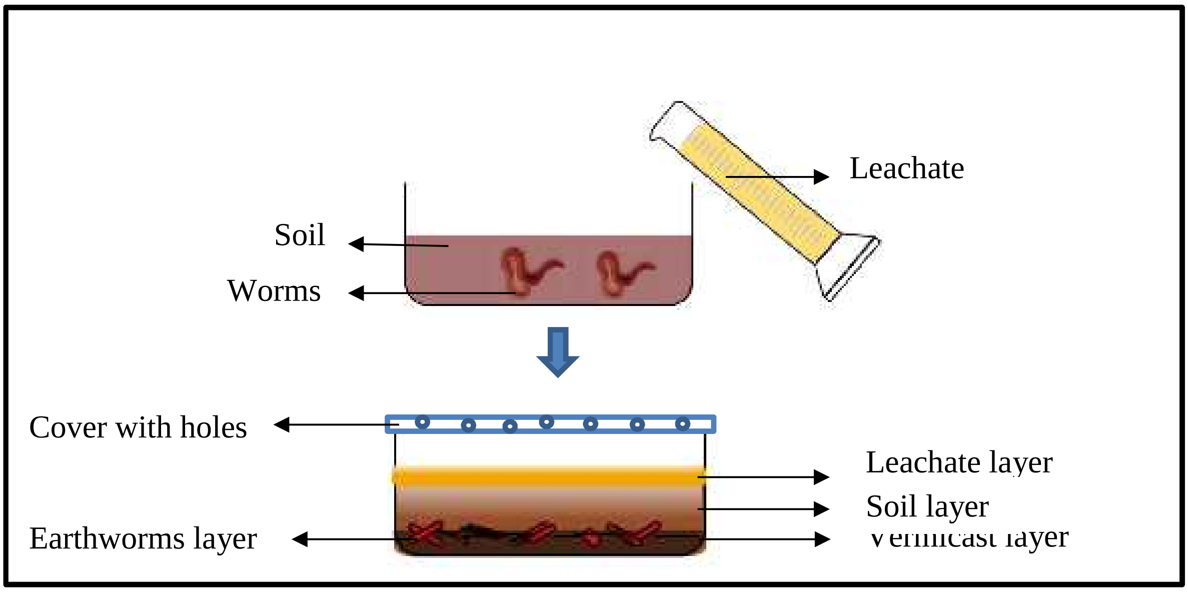

Figure 3.2: Schematic design of vermibin and experiment setup.

Source: (Zularisam, et al., 2010)

3.4 Physical Analysis

In this section, analysis using equipment such as Atomic Absorption Spectrometry and Total Solid Suspended were conducted for soil and leachate. Zainol, et al., (2012) reported in Characterization of Leachate from Kuala Sepetang and Kulim Landfill, leachate mainly consists of a large amount of organic matter including ammonical-nitrogen, phosphate, heavy metals and other toxicant. Thus, AAS able to analyze the concentration of heavy metals and micro and macro nutrient present in soil and leachate.

3.4.1 Atomic Absorption Spectrophotometry (AAS) for heavy metals identification

50 ml of leachate was measured using measuring cylinder and diluted with 100 distilled water. The mixture was filtered using a vacuum pump and ready for AAS analyses. The selected heavy metals such as Ni, Cr, Fe, Cu, and Zn were measured using AAS.

3.4.2 Total Suspended Solid (TSS)

Solids suspended in leachate consist of inorganic and organic particles or of immiscible liquids. Howard et. al., (1985) in "Environmental Engineering" book, claimed that leachate contains inorganic solids (silt, clay, soil, etc.) and organic matters such as biological solids (bacteria, pathogens, etc.) and plant fiber are familiar constituents in surface water. These suspended are exist due to the flow of water leachate from the accumulation of waste in the landfill. Therefore, analyze of leachate was conducted using TSS to determine the concentration of solids suspended in leachate.

Filter disk was dried in the oven at 105°C for 1 hour, cooled in a desiccator and weighed. Along the time of drying, filtering apparatus was assembled, filtered and the suctioned was begin. The filter was wet with a small volume of distilled water. After that, 50mL of leachate was pipetted onto the center of filter disk in Buchner flask under vacuum condition. The filter was washed with 10mL of distilled water three times and suction continue for 3minutes. The wet filter was dried in the oven for 1 hour at 105°C and cooled in a desiccator and ready for weighed.

3.5 Chemical Analysis

Vermicompost contains plant nutrients such as N-P-K. In this research, chemical analyses were done on vermicompost to determine the concentration of N-P-K and ammonium. Moreover, N-P-K are the major nutrients uptake by the plant for better performance in growth and productivity. The vermicompost were analyzed before and after vermicomposting as well determine the suitable ratio for the tomato plants. Thus, chemical analyses for each vermicompost were done on soil once per week during vermicomposting. This is to study the degradation of organic matter in a mixture of soil and leachate by earthworms on the concentration of N-P-K. The concentration of N-P-K was determined by Hach spectrometry method DR/2400.

3.5.1 Measurement of soil pH

pH is greatly tied with the availabilities of plant nutrients intake for plant growth (Lucas, & Davis, 1961). Thus, in this research, observation on pH changes during the vermicomposting process and the effect of end-product, vermicompost on the plant are conducted. Ideal pH for vermicompost is 5.0 to 9.0 as for better performance in plant growth (Narkhede, et al., 2011; Zularisam, et al., 2010).

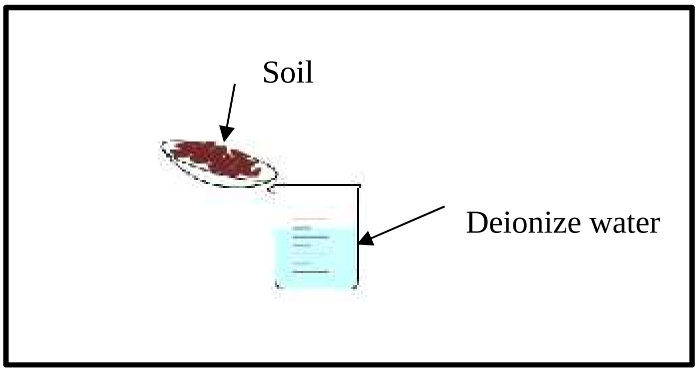

Figure 3.3: Soil pH analysis setup

Referring to Figure 3.2, 1 g of soil into the 1000 ml beaker, distilled water 5 ml was added to soil sample (1:5 diluted) (Marangon, et al., 2014). pH meter was used for measurement and data was recorded. The above steps were repeated for V2, V3, V4, V5, and V6 (Table 1).

3.5.2 Determination of Biochemical Oxygen Demands (BOD)

By referencing to Howard et. al., (1985) in "Environmental Engineering" book, dilution water was prepared by adding 1ml each of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solution. Distilled water was added until 1L in a volumetric flask. The leachate was measured up to 10ml and dilution prepared were added until 300ml in incubation bottle. pH leachate mixture was adjusted between 6.5 to 7.5 by adding acid/alkali. 300ml of dilution water was poured into another incubation bottle as a control. Measurement of dissolved oxygen concentration (DO_1) was performed using Dissolved Oxygen meter and the data was recorded. Before incubate both bottles labeled as 'Blank' and 'Sample' in BOD Incubator for five days at a temperature of 20°C. Reading DO_2 was taken after five days of incubation.

3.5.3 Determination of Chemical Oxygen Demands (COD)

COD analysis was tested on leachate before vermicomposting started and soil after the process. High levels of COD in leachate often correlate with threats to human health including seafood contamination and toxic algae blooms bacteria from organic wastes when released it to water stream (StormwateRx LLC, 2016). Therefore, vermicomposting was an alternative to decrease the levels of COD in leachate aside of leachate treatment. The measurement was performed using Hach method (DR/2400, method8000)(Anon 2004). The analysis was done before and after vermicomposting and the data was recorded.

3.5.4 Determination of Nitrogen, Phosphorus, Potassium and Ammonia

Nitrogen, potassium, and phosphorus are important nutrients for fertilizers as it enhances the plant's growth. However, vermicomposting is involved with earthworms that will be the emission of odor during the process. Therefore, soil and vermicompost were performed using Hach Spectrometry DR/2400; nitrate (N) (method8039), available phosphorus (P) (method8190), exchangeable potassium (K) (method8049) and ammonia nitrogen (NH_3) (method8155).(Anon 2004).

3.6 Phytotoxicity test on tomatoes plant

Phytotoxicity test is to evaluate the potential of vermicompost as a biofertilizer. These experiments were held for 60 days (November to December). Each treatment was conducted in duplicate. Eighteen tomatoes seed were planted in garden pot filled with 2-inch depth of vermicompost mixed with soil. Plants were grown in the pot with one control without vermicompost. All plants were irrigated with tap water ranging from 300 to 500 ml per day depending on crop maturity and soil water content till the tomato plants were grown fully. After that, the tomato plants height was measure every week for a month, and 2 weeks for the next months.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In this present study, the results show the mixture of soil and leachate enriching in micronutrients (NPK) after vermicomposting. This is because their nutrients in plant-available forms are released slowly during vermicomposting period. Thus, vermicomposting of leachate appears to be high value biofertilizer beside vermicomposted from sewage sludge, kitchen waste and animal manure which not only increases the plant growth in term of plant height but also pollution free and cost effective. The use of vermicompost promotes soil aggregation where it is improves the air-water relationship of soil, thus encourages extensive development of root system of plants and increasing the water retention capacity (Khan, & Ishaq, 2011).

4.2 Characterization of leachate

Table 4.1 shows the characterization of leachate at Sg. Ikan, Terengganu. Data were presented for the chosen parameters. Values of discharge limit EQA 1947 were listed as a comparison. As a rules of thumb, leachate presented by high values of pH, COD, ammonia nitrogen and heavy metals, as well as strong color and bad odor. However, the composition of waste, climate, rainfall, seasonal variations, solid waste management practices and biodegradable matter present in the leachate over the time influence the characteristics of the leachate (Meeroff, et al., 2015).

The pH values for leachate at Sg. Ikan was recorded at 8.41. This indicate that this leachate was in the methane fermentation phase (Mohd Zin, et al., 2012). Fortunately, the result analysis in Table 4.1 was found that the elements of heavy metal such as Cr, Ni, Fe, Cu and Zn recorded low value in the leachate and passed the permissible for leachate discharge. At these phase, the solubility of heavy metal to soil solution decreasing as the pH of leachate at alkalinity.

Table 4.1: Chemical-physical characteristic of leachate used in the experiment (the value in ppm)

Parameter	Unit	Value	Standard
pH	-	8.41	6.0 – 9.0
BOD_5	° C	155.11	20
COD	mg/L	5,443.5	400
SS	mg/L	20.88	50
Fe	mg/L	34.65	5.0
Cu	mg/L	0.03	0.20
Zn	mg/L	0.23	2.0
Ammonia Nitrogen	mg/L	155	5
Cr	mg/L	Undetected	0.20
Ni	mg/L	Undetected	0.20

The pond has been in operation since five year ago. The leachate was characterized by high BOD, and low dissolved solids as well as heavy metals. BOD was chosen as a measure of the amount of oxygen uptake by microorganism during biological degradation of organic compound (Mohd Zin, et al., 2012). It used as indicator in measuring the strength of organic matter. Table 4.1 recorded about 87 % of BOD leachate exceed the permissible from EQA 1974 for discharge. This shows that leachate has high organic matter and able to be used as feeding stock for earthworms during vermicomposting process.

Landfill leachate Sg. Ikan containing COD of 5,443.5 mg/l, N 75 mg/l and P 33.5 mg/l. Sg. Ikan was found being rich in COD which presents great toxicity risks on natural water bodies; however, the toxicity can be remove once the pH rise. This is due to the conversion of volatile acids to methane (CH_4) and carbon dioxide (CO_2) throughout the leachate recirculation phase (Tittlebaum, 2010). The COD: N: P ratio is 162:2.2:1. The nutrient content is low for aerobic treatment processes but vermicomposting treatment processes has been used widely to transform waste in to value added product. Vermicomposting technology is efficient to enhance the nutrient content by decompose the organic matter using microorganisms.

Prior to this characterization of leachate, the vermicomposting technology may be possibly to decompose the organic matter in leachate into valuable fertilizer for agriculture purpose.

4.2 Earthworm biomass

The number of earthworm before and after vermicomposting is shown in Figure 4.2. The number of earthworms decrease in all vermibin. Decreasing the number of earthworms from 90 to 78 per kg of vermicompost in V1 by 13 % is greater than V2
(5 %) and V3 (7 %).

Table 4.2: The number of earthworm before and after vermicomposting

Vermibin	Initial	Final
	Number of worms	Number of worms
V1	90	78 ± 2.82
V2	39	37 ± 1.41
V3	15	14 ± 0.70

The decrease in the number of earthworms from each vermibin is may due to short-period of vermicomposting; young earthworms takes many months to grow into mature adults and they are easily affected by the toxicity of the waste (Frederickson, & WRC, 2002).

The number of earthworms shown the same trend of reduction in the final weight of earthworms. However, the final average weight of individual earthworms was found to be merely equivalent with the initial weight even though, the final total weight of earthworm was found to be lower by the end of vermicomposting period. As recorded in

Table 3, earthworm has lost their by 34 % (V1), 52 % (V2) and 41 % (V3) from their initial number reading. The possible cause is the earthworms might lack of nutrient. According to Mississippi Department of Environmental Quality, the earthworms need to eat about over half their weight in food each day. In this studied, the earthworms only being feeding 150 ml of leachate at the initial of the experiment setup and followed by deionizer water for the next 30 days (MDEQ, 2007).

Table 4.3: Growth rate of earthworms in vermibin

Vermibin	Period (days)	Initial weight of worms (g)	Final weight of worms (g)
V1		52.1 ± 1.697	34.62 ± 1.754
V2	31 days	32.4 ± 0.495	15.46 ± 3.917
V3		7.7 ± 0.424	4.52 ± 1.895

The main factors influencing the growth and activity of earthworm is the physio-chemical characteristics of soil; like soil texture and presence of organic matter. Decreased supply of food as the organic matter content gradually decreases with repeated cultivations, growth (mature, formative, immature) and mechanical damage during vermicomposting (Clive, & P.J, 1996). Department of Primary Industries reported that worms can lose 20% of their body weight each day in mucus and castings, so the soil must be kept moist at the optimum moisture content. However, the moisture content in this study was about 46% which did not achieved the optimum standard moisture at 60 % -80% (Singh, et al., 2016). This is because the bedding depth in this research did not achieved the standard height, which in the range of 4 – 6 inches ("Worm Care and Feeding", 2017). Thus, this resulted flood in the vermibin due to less amount of soil to absorb the water. Not only that, this effected in the weight loss of earthworms. Therefore, the moisture content plays an important role in the distribution of earthworm species.

4.3 *pH*

From the results in Figure 4.1, the effect decreased after 3-weeks of vermicomposting did not differ for V2 and V3 except for V1. pH of vermicompost in V1 recorded the highest pH from the range of 6.42 to 6.13, compared to control (6.7 to 6.1). V3 showed the highest reduction by 14 % while control reduce by 8 % after 31-days of experiment. The application of earthworms may produces acidic compound by-product, which resulted in lowering of pH. Moreover, pH has a significant role in concentration of nitrate, where high pH cause nitrogen lost as volatile ammonia, while neutral pH stabilizes the content of nitrates (Sharma, et al., 2011).

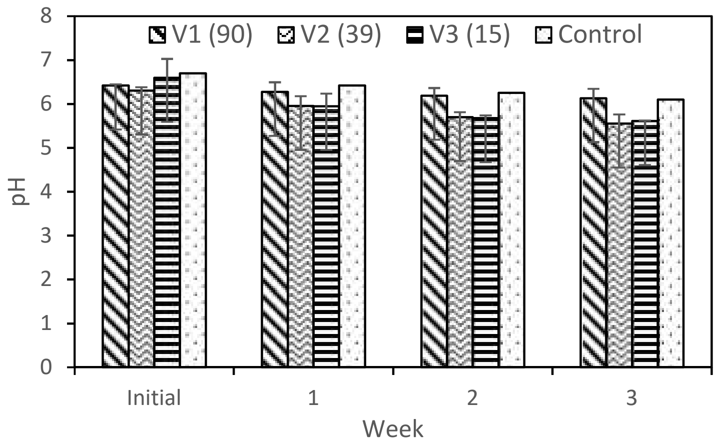

Figure 4.1. Changes of pH during vermicomposting.

The reduction in pH (Figure 4.1) might be due to the anaerobic process and acidification phase occurred during vermicomposting (Rostami, 2011). In fact, the reduction in pH may due to production of CO_2, NH_3, NO_3^- , and organic acids by microbial decomposition during vermicomposting (Sharma, et al., 2011) (refer Figure 4.1). Arancon, et al., (2004) also reported that vermicompost tended to have pH values near neutrality due to organic acids produced during microbial metabolism and production of CO_2.

4.4 Nitrates (N)

The concentration of ammonium in leachate utilized in the experiments showed comparatively low value (155 mg/l) which indicate low nitrogen intensification process (Bityutskii, et al., 2007; Domínguez, et al., 2000). However, the results in Figure 4.2 showed that the highest concentrations of $NO_3^- - N$ corresponded to the vermicompost and the lowest to the control, where $NO_3^- - N$ in all vermibins increase at the end of day 31. The increase of nitrate concentration in vermicompost may due to the additional of earthworms in the process of degradation. As shown in Figure 4.2, the application of 90 earthworms to 150 ml leachate was found to increase 54-fold of nitrate concentration; while control has the lowest nitrate concentration with increase 2-fold from initial reading. The number of earthworms in V1 reduce from 90 to 78 (Table 4.2) indicating that microbial communities used the available energy more efficiently. As a consequence, the large increase V1 in the rate of decomposition of organic matter and in the rates of nitrogen mineralization (Dominguez, 2011).

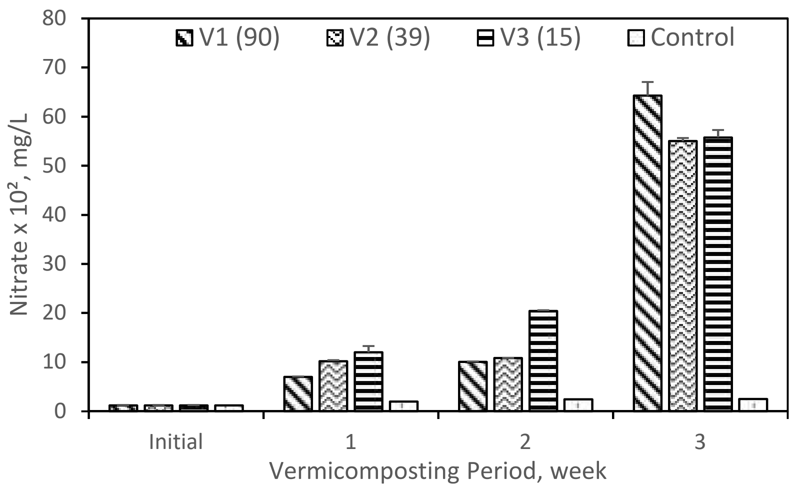

Figure 4.2 Concentration of Nitrates of Vermicompost and Control

The increased in concentration of nitrates values in vermicompost might be due to an increased surface to volume ratio which favoured microbial activity, and consequent N mineralization (Tognetti, et al., 2007). Other research claimed that the increased in $NO_3^- -$ N in vermicomposted indicates that these was no loss of $NO_3^- - N$ during the process of vermicomposting (Francou, et al., 2005; Sharma, et al., 2011). Therefore, retention time of N might be facilitated in the phytotoxicity process. Nitrate content (plant-available form of nitrogen) was high when vermicompost from sewage sludge (35,700 mg/L) compared to cow manure (600 mg/L) after one month vermicomposting. This confirms that the different wastes as a substrate for feeding the earthworms affects the amount of nitrogen from the compound and mineralization of nitrogenous organic compound. Furthermore, nitrogen was released by earthworms during vermicomposting possibly from the formation of various metabolic products such as growth stimulating hormones and dead tissues get attached to the available nitrogen (Shamini, & Fauziah, 2014).

4.5 Phosphorus content (P)

As shown in Figure 4.3, Phosphorus content in vermicompost was enhanced within 31-day vermicomposting, whereas control decrease after 2-week of vermicomposting. Among the vermicomposts, V1 (291.62 mg/L) containing 90 earthworms recorded the highest phosphorus content followed by V2 (218.25 mg/L) and V3 (165.225). Phosphorus in V1 increase 194-fold from 1.5 to 291.62 mg/L at the end of vermicomposting. This results showed that the vermibin containing earthworms enhance the phosphorus content in the vermicompost by degradable the organic matter in the leachate compared to control.

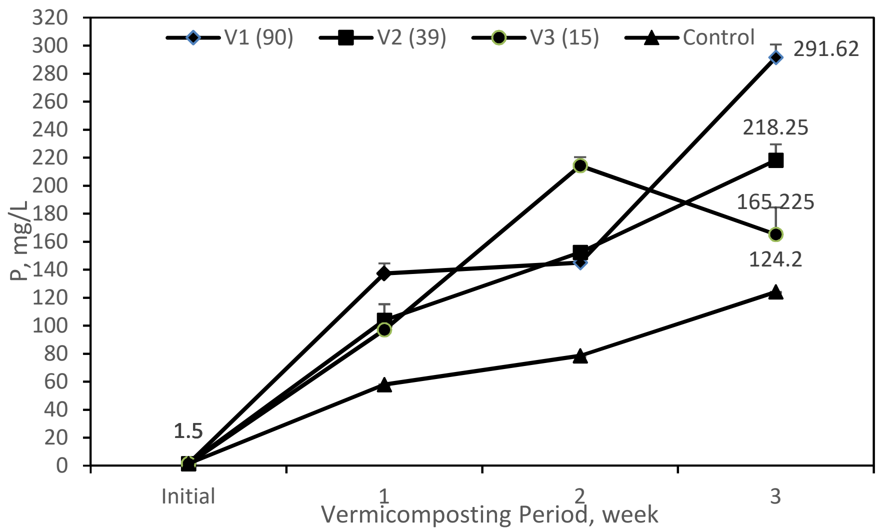

Figure 4.3 Concentration of Phosphate of Vermicompost and Control after 31-days of vermicomposting process. Values ± SE.

Moreover, the earthworms degrade the phosphorus contents in organic matter to the available form for plant uptake. Adhikary, et al., (2012) reported that earthworms consumed large amount of soil with organics, grind them in their gizzard and digest them in their intestine with aid of enzymes. Phosphorus solubilizing microorganisms living the earthworms gut produce enzyme, which will react on the substrate and then releasing phosphorus via secretion (Zularisam, et al., 2010). About 5-10 percent of the chemically digested and indigested material is absorbed into worms' body and the rest is excreted as vermicast; a fine mucus coated granular which are rich in NPK and beneficial soil microbes.

4.6 Potassium content (K)

Figure 4.4 recorded that the highest values were after 31-days of vermicomposting than initial feed mixtures (Figure 4.4). In week 1, vermicompost showed almost the same value for K. However the concentration of K increased significantly in V1 from 327 to 1263 mg/L compared to control which slightly decrease from 100 to 84 after 2-weeks of vermicomposting. Higher amount of earthworm in V1 (90 earthworms) resulted in higher amount of K. This finding were supported by Zularisam, et al., (2010), who reported an increased in K in sewage sludge; Das, et al., (2014), who also reported an increased in K when vermicomposting with different substrates (cattle manure, paddy straw, municipal solid waste and fly ash).

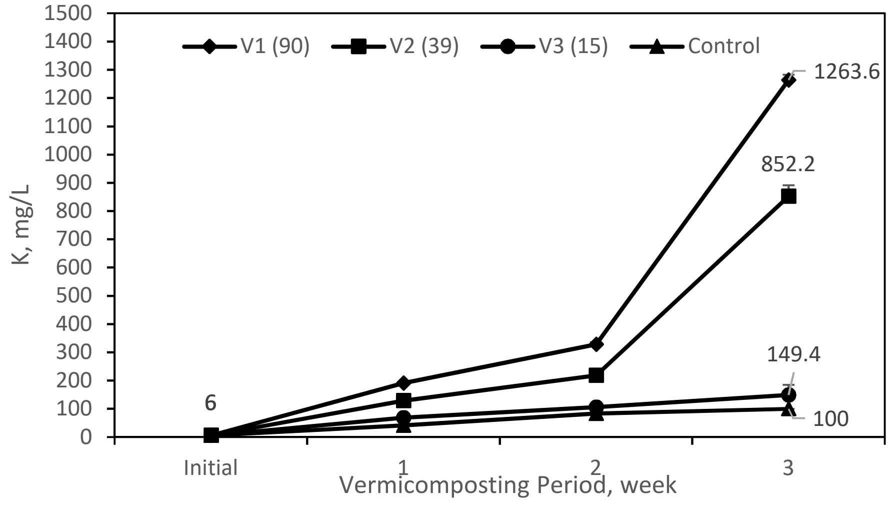

Figure 4.4 Concentration of Potassium of vermicompost and control (means±SE). Standard deviation are given at the top of the column.

Earthworm secreted mucus and water to increase the degradation of ingested organic matter and released the exchangeable potassium, thus enhancing K content in vermicompost (Suthar, 2007). In addition, organic acids produced by these microorganisms in earthworms influences the status of K (Das, et al., 2014; Sharma, et al., 2011). According to Fulton, et al., (2010), potassium undergoes exchange reactions with other cations in the soil such as sodium, calcium, magnesium, and hydrogen. The present of heavy metals in leachate might as well react, thus resulted in the enhancing of K content in vermicompost compared control which recorded lowest K (100 mg/L).The colour and texture of the vermicompost started changing to the dark-brown color of soil which indicated of the stabilization process that started in week 2. Similarly, Sinha, et al., (2010) claimed if the vermicompost changed to the color of soil, then the stabilization process occurred.

4.7 Phytotoxicity test

According to Figure 4.5, the plant height was significantly higher in the tomato plants that were added with vermicompost as compared to control after 4-weeks of planting. This probably increase in soil fertility, as vermicompost is rich in nitrate content. The leaf number of tomato plants did not show any sign of disease. V1 consists of 90 earthworms has high NPK compared to V2 and V3. Thus result, the highest plant height in V1 (14.4 cm) while the lowest value (4 cm) was recorded for the control. In addition, V1 was measure to have 32-leaf number compare to V2 and V3.

This indicate N-P-K (6,247- 291 -1263 mg/L) of V1 is suitable for tomato plant.

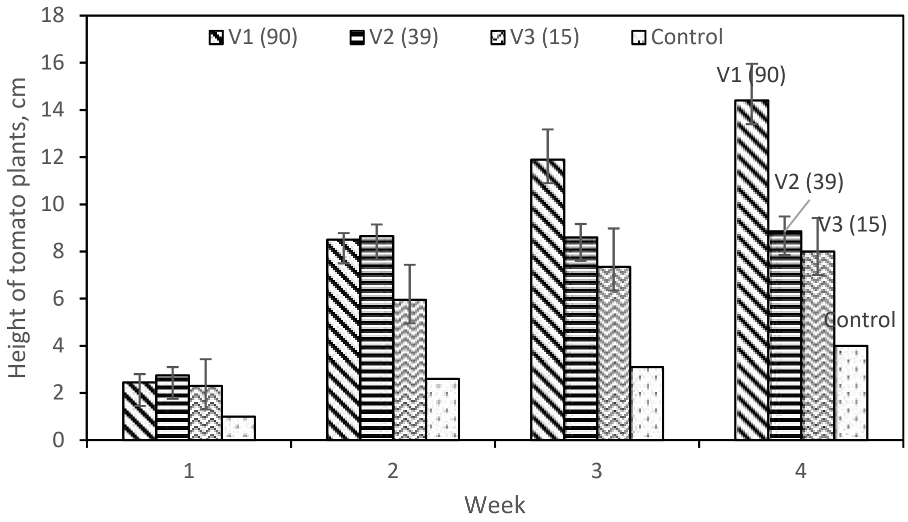

Figure 4.5. Height of tomatoes stem (means±SE). Standard deviation are given at the top of the column.

The tomato plants resulted from the vermicompost was significantly higher than control. These results were agreed with the finding of Mengistu, et al., (2017) who reported the significant impact in height of tomato after the application of vermicompost.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Leachate production may contaminate the soil and underground water as well as the releasing of unpleasant smell. A way of handling this leachate is by converting into vermicompost or biofertilizer, which is rich in important nutrients required for plants. In this research, biofertilizer production by vermicomposting of leachate showed significant result in enhancing NPK and the texture of the soil. Vermicompost in V1 showed the best biofertilizer, which contain high N-P-K with pH of 6.13. In our present study, V1 containing 90 earthworms enhance the nutrient content of N-P-K (6,247-291-1263 mg/L). This indicate that the number of earthworms influence the enhancement of NPK during vermicomposting period. The phytotoxicity test showed remarkable height of tomato plant of 14.4 cm with 32-leaf number after 4-weeks from the application of vermicompost.

5.2 Recommendation

For the further research, the moisture content need to reach at least 60 % – 80 % and maintain during vermicomposting process (Mehta, & Karnwal, 2013). This is to help earthworms in retaining moisture and enhance their activity to produce vermicompost that exceptionally valuable fertilizer. Considering the moisture content, height of soil should be 8 to 12 inches to avoid the soil become muddy and to increase the mobilization of earthworms in soil (Hashemi, et al., 2004). Optimum condition will help production of earthworm besides enriching the element such as nitrate, phosphorus and potassium.

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APPENDIX

Electrode must be totally immersed in the buffer to get the precise and accurate pH reading.

Analysis species earthworm purchased using Illustration 1.

Steps for COD analysis

Preparation for diluting leachate with dilution factor 67 for COD analysis.

Blank and leachate sample at 150°C for two hours.

Reading COD for raw leachate

Sample (different sources)	COD, mg/L
Landfill Leachate	50 317
Blank	0