DETERMINATION OF FORMALDEHYDE CONTENT FROM DISPOSAL OF FORMALIN FIXED BIOLOGICAL SPECIMEN BURIED IN SOIL BY ULTRAVIOLET-VISIBLE SPECTROPHOTOMETER

by

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Sustainable Science) with Honours

FACULTY OF EARTH SCIENCE

UNIVERSITI MALAYSIA KELANTAN

2020

DECLARATION

I declare that this thesis entitled "Determination of Formaldehyde Content from Disposal of Formalin Fixed Biological Specimen Buried in Soil by Ultraviolet-Visible Spectrophotometer" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ________________________________

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Date : 7th January 2020

ACKNOWLEDGEMENT

Firstly, the most important is I want to thanks to Allah SWT and not forgettable His Messenger, Muhammad SAW because without of His will, I could not finish my last work in University Malaysia Kelantan which is my final year project (FYP). All of my effort in this project will not finish well without help and support from several people who are always be in by my side either far from me or the person that are closed with me. I would like to thank my supervisors, Dr. Musfiroh Binti Jani and Dr. Nor Shahirul Umirah Binti Idris for their continued mentorship, guidance and support throughout this lengthy project. Their enthusiasm for this research has kept me going over the years. All of their effort, time, ideas, and instruction will be my valuable memory. I would not forget all their tireless effort that helps me a lot to finish my FYP. My highest appreciation for you and hope that one day god will reward you for all your works. I hope that we will work together again in the future.

Special thanks to our laboratory technician Mr. Rohanif, Mrs. Izzati and Mrs. Syahida for all their help with the instrument and handling chemical in laboratory and also to all technicians from the Faculty of Earth Science departments for letting me use all the facilities like instrument. Hence, thanks to University of Malaysia Kelantan for allowing me to use all the facilities such as Wi-Fi, laboratory and library.

I could not finish my FYP without support and encouragement from all of my friends in this university. Thank you very much friends. I am truly grateful that you shared your time and knowledge with me. My prayed were also for all of you and hope you guys also will succeed in whatever you do.

I wouldn't be where I am without my family member who have always believed in me. Thank you for your support throughout the years. Lastly but most importantly, thank you to my father Mohammad Zakaria Bin Alang Zahari and my mother Rosna Binti Abdul Rahman for given their 100% full of support and prayed for my successful in completing my FYP. Without both of you none of this would have been possible. Your endless love and encouragement have kept me going over the years. Thank you for always believing in me and for your many sacrifices.

Determination of Formaldehyde Content from Disposal of Formalin Fixed Biological Specimen Buried in Soil by Ultraviolet-Visible Spectrophotometer

ABSTRACT

Incineration technology is the best way to eliminate hospital waste, however, usage of incineration would lead to environmental pollution. Therefore, University Science of Malaysia Hospital (HUSM) has used formalin in their dead biological specimens to provide high resistance from decaying quickly before buried at cemetery area. Thus, this study aims to evaluate the concentration of formaldehyde in soil either give adverse impact to the soil or not. It was an important information to the community at the HUSM on the suitability of burying biological specimens that have been treated by formalin, thus can help them in selecting and designing proper way of biological waste disposal for references. In this study, soil fertility associated with pH, moisture content and organic matter were evaluated. The amount of soil elemental distribution and formaldehyde concentration of pre-burial and post-burial of biological specimen also have been evaluated by using Energy Dispersive X-Ray Fluorescence (EDXRF) and Ultraviolet-Visible Spectrophotometer instrument respectively. The absorbance value for standard curve of formaldehyde was determined at reflectance 412 nm. The results showed that the soil fertility in terms of pH at Point C (closest point to burial) indicates neutral pH (even though formalin was an acidic) while its moisture content in optimum condition and the organic matter percentage was medium before burial and become low after burial within a period of three months. For X-Ray Fluorescence (XFR) analysis at Point C, soil elemental distribution after burial of dead biological specimens has higher concentration compared to before the burial. According to United States of Environmental Protection Agency (USEPA) Regulatory, all the elemental distribution detected before and after burial were still below the recommended limits. Lastly, the concentration of formalin at Point C was higher after the burial of dead biological specimen compared to before burial. The highest and lowest concentration of formalin at Point C were 175.10 mgL^{-1} and 148.83 mgL^{-1} respectively, which exceeds the tolerable concentration recommended by the World Health Organisation (WHO).

Penentuan Kandungan Formaldehida dari Pelupusan Spesimen Biologi Tetap Formalin yang Dikebumikan dalam Tanah oleh Spektrofotometer Ultraviolet-Terlihat

ABSTRAK

Teknologi pembakaran adalah cara terbaik untuk menghapuskan sisa hospital, namun penggunaan pembakaran akan membawa kepada pencemaran alam sekitar. Oleh itu, Hospital Universiti Sains Malaysia (HUSM) telah menggunakan formalin dalam spesimen biologinya yang mati untuk memberikan ketahanan yang tinggi dari pembusukan cepat sebelum dikebumikan di kawasan perkuburan. Oleh itu, kajian ini bertujuan untuk menilai kehadiran formaldehid di dalam tanah sama ada memberi mudarat kepada tanah atau tidak. Ini adalah maklumat penting kepada komuniti di HUSM mengenai kesesuaian menguburkan spesimen biologi yang telah dirawat oleh formalin, dengan itu dapat membantu mereka dalam memilih dan merekabentuk cara pembuangan sisa biologi untuk rujukan. Dalam kajian ini, kesuburan tanah yang dikaitkan dengan pH, kandungan lembapan dan bahan organik dinilai. Jumlah pengedaran elemen tanah dan kepekatan formaldehid spesimen biologi sebelum dan selepas pengebumian juga telah dinilai masing-masing dengan menggunakan instrumen Penyebaran Tenaga Pendarfluor Sinar-X dan Spektrofotometer Ultraviolet-Terlihat. Nilai penyerapan untuk lengkung standard formaldehid ditentukan pada refleksi 412 nm. Keputusan menunjukkan bahawa kesuburan tanah dari segi pH pada Titik C (titik paling dekat dengan pengebumian) menunjukkan pH neutral (walaupun formalin adalah berasid) manakala kandungan kelembapannya dalam keadaan optimum dan peratusan bahan organik adalah sederhana sebelum pengebumian dan menjadi rendah selepas pengebumian dalam tempoh dua bulan. Untuk analisis Pendarfluor Sinar-X di Titik C, taburan unsur tanah selepas pengebumian spesimen biologi yang mati mempunyai kepekatan yang lebih tinggi berbanding sebelum pengebumian. Menurut Peraturan Agensi Perlindungan Alam Sekitar Amerika Syarikat, semua pengagihan elemen yang dikesan sebelum dan selepas pengebumian masih di bawah had yang disyorkan. Akhir sekali, kepekatan formalin di Titik C lebih tinggi selepas pengebumian spesimen biologi mati berbanding sebelum pengebumian. Kepekatan formalin tertinggi dan terendah di Titik C adalah 175.10 mgL^{-1} dan 148.83 mgL^{-1} masing-masing, dimana melebihi kepekatan yang boleh diterima oleh Pertubuhan Kesihatan Sedunia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Al^{3+}	Aluminium Ion
Al	Aluminium Element
Ba	Barium Element
BC	Before Christ
Ca^{2+}	Calcium Ion
Ca	Calcium Element
CEC	Cation Exchange Capacity
Cl	Chlorine Element
cm	Centimetre
Cr	Chromium Element
Cu	Copper Element
EC	Electrical Conductivity
EDXRF	Energy Dispersive X-Ray Fluorescence
EPA	Environmental Protection Agency
Fe^{2+}	Iron (II) Ion
Fe^{3+}	Iron (III) Ion
Fe	Iron Element
g	Gram
GPS	Global Positioning System
H^{+}	Hydrogen Ion
HBV	Hepatitis B Virus
HCHO	Formaldehyde
HIV	Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HUSM	University Science of Malaysia Hospital
I	Iodine Element
IARC	International Agency for Research on Cancer
K^{+}	Potassium Ion
K	Potassium Element
M	Mass
mg/kg	Concentration (Milligrams Per Kilograms)

mg/L	Concentration (Milligrams Per Litre)
Mg^{2+}	Magnesium Ion
mL	Millilitre
mm	Millimetre
Mn	Manganese Element
µL	Microlitre
µm	Micrometre
N.D.	Not Determined
Na^{+}	Sodium Ion
Ni	Nickel Element
nm	Nanometre
P	Phosphorus Element
Pb	Lead Element
pH	Potential of Hydrogen
PIC	Products of Incomplete Combustion
ppm	Parts Per Million
R^2	Correlation Coefficient
Rb	Rubidium Element
Si	Silicon Element
Sr	Strontium Element
Te	Tellurium Element
Ti	Titanium Element
TLC	Thin Layer Chromatography
USEPA	United States of Environmental Protection Agency
USM	University Science of Malaysia
UV	Ultraviolet
VOC	Volatile Organic Compound
V	Volume
WHO	World Health Organisation
XRF	X-Ray Fluorescence
Zn	Zinc Element
Zr	Zirconium Element

LIST OF SYMBOLS

%	Percentage
℃	Temperature (Degree Celsius)
<	Less Than
+	Addition
×	Multiplication
( )	Parentheses

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of Study

Formalin is a chemical substance and is about 40% of the formaldehyde solution (HCHO). Formaldehyde is a pungent gas. However, some scientists say that formalin is a chemical of 37% of the formaldehyde solution (HCHO). Typically, this formalin acts as an agent used in the preservation process known as embalming. Formalin is always used in biological laboratories to provide high resistance to dead biological specimens from decaying quickly. It will make the dead biological specimen to not decay over a prolonged period of time (Subramanian & Kumar, 2018). Decomposition and decay processes will occur when an organism dies and is buried. However, the rate of decomposition process and decaying process varies depending on the composition of the organism and is influenced by environmental conditions or surrounding conditions. For example, when buried without coffin and / or embalming fluid formaldehyde, the both processes would take 8 to 12 years. However, the decomposition and decaying process will take place for a longer period of time if there is adding of coffin and / or embalming fluid and also depends on funerary box. Decaying and decomposition processes will occur 4 times faster in the sea. Therefore, formalin and / or formaldehyde can act as a good preservative agent (Wisti, 2019).

Formaldehyde is also very important in histology. Histology is a study on tissue under the microscope. In addition, it also acts as an antiseptic and disinfectant.

Exposure to formaldehyde will cause immediate eye inflammation. Other than that, a study has been informed that exposure to 2 ppm of 10 ppm formaldehyde will cause sharp burning sensation of the nose and throat which may occur due to sneezing, difficulty in deep breathing and coughing (Martínez-Aquino, Costero, Gil, & Gaviña, 2018). The recovery will be faster with temporary effects as compared to the prolonged effects of higher ppm, where if one is exposed to a range of 25 to 100 ppm over a period of 5 minutes to 10 minutes, the probability of serious injury in respiration may occur. Even in 2011, the US State of the Toxicology Program has listed formaldehyde chemicals as a carcinogenic ingredient for human health (Martínez-Aquino et al., 2018). Formaldehyde also affects the environments such as plants, animals, water quality and soil fertility. In this study, the probability of the presence of formaldehyde in the soil is due to the disposal of biological specimens treated by formalin. This is because the disposal of biological specimens treated with formaldehyde has been buried near the sampling area. It aims to provide a resilient (non-decay) resistance to the specimen before being buried as before this specimen is only burned and the ashes are buried.

Typically, medical waste such as patient diagnoses or human treatment that has been collected may be deduced as infectious material only if it is not collected separately. If all hospital waste materials such as medical waste, domestic waste and infectious waste are not isolated, it can cause negative impact on humans and the environment. In the past, residues from hospitals and municipal waste were disposed of together at landfills. In the 1980s, viruses such as immunodeficiency virus (HIV), hepatitis B virus (HBV) and other agents associated with blood bones disease has increased rapidly and became a trend (Altin et al., 2002). Hence, this incident has given rise to awareness and has raised the public's concern about the proper disposal of hospital waste and more environmentally friendly. This will indirectly lead to positive developments in the management of hospital waste and municipal waste. Hospital waste is recommended not to be disposed-off mixed with municipal waste, but using the alternative method. The method called as incineration. Previous studies have suggested that incineration technology is the best way to eliminate hospital waste. Proper hospital waste disposal will reduce the amount of infectious waste and also the cost of treatment. However, incineration or combustion methods are found to be non-sharia and contrary to the way of life of the Muslim community especially in Malaysia (Universiti Sains Malaysia, 2019). Other than that, incineration would lead to the exposure of heavy metal and other pollutant such as hydrogen chloride, sulphur dioxide, fine dust particles and nitrogen oxides which can lead to air pollution (Jorge et al., 2004). Then this study should be conducted to see the effect of formalin on environment in terms of soil fertility.

This study required assessment of soil fertility, soil elemental distribution of pre-burial and post-burial and the presence of formalin in the soil. The soil fertility was identified in term of pH, moisture content and its organic matter. Next, the amount of soil element distribution was determined by using Energy Dispersive X-Ray Fluorescence (EDXRF) instrument while the presence of formalin has been tested by using Ultraviolet-Visible Spectrophotometer.

The importance of this study is to monitor soil fertility so that it is not contaminated by formaldehyde. Besides, it had answer to the question of non-compliance toward sharia in Islam through the method of combustion or incineration of biological specimens. Furthermore, it also had answer questions to the community at the University Science of Malaysia Hospital (HUSM) on the suitability of burying biological specimens that have been treated by formalin, either not giving any effect to the environment (soil) or otherwise, rather than uses incineration method again.

1.2 Problem Statement

In general, biological specimens or referred to as anatomical specimens was burned by local combustion facilities. As well as anatomical specimens, former anatomy specimens and body parts was also burned by local combustion facilities (Human Tissue Authority, 2015). This specimen has been preserved using formalin first. However, the process of incineration of biological specimens treated with formalin in most hospitals was brought harmful effects to humans, animals, plants and the environment such as water quality and soil fertility. The incineration process of medical waste has been identified by the Environmental Protection Agency (EPA) as the sole largest source of dioxin air pollution ever that have occurred in the United States (Jorge et al., 2004). Organic compounds such as dioxins and furans will emerge if an incomplete combustion or incineration at a predetermined minimum temperature of 1200°C happened. In addition, chemical compounds such as heavy metals including lead, cadmium and mercury, hydrogen chloride, sulphur dioxide, fine dust particles, nitrogen oxides, products of incomplete combustion (PIC), carbon monoxide and many other pollutants will be emitted by medical waste incinerator into the atmosphere (Jorge et al., 2004). The air pollution might happen. The International Agency for Research on Cancer (IARC) has categorized the most toxic dioxins is the dioxin that is in group 1 because it contains carcinogenic substances to humans. Dioxins are often associated with diseases such as abnormal hormonal systems, damaging the stamina of the organism, genetic defects, diabetes, endometriosis and other diseases.

The methods by which biological specimens can be disposed of with respect are also very limited. Therefore, the economic way to remove specimens remains a formalin anatomy is required. In this study, environmentally friendly means of burial of biological specimens treated by formalin into the soil was introduced. In this study, the probability of the presence of formaldehyde in the soil is due to the disposal of biological specimens treated by formalin. This is because the disposal of biological specimens treated with formaldehyde has been buried near the sampling area. It aims to provide a resilient (non-decay) resistance to the specimen before being buried as before this specimen is only burned and the ashes are buried. However, incineration method was found to be non-sharia and contrary to the way of life of the Muslim community especially in Malaysia (Universiti Sains Malaysia, 2019). Therefore, the study of the effects of formalin on the environment especially on the soil was important to ensure that this method (burying of biological specimen that treated with formalin) was safe and did not pose any risk to the environment and so on.

1.3 Objectives

1. To investigate the soil fertility in terms of pH, moisture content and organic matter.
2. To analyse the amount of soil elemental distribution of pre-burial and post-burial of biological specimen which treated by formalin.
3. To quantify the concentration of formaldehyde in pre-burial and post-burial soil.

1.4 Scope of Study

This research required assessment of soil fertility, soil elemental distribution of pre-burial and post-burial and the presence of formalin in the soil. The soil fertility was identified in term of pH, moisture content and its organic matter. Next, the amount of soil element distribution was determined by using Energy Dispersive X-Ray Fluorescence (EDXRF) instrument while the presence of formalin has been tested by using Ultraviolet-Visible Spectrophotometer.

1.5 Significance of Study

The importance of this study was to maintain the soil fertility so that it did not contaminated by formaldehyde as it uses soil parameter like pH, moisture content and organic matter to know the current condition of soil fertility. At the end of this study, it was able to know whether the method of burying of biological specimen that treated with formalin was safe and not pose any risk to the environment especially in soil and so on. It was an important information to the community around the world since this research is still in developed among the other researcher. Besides, it had answer to the question of non-compliance toward sharia in Islam through the method of combustion/incineration of biological specimens. Furthermore, it also had answers questions to the community at the USM Hospital on the suitability of burying biological specimens that have been treated by formalin, either not giving any effect or impact to the environment (soil) or otherwise. So, this study might help them to select and design proper way of biological waste disposal for references.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction to Formaldehyde

Formaldehyde also known as methanol was first identified by chemists from Germany (Garcia, 2019). A chemist named August Wilhelm von Hofmann has been researching this formaldehyde in 1867. Formaldehyde has its own distinctive features. It exists in the form of flammable gases. It can also dissolve in water. Commercially, it can be purchased as formalin. Formalin is a chemical substance and is about 40% of the formaldehyde solution (HCHO). Formaldehyde is a pungent gas. However, some scientists say that formalin is a chemical of 37% of the formaldehyde solution (HCHO) (Weiler, 2016).

2.2 Source of Formaldehyde

Formaldehyde is one of the types of volatile organic compound (VOC) that is usually found in both outdoor or indoor air. Formaldehyde can be formed naturally from vegetables and fruits. In addition, in human and animal bodies, formaldehyde will be produced in small quantities to synthesize amino acids. Formaldehyde does not actually pose a threat or health problem to humans if the concentration is at 0.03 parts per million (ppm). 0.03 ppm is actually its natural background level or called as intermediate minimal risk levels in the air (Nelson, 2015), while 0.02 ppm and

0.04 ppm are chronic minimal risk levels and acute minimal risk levels respectively (Agency for Toxic Substances and Disease Registry, 2015). Formaldehyde sources such as higher formaldehyde concentrations due to smoke from car exhaust, industrial pollution and by-products combustion, can trigger harmful toxic levels to humans, especially in urban areas. However, the highest level of formaldehyde is in indoor as home, office and school. It is due to the production of products that contains formaldehyde that has been widely used nowadays. For example, in products such as clothes, air fresheners, pillows, toothpaste, furniture and so on.

2.3 Technique for Identifying Formaldehyde

There are various methods for identifying the presence of formaldehyde. Among others are, capillary electrophoresis, high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), laser induced fluorescence spectroscopy, conductometry, enzyme-based biosensors and spectrophotometry. Although there are so many ways to detect formaldehyde, there are still some constraints or weaknesses in using these methods. Examples such as selectivity restrictions, complexity of operations, non-mobile or difficulties in real-time monitoring (Martínez-Aquino et al., 2018).

2.4 Effect of Formaldehyde on Environment and Human Health

2.4.1 Environment

Typically, formaldehyde does not seem to have serious effects on plants and wildlife around it as it will be quickly removed from the air. The reaction process with other species in the atmosphere will cause the formaldehyde to be rapidly removed from the air (Scottish Environment Protection Agency, 2019). It is also easy to decompose in water and soil in a few days. Formaldehyde in the air will be destroyed when released into the environment to create formic acid and carbon monoxide that can be harmful substances (National Pollutant Inventory, 2009). It will be released in the form of gas. It will be released from dry or damp soil, as well as from the surface of the water. However, formaldehyde can also absorb into the water again as it is soluble in water (Toxicology Data Network, 2015). Formaldehyde will move with water through the soil. As one of the members of the VOC group, formaldehyde can also damage plants and materials. This is because it acts in the formation of ground level of ozone. Besides, animals will get diseases such as difficulty in producing offspring and decreases in life expectancy if exposed to formaldehyde. Formaldehyde also is able to change the way of behaviour and physical characteristics of animals (National Pollutant Inventory, 2009). The high toxic contains of formaldehyde towards the aquatic life such as fish, crab, shrimp, shellfish and others will indirectly affect the composition and quality of rivers, lakes and seas such as acidification. However, formaldehyde pollution is still considered unlikely to cause major problems in the global environment.

2.4.2 Human Health

There are three roads or pathways on how formaldehyde can enter into the human body. Formaldehyde can easily infect the body through air inhalation, ingestion of contaminated food with formaldehyde or through direct skin contact. Inhalation of air containing formaldehyde directly will irritate eyes, nose and throat. More than that, formaldehyde can cause death if exposed to a high level of concentration. In addition, high concentration of formaldehyde also causes spasms throats and accumulation of fluid in the lungs. Other than that, respiratory problems such as asthma and bronchitis also can occur if the person is exposed to persistent airborne of formaldehyde. This kind of person become more easier infected with the disease such as vomiting and severe pain which is due to the air surrounding that contains formaldehyde chemicals. In some extreme cases, coma and death also can happen due to ingestion of formaldehyde that contained in food. Direct skin contact with formaldehyde will cause skin irritation and burnt skin. The effect is same going to eyeball as well. The effects on both skin and eyeball will appear within a few hours after exposure to formaldehyde. The International Agency for Research on Cancer has agreed and declared that formaldehyde can be categorized as carcinogenic chemicals (Scottish Environment Protection Agency, 2019). However, exposure to formaldehyde at the permissible level according to its standards, will not adversely affect human health.

2.5 History of Preserved Human Bodies

In ancient times, some individuals have perpetuated their bodies for thousands of years using preservation techniques. This technique will enable autopsy on the body to be as if the body had just died. The fact is, the body has died many years ago. Even more than that. The autopsy is still possible if the dead body is preserved. In ancient times before 5000 BC or about 7000 years ago, the process of preservation of human flesh has been made (Ryder, 2018). In fact, on ancient Egypt, mummies were introduced and this has become a benchmark for the process of preserving of death human body to be more easily understood today. Normally, the human body is made up of 26 elements whose main elements were carbon, oxygen, hydrogen and nitrogen, while the rest are aluminium, boron, calcium, chlorine, cobalt, chromium, copper, fluorine, iron, iodine, potassium, magnesium, manganese, molybdenum, sodium, phosphorus, sulphur, selenium, silicon, tin, vanadium and zinc (Niziolomski, Rickson, Marquez-Grant, & Pawlett, 2016).

2.6 Type of Preservation

2.6.1 Formaldehyde Preservation

The use of formaldehyde to treat corpses has become a warm conversation since it was discovered by German chemist August Wilhelm von Hofmann in the 1860s. This method is also the main method for preserving the body. Formaldehyde materials are used as a base material for preserving corpses. A chain of chemicals introduced into the body system through injection and usually involves the drying or freezing process after flushing the body with liquids and gases (Ryder, 2018). Then, most of the bodies will be sprayed with a waxy mixture to create an impermeable seal around the dead bodies. Hence, colonies of microorganisms cannot reproduce. This kind of method can be categorized as one of the most commonly method that have been used before. Although, this method was use for preserving corpses today but it also has its own constraints where formaldehyde is a toxic substance for the environment. It is not safe to be used by human especially when they exposed to the high concentration.

2.6.2 Egyptian Mummification

One of the most popular methods popularized by ancient Egyptians after was mummification. This mummification process is very unique and the technique used is amazing. First of all, palm wine and water from the river will be used for the purpose of washing the dead body. Then, slices are made on the side of the body (Ryder, 2018). Most internal organs will be removed except the heart. The heart will not be discarded due to the existence of spiritual elements. After that, a thin hook is used to remove the brain from the head through the nose while the body of the corpse will be placed with natron (salt mixture) and let it dry for more than 40 days. After the part of the body was washed for the last time, it would be wrapped in a garment outfit known as oil-lined linen (Ancient Egypt, 2019).

2.7 Soil Fertility

2.7.1 Soil pH

The concentration of hydrogen ions in the soil will determine the soil pH level. It is a determinant of the acidity and alkalinity of soil samples based on a scale of 0 to 14 as shows in Figure 2.1: pH Scale.

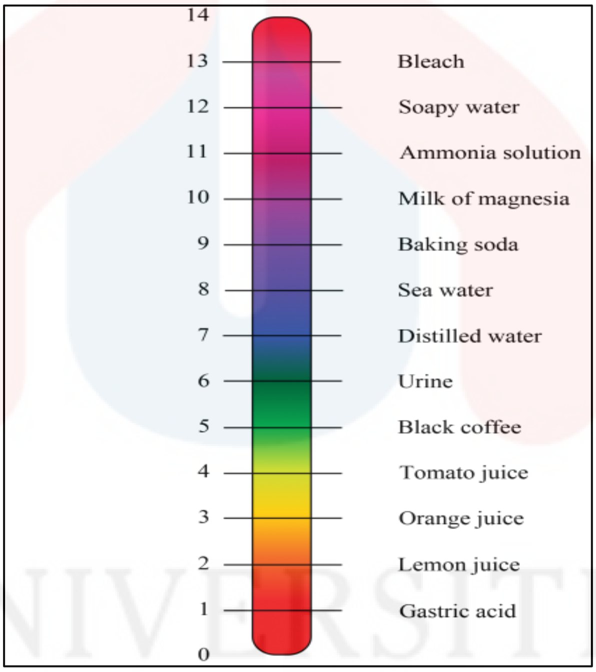

Figure 2.1: pH Scale (Kosto, 2018).

The acidic soil has a pH of less than 7, while the alkaline soil has a pH of more than 7 and then pH 7 is a neutral pH point. According to the definition, the negative logarithm scale for the concentration of hydrogen ions of pH will be measured as pH equal to -log[H+]. Thus, if the concentration of hydrogen ions increases, the pH level will decrease (McCauley, Jones, & Olson-Rutz, 2017). Each scale at the pH levels is

10 times more acidic than the pH scale on above. For example, pH 5 has a hydrogen ion concentration of 10 times more than pH 6 and 100 times more than pH 7.

The pH levels in the soil are influenced by both the formation of acid and base where it will form cations (positively charged dissolved ions) in the soil. The common-formed acidic cations are hydrogen (H^+), iron (Fe^{2+} or Fe^{3+}) and aluminium (Al^{3+}), while the common-formed basic cations are magnesium (Mg^{2+}), potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) (McCauley, Jones, & Olson-Rutz, 2017).

The pH of the soil is very important to determine soil fertility because its acidity and alkalinity will determine how easily the plant will absorb the nutrients from it (Maximum Yield Incorporated, 2019). Plants can only take nutrients from the soil if the nutrients have been dissolved in water. However, the nutrient extraction process from the soil is somewhat impossible if the pH of the soil is too acidic or too alkaline. Impressed nutrients are like nitrogen, magnesium, phosphorus, iron, potassium and others. These nutrients are not be able to dissolved efficiently. Too acidic soils are within the range of pH 6 and below which are nutrients such as potassium, nitrogen and phosphorus will not be dissolved and absorbed efficiently. Next, too alkaline soils are located within the pH range 7.5 and above will cause nutrients such as manganese and iron to not be absorbed and absorbed efficiently. Therefore, the pH range between 6.0 to 7.5 is the ideal pH for healthy plant growth (Maximum Yield Incorporated, 2019).

There are many factors that affect the pH of the soil. Among them are the temperature of the environment, the types of plants that have previously grown in the area before, the number of rainfall and others. Typically, the areas with the highest rainfall or damp regions have more acidic soil compared to dry climate areas. Areas lacking rain or dry areas have more alkaline soil. Farmers and horticultural experts can stabilize the desired pH level by using many techniques such as crop rotation and fertilizers to get the ideal pH for healthy plant growth.

2.7.2 Soil Moisture Content

In general, the moisture content of the soil is the quantity of water contained in it. The soil moisture content has been widely used in technical and scientific fields and is expressed in terms of ratio. The value of 0 is really dry (Van Walt Limited Companies, 2015). The moisture content of the soil is a moisture present on the inner surface of the soil and will form as a capillary of water in the small pores in the soil. Soil moisture content plays an important role in the drainage of groundwater, agriculture and soil chemistry in hydrology, agricultural science and soil science.

Plants will be very easy to absorb water from the soil if soil moisture content is at optimum levels for plant growth. Sufficient and balanced (optimum) soil moisture content is essential for plant growth. Normally, moisture content of soil and soil nutrients will be taken by plants to grow well, quickly and healthy and eliminated by the transpiration process. Inadequate soil moisture content combined with transpiration will slow the growth rate of the crop and reduce the yield of the final crop (Shittua, Oyedelea, & Babatunde, 2017). Erosion of wind and water is influenced by soil surface roughness. Soil surface roughness will reduce soil detachment and transport due to both erosions. The low moisture content of the soil can make the cohesion between the soil particles and make it very strong. This will cause a lot of energy used to overcome this during the tillage (Shittua, Oyedelea, & Babatunde, 2017).

Soil particles with smaller particles have a larger combined surface area or pore, where the surface tension can hold more water. When water enters the clay soil, large volume of water attaches to the soil particles due to the surface tension, while only small remainder dissolves into deep soil. The clay soil has a strong water holding capacity. Soils that have a strong water holding capacity, can store higher amounts of water than their own volume after watering or rainfall events (Pitts, 2016). Under the right circumstances, water stored in the soil can remain available for plants to use. Although soil with a small particle size has a strong surface tension and water holding capacity, but this situation makes it difficult for plant to extract and use water to grow and survive too. It is quite difficult for water to move through fine particles of soil by itself. In order for water to move easily into the soil and allow the plants to use it, it needs a certain amount of energy. The energy of the plant itself must be applied to the water. This allows water to be separated from the soil particles and absorbed back into the plant's root system. It is known as 'tension'. Although the mixture of soil contains water, if the tension required to extract water from the soil is greater than the plant's ability to extract water, they will die (Pitts, 2016). The condition of the soil is arguably unsuitable for most crops. The smaller the particle size of soil, the more pore space to hold water. The more pore space to hold water, the higher the surface tension of the soil. The higher the surface tension of the soil, the less the availability nutrient of the soil for healthy plant growing.

Not all the water contained in the soil will be absorbed by the plant and available to it. Most of the water that remains in the ground acts as a thin film. Soil solutions are formed due to groundwater dissolving salts in the soil which will act as an important medium for supplying nutrients to growing plants. The amount of soil moisture content is appropriate to be monitored due to several important factors.

Among them are soil moisture content acts as a solvent and nutrient carrier for plant growth, controlling soil temperature, essential for photosynthesis, plant yields are often determined based on the amount of water contained and no other nutrients of other foods, water in the soil is also its own nutrients, the process of soil formation and weathering depends on water, microorganisms need water for their metabolic activity, water in the soil help in chemical and biological soil activity and it is the main constituent of plant growing (Van Walt Limited Companies, 2015).

2.7.3 Soil Organic Matter

Living organisms play an important role in the formation of soil organic matter. Living organisms such as flora and fauna all contribute to the formation of soil organic matter. Examples such as plant roots, worms, microorganisms, decomposers and others contribute to the formation and breakdown of organic matter in the soil. It will form organic waste decomposition, nutrient or organic carbon which will be released either for plant extraction or brought to the soil organic matter pool as shows in Figure 2.2: Organic Matter Decomposition Cycle. Indirectly, this process will form carbon dioxide that is released through chemical oxidation and microbial respiration which ultimately will be released into the air (Maximum Yield Incorporated, 2019).

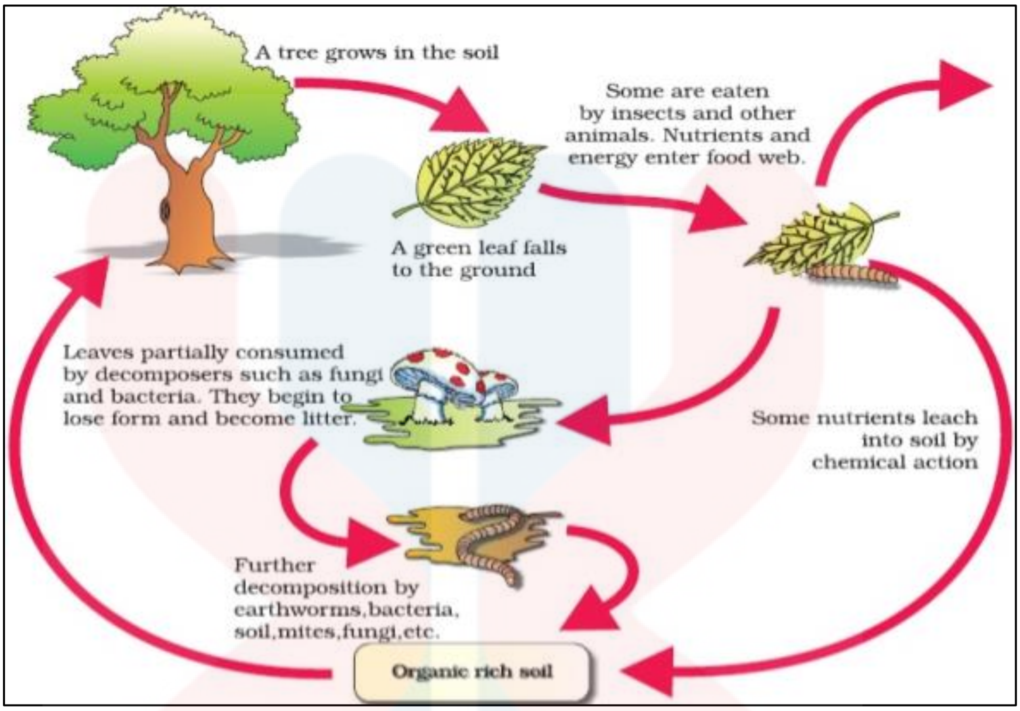

Figure 2.2: Organic Matter Decomposition Cycle (Najiha, 2013).

Soil organic matter is a measurement of soil fertility. Soil organic matter can help increase soil fertility in terms of chemical, physical and biological properties such as chelating of micronutrients, cation exchange capacity, pH buffering capacity and water holding capacity (University of Massachusetts Amherst, 2019). If soil organic matter decomposes properly, it can help improve soil structure by maximizing biological activity in the soil, slowly releasing nutrients, increasing aggregation and preventing some diseases. Incidents such as soil erosion, general soil degradation, loss of fertility and compaction can be due to the absence or lack of organic matter in the soil. The fertile soil contains the percentage of soil organic matter in the range of 3.5% to 4% and is suitable for healthy plant growth (Hossain, 2014). Besides, organic materials are able to absorb heavy metals such as arsenic, nickel, lead, chromium, cadmium, copper and others which are toxic to plants and pollute the soil and thus lead to a decrease in soil fertility (Fageria, 2012).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Research Flow Chart

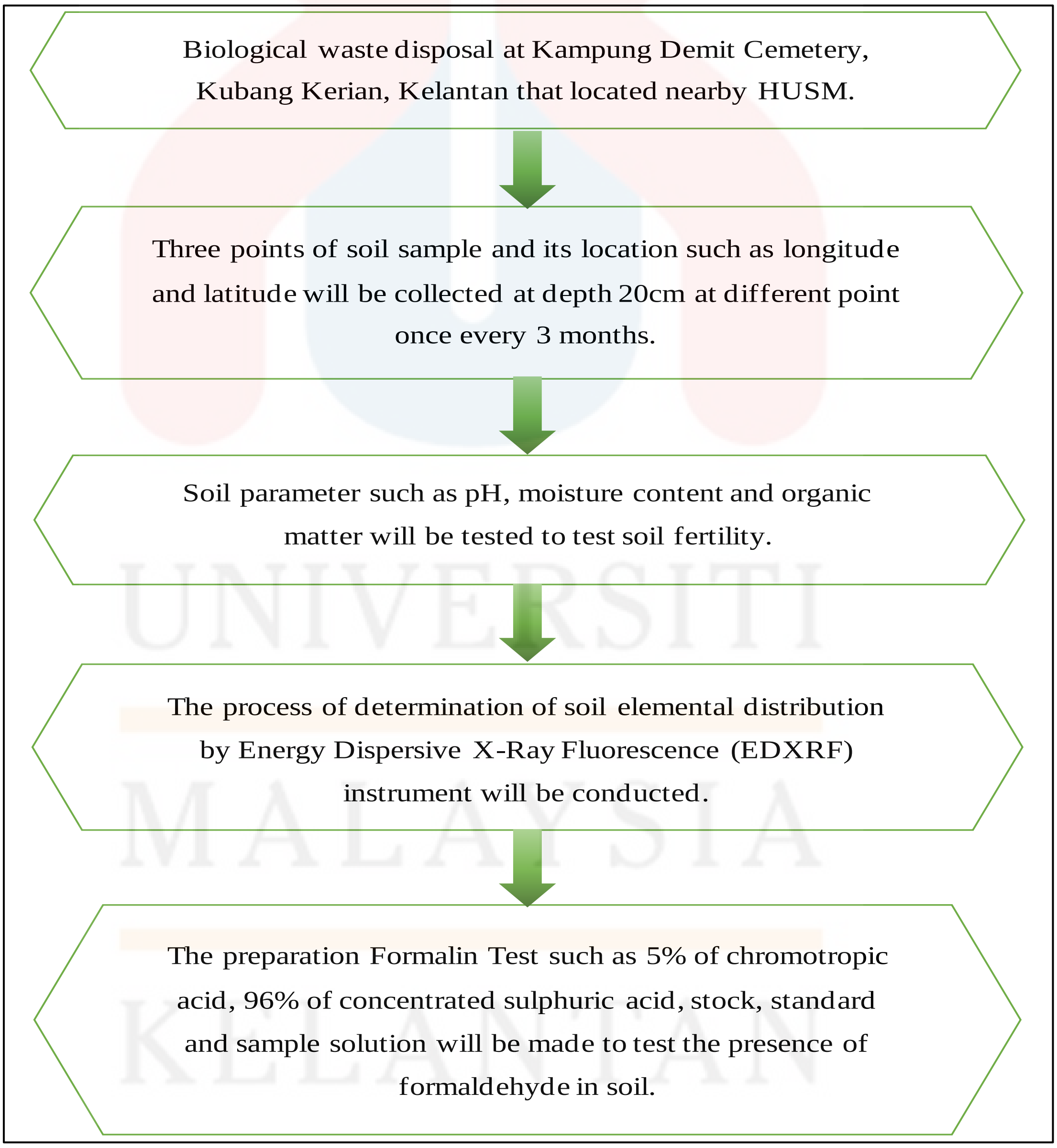

Figure 3.1: The Flow of Research Methodology.

3.2 Materials

3.2.1 Apparatus and Instrument

Table 3.1: The List of Apparatus and Instruments for Each Methods.

Methods	Apparatus and Instrument
Sampling	Auger, zip lock bag, tagging sticker, marker pen, glove and refrigerator while Garmin GPSMAP 64s instrument made in United States of America will be used to determine the location position.
Soil Parameter	Takemura Electric Works DM-15 Soil PH & Moisture Tester Hygrometer made in Japan, Muffle Furnace, glove, porcelain crucibles, tongs, electronic balance and spatula.
Soil Elemental Distribution	Aluminium foil, glove, oven, mortar, pestle, sieve 1 mm, grinder, Bruker S2 Ranger Energy Dispersive X-Ray Fluorescence (EDXRF) instrument made in Germany, and porcelain crucible.
Formalin Test	Hach DR6000™ Ultraviolet-Visible Spectrophotometer made in United States of America, fume hood, cuvette, analytical balance, glove, facemask, beaker, hot plate, dropper, glass rod, volumetric flask, measuring cylinder, micropipette, centrifuge, centrifuge tube, aluminium foil, spatula, airtight bottle, mortar, pestle, sieve 1 mm, and culture tube.

3.2.2 Chemicals

Chemicals that have been used for sampling was distilled water. Next, chemicals that have been used for detecting presence of formaldehyde were distilled water, boiling water bath, 98.5% chromotropic acid powder, 96% concentrated sulphuric acid solution and 37% formaldehyde solution.

Figure 3.2: 5% Chromotropic Acid Solution (Prepared).

3.2.3 Sample

The soil samples were collected at Kampung Demit Cemetery, Kubang Kerian, Kelantan where it serves as biological specimen disposal site. This location is nearby with HUSM. The soil sampling has been done for five times starting from 26 September 2018 and ending on 3 October 2019. The first sampling was conducted before the burial process in order to get the baseline reference for the investigation of formalin treated organs while the rest four sampling were conducted after the burial process.

3.3 Methods

3.3.1 Sampling

The sampling site was conducted at Kampung Demit Cemetery, Kubang Kerian, Kelantan where it serves as biological specimen disposal site once every three months as illustrated in Figure 3.3: The Location of Sampling. Two soil samples were taken near the biological specimen disposal site containing formalin at different point, while one sample was taken away from the biological specimen disposal area containing the formalin material. Each of the soil samples were taken at depth 20 centimetres (cm). The sample taken away from this distance was used as the control of other soil samples. The soil sample had collected before and after the disposal of the biological specimen that might be treated by the formalin. Then, this soil sample was dug by using auger equipment with a depth of 20 cm per hole as shown in Figure 3.5: The Process of Collecting the Soil Sample at Sampling Site. After that, the soil samples had been put into a zip lock bag and labelled as point A, B, and C, where Point C was the closest point (Figure 3.4). These soil samples were taken to the laboratory to maintain or preserve its composition in the refrigerator at cool temperature.

Figure 3.3: The Location of Sampling.

Figure 3.4: The Closest Point of Soil Sampling at The Location of Biological Specimen Disposal Site (Point C).

Figure 3.5: The Process of Collecting the Soil Sample at Sampling Site.

3.4 Global Positioning System (GPS)

The location of the sampling was determined in terms of latitude and longitude by using Garmin GPSMAP 64s instrument. The information of this location also be recorded on Table 3.2 below.

Table 3.2: Coordination of Sampling Point.

Points	Latitude	Longitude
A 	N 06°06’10.5”	E 102°17’25.9”
B 	N 06°06’14.4”	E 102°17’26.0”
C 	N 06°06’14.2”	E 102°17’25.9”

There are three predefined locations for this sampling, namely as Point A, Point B and Point C. The altitude of all these points were 10 meters from Mean Sea Level.

Point A was the farthest point from the biological waste disposal site with its GPS coordinates for latitude of 6 degrees (6°), 6 minutes (6'), 10.5 seconds (10.5”) north, while longitude is 102 degrees (102°), 17 minutes (17'), 25.9 seconds (25.9”) east.

Point C was the closest point to the biological waste disposal site with its GPS coordinates for latitude of 6 degrees (6°), 6 minutes (6'), 14.2 seconds (14.2”) north, while longitude is 102 degrees (102°), 17 minutes (17'), 25.9 seconds (25.9”) east.

Point B was the intermediate point between Point A and Point C where its GPS coordinates for latitude of 6 degrees (6°), 6 minutes (6'), 14.4 seconds (14.4”) north, while longitude is 102 degrees (102°), 17 minutes (17'), 26.0 seconds (26.0”) east.

3.5 pH and Moisture Content

The reading of soil parameter such as pH and moisture content has been taken first by using Soil pH and Moisture Meter instrument before the soil was dug (Figure 3.6). The reading also be taken and recorded.

Figure 3.6: Soil pH and Moisture Meter Used in In-Situ Analysis of pH and Moisture Content of the Soil.

3.6 Organic Matter

Three empty porcelain crucibles (M_A, M_B and M_C) were weighed by using electrical balance and recorded respectively. These three porcelain crucibles were labelled as A, B and C using marker pen. As much as 500 grams of soil samples was taken for each porcelain crucibles and weighed again (Figure 3.7). All porcelain crucibles were placed safely into Muffle Furnace by using tongs. The temperature of the Muffle Furnace was set to 440℃ and all porcelain crucibles was left in the Muffle Furnace for one night (Figure 3.8 & Figure 3.9) (Reddy, 2015). The next day, the Muffle Furnace was turned off and left cool for at least an hour. All dry soil samples were removed carefully from Muffle Furnace. The porcelain crucibles may be still hot and should be careful as the ash may suddenly come out of the porcelain crucibles (Figure 3.10). Soil samples that have been cooled must be weighed once again. The percentage of organic matter were calculated based on weight difference as Equation (3.1) – (3.6) below:

i) The mass of empty porcelain crucibles which labelled as M_A, M_B and M_C were determine respectively. (3.1)

ii) The mass of each porcelain crucibles (M_A, M_B and M_C) and soil sample before combustion were determined. (3.2)

M_A + Mass of soil sample before combustion = $M_{before\ A}$

M_B + Mass of soil sample before combustion = $M_{before\ B}$

M_C + Mass of soil sample before combustion = $M_{before\ C}$

iii) The mass of each soil before combustion were determined. (3.3)

Mass of soil sample before combustion = $M_{before\ A} - M_A$

Mass of soil sample before combustion = $M_{before\ B} - M_B$

Mass of soil sample before combustion = $M_{before\ C} - M_C$

iv) The mass of each porcelain crucibles (M_A and M_B) and soil sample after combustion were determined. (3.4)

M_A + Mass of soil sample after combustion = $M_{after\ A}$

M_B + Mass of soil sample after combustion = $M_{after\ B}$

M_C + Mass of soil sample after combustion = $M_{after\ C}$

v) The mass of each soil after combustion were determined. (3.5)

Mass of soil sample after combustion = $M_{after\ A}$ - M_A

Mass of soil sample after combustion = $M_{after\ B}$ - M_B

Mass of soil sample after combustion = $M_{after\ C}$ – M_C

vi) The percentage of Organic Matter (%) was determined. (3.6)

$$\frac{\text{Mass of Soil Before Combustion - Mass of Soil After Combustion}}{\text{Mass of Soil Before Combustion}} \times 100\%$$

Figure 3.7: Soil Sample and Crucible Weighed Using Electronic Balance Instrument.

Figure 3.8: Muffle Furnace Instrument Used in Organic Matter Analysis.

Figure 3.9: Soil Samples and Crucibles Placed in Muffle Furnace for Combustion Process.

Figure 3.10: Soil Samples and Crucibles After the Combustion Process.

3.7 Soil Elemental Distribution

Five soil samples of Point C that has been taken were transferred about 35 g into aluminium foil. The soil sample was dried in air-oven at 80℃ within a day of 24 hours. Small rocks, grass and any unnecessary sediments on soil samples was discarded before being crushed into small particles using mortar and pestle. Furthermore, the small particle of soil sample was passed through sieve (1 mm), so that the uniform size of soil sample can be obtained (Figure 3.11). To prepare the finer particle size of soil sample, it was placed in grinder machine.

All soil samples before and after the burial of the biological specimen treated by formalin was analysed according the distribution of soil elements. The amount of soil element distribution has been determined by using Energy Dispersive X-Ray Fluorescence (EDXRF) instrument. The finely ground soil samples were placed in a sample analysis cup. Sample analysis cup of this sample was filled as three-quarter by the soil samples and compressed in pellet (Figure 3.12). The sample analysis cups were wrapped with Mylar Film. After that, it was inserted into the EDXRF instrument for elemental distribution analysis.

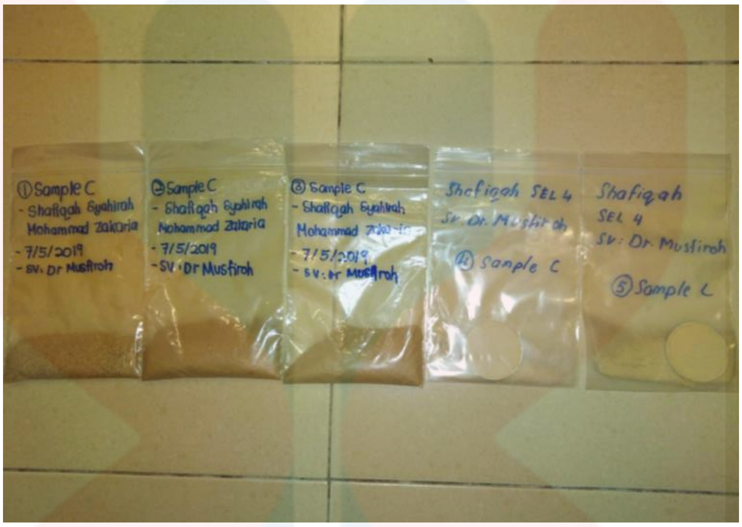

Figure 3.11: Soil Samples for X-Ray Fluorescence (XRF) Analysis.

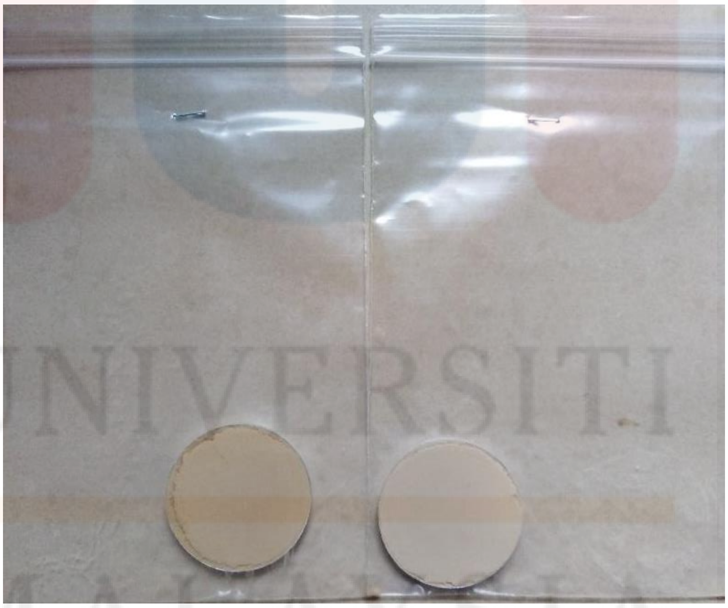

Figure 3.12: Pellet Used in XRF Analysis.

3.8 Formalin Test

3.8.1 Stock Solution, Standard Solution and Calibration Curve

Stock solution of formaldehyde (1000 mg/L) has been prepared by diluting 37% formaldehyde solution with proper quantity of distilled water until reach 1000 mL in volumetric flask. As shown in Table 3.3, standard solutions of desired concentrations such as 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L were prepared by appropriate dilution of the stock solution and distilled water. The standard solutions were calculated based on formula as Equation (3.7) below:

$$M_1V_1 = M_2V_2 \quad (3.7)$$

The blank sample for calibration curve was only distilled water. Each standard solutions and blank sample that have been prepared earlier, were inserted into cuvette for analysis by using ultraviolet-visible spectrophotometer instrument. Every absorbance reading in ultraviolet-visible spectrophotometer at reflectance of 412 nm were recorded. 412 nm reflectance was used as it was the wavelength for formaldehyde. Figure 4.5 showed the calibration curve that has been created by using Microsoft Excel 2019.

Table 3.3: Calculation of Standard Solution from Stock Solution of Formaldehyde.

Desired Concentration of Formaldehyde (Standard Solution)	Calculation
20 mg/L	(1000 mg/L) (V_1) = (20 mg/L) (0.1 L) V_1 = 0.002 L = 2 mL 100 mL Volumetric Flask = 2 mL of stock solution + 98 mL of distilled water
40 mg/L	(1000 mg/L) (V_1) = (40 mg/L) (0.1 L) V_1 = 0.004 L = 4 mL 100 mL Volumetric Flask = 4 mL of stock solution + 96 mL of distilled water
60 mg/L	(1000 mg/L) (V_1) = (60 mg/L) (0.1 L) V_1 = 0.006 L = 6 mL 100 mL Volumetric Flask = 6 mL of stock solution + 94 mL of distilled water
80 mg/L	(1000 mg/L) (V_1) = (80 mg/L) (0.1 L) V_1 = 0.008 L = 8 mL 100 mL Volumetric Flask = 8 mL of stock solution + 92 mL of distilled water
100 mg/L	(1000 mg/L) (V_1) = (100 mg/L) (0.1 L) V_1 = 0.010 L = 10 mL 100 mL Volumetric Flask = 10 mL of stock solution + 90 mL of distilled water

3.8.2 5% Chromotropic Acid Solution

Ingredients such as 0.5 g of 98.5% chromotropic acid powder, 52 mL of 96% concentrated sulphuric acid solution and 48 mL of distilled water were dissolved and diluted together (Figure 3.13). The reagents that have been prepared was placed in beaker and stored in fume hood (Figure 3.2 & Figure 3.14).

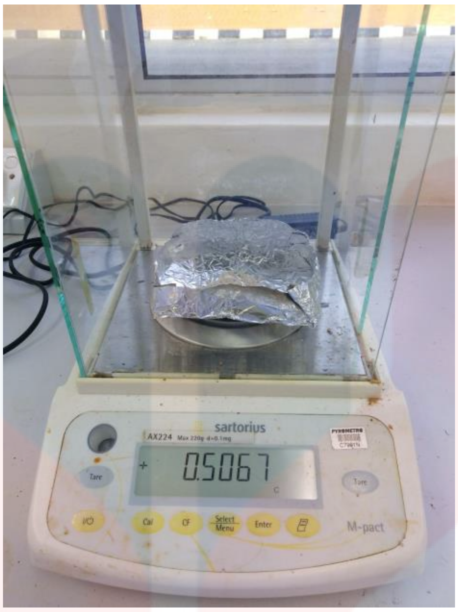

Figure 3.13: 0.5 g of 98.5% Chromotropic Acid Powder Weighed Using Analytical Balance Instrument in Preparation of 5% Chromotropic Acid Solution.

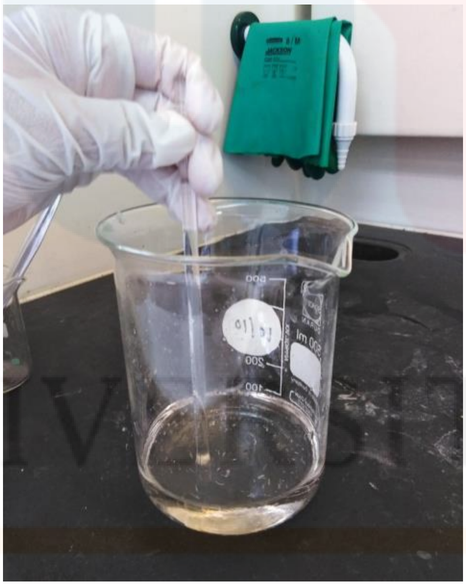

Figure 3.14: Preparation of 5% Chromotropic Acid Solution.

3.8.3 Sample Solution

Fifteen soil samples were crushed into small particles by using mortar and pestle. Furthermore, the small particle of soil sample was passed through sieve (1 mm), so that the uniform size of soil sample can be obtained. Then, this soil sample was weighed exactly 1.0 g (Figure 3.15). About 3.0 mL of 96% concentrated sulphuric acid solution and 300 μL of 5% chromotropic acid solution (prepared) were mixed together with soil samples that were weighed earlier in culture tube (Figure 3.16). Then, warmed-over culture tube using beaker in boiling water bath for 1 hour at 100℃ (Figure 3.17) (Georghiou & Ho, 1989). After that, left it until achieve room temperature around 20℃ to 25℃. Then mix the mixture to ensure that it is completely mixed. Then, it was centrifuged. The liquid to be produced on the top of the centrifuge tube after centrifugation, is called as supernatant. This supernatant has been used as a sample solution for next step of this experiment. The blank sample for sample solution was the mixture of 2 mL of distilled water, 3.0 mL of 96% concentrated sulphuric acid solution and 300 μL of 5% chromotropic acid solution (prepared) (Georghiou & Ho, 1989). Sample solutions and blank sample that have been prepared earlier, were inserted into cuvette for analysis by using ultraviolet-visible spectrophotometer instrument. Every absorbance reading in ultraviolet-visible spectrophotometer at reflectance of 412 nm were recorded (Hayun, Harmita, & Pramudita, 2017). 412 nm reflectance was used as it was the wavelength for formaldehyde.

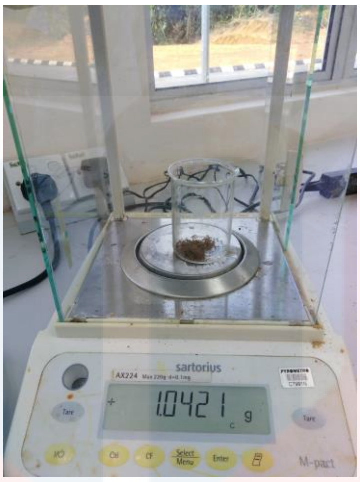

Figure 3.15: Soil Sample Weighed in Beaker Using Analytical Balance Instrument.

Figure 3.16: Culture Tube Filled with Soil Sample that Weighed Earlier.

Figure 3.17: Warmed-Over the Mixture of Soil Samples, 96% Concentrated Sulphuric Acid Solution and 5% Chromotropic Acid Solution Boiling Water Bath For 1 Hour At 100℃ Using Hot Plate Instrument.

3.8.4 Formaldehyde Analysis in Soil Samples

The absorbance of 15 soil sample solutions and standard solution were compared and linked together according to linear equation: Y = 0.0198X – 0.017 with correlation coefficient (R^2) = 0.999. By comparing the similarity of the absorbance reading between sample solution and standard solution in calibration curve, formaldehyde identification can be determined (Hayun, Harmita, & Pramudita, 2017). The concentration of formaldehyde in soil sample can be obtained from peak area of the absorbance reflectance at 412 nm (linear line) against the standard calibration curve.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Soil Fertility

The soil fertility was identified in term of pH, moisture content and its organic matter.

4.1.1 Soil pH

The in-situ pH measurement of soil samples has conducted at three sampling points. Referring to Table 4.1, the results show that the highest pH value is 7, where almost every samples were taken, the reading of pH value showed at Soil pH and Moisture Meter instrument were 7. Whereas the lowest recorded pH value is 5, which located at Point A sample on the fifth sampling. The majority of the soil pH shows neutral pH. From the pie chart on Figure 4.1, it was clear that 67% of the soil sample showed a pH value reading of 7, 20% of the soil sample showed a slightly acidic pH value of 6 and only 13% of the soil sample showed a pH value of 5. Next, the pH values were found consistent at three sampling points for first three sampling with the pH value ranging from 6 to 7 which indicate neutral to slightly acidic condition. However, there were two soil samples on the fourth and fifth sampling show an acidic reading which was pH 5 (Point A). There were several factors that have been identified. It was not due to the contamination of formalin itself, since it was the farthest point from the disposal site of biological specimens where about 300 meters away. As seen from Table 4.1, Point B and Point C indicate pH values of 7 respectively. Point C was the closest point to the formalin-treated biological specimen disposal site.

Table 4.1: Measurement of pH Value.

Sampling	pH Values		
	Point A (Loamy Soil)	**Point B (Sandy Soil)**	**Point C (Sandy Soil)**
First (Pre-Burial) 26/9/2018 2.00 pm	6	7	7
Second (Post-Burial) 27/12/2018 11.30 am	6	7	7
Third (Post-Burial) 3/4/2019 5.30 pm	6	7	7
Fourth (Post-Burial) 23/7/2019 9.00 am	5	7	7
Fifth (Post-Burial) 3/10/2019 7.42 am	5	7	7

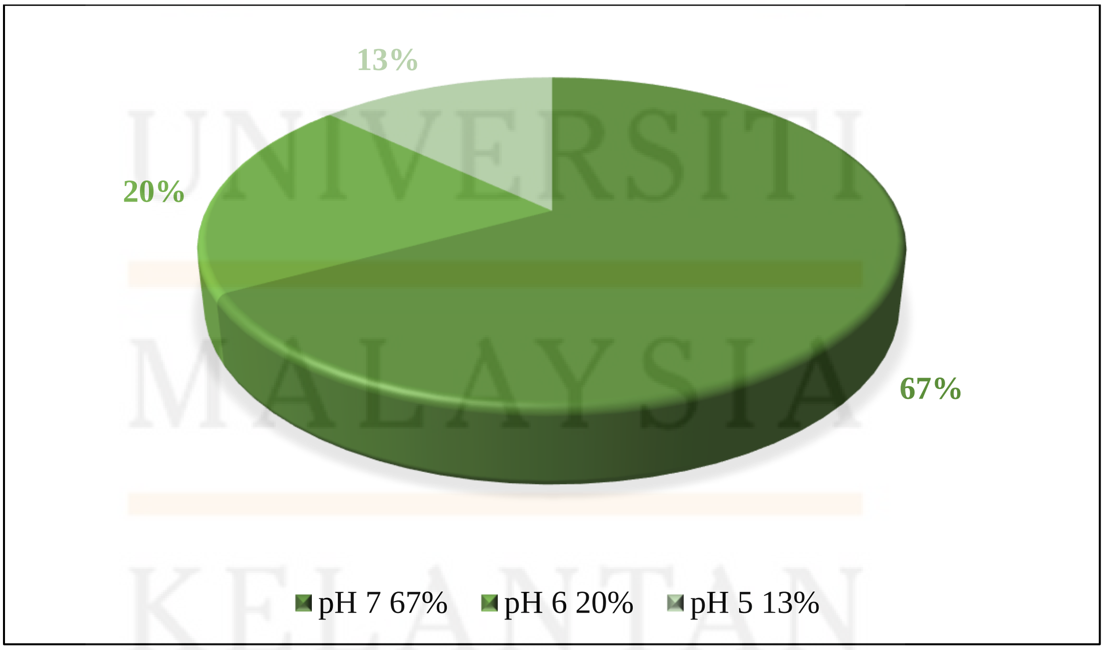

Figure 4.1: Pie Chart of The Percentage of Soil pH Value.

The pH value of Point A were found acidic due to pesticides that have been applied in order to kill weeds and shrubs in the cemetery. It can be seen from the leaves itself showed yellowish colour and withered. Soil pollution is a result of penetration of harmful pesticides that deteriorate the soil quality making it unfit for later use (Johnson & Zhang, 2019). Pesticides would react according to their target, mode, period of action or their chemistry. However, the usage of huge amount of pesticides make it poison and toxic to non-target organisms that include the plant itself and also human health (Rao, Bellin, & Brusseau, 1993). Non-target pesticide poisoning has been identified as the lead to kills of fish, reproductive failure in birds and illness in humans (Rao, Bellin, & Brusseau, 1993). In fact on previous, studied has shown that less than 0.1% of the pesticide applied to crops actually reaches the target pest while the rest enters the environment gratuitously, contaminating soil, water and air, where it can poison or otherwise adversely affect non-target organisms (Rao, Bellin, & Brusseau, 1993).

According to Johnson and Zhang (2019), there are four main factors that influence the acidity of the soil include rainfall and leaching, organic matter decay, acidic parent material and harvest of high yielding crops. Heavy rainfall will cause alkaline cations to be easily removed from the soil for thousands of years. For example, a study in Oklahoma could conclude that rain that falls more than 30 inches a year is an effective agent for acidic soil on its own (Johnson & Zhang, 2019). If large volumes of water flow through the soil quickly, the soil will quickly become acidic. The second factor of acidic soil is due to the different chemical composition of the parent material (Johnson & Zhang, 2019). It is believed that the soil derived from the granite parent material is more acidic than the soil derived from calcareous shale or limestone parent material. The third factor is due to decomposition of organic matter (Johnson & Zhang, 2019). The decomposition of organic matter will indirectly release hydrogen ions which has led to soil acidity. As illustrated at Figure 4.3, organic matter at Point A were recorded as the highest value compared to Point B and Point C. During the decomposition of organic matter, carbon dioxide gas is produced. This carbon dioxide gas will produce carbonic acid which is a kind of weak acid, if it reacts with the water in the soil. The last factor is the production of crops that are too high and too large at a time at Point A. This is because the plant at Point A will absorb elements that have properties such as lime as cation for its nutrition (Johnson & Zhang, 2019). There were many weeds and shrubs on soil at Point A. These plants also act as agents who is responsible for controlling the acidity of the soil due to the presence of alkaline cations in the soil. But in the absence of this plant, the basic material that controls the acidity of the soil will be lost from the soil which will cause the soil to become more acidic. The more plants, the more basic materials will be absorbed through the roots. The more basic materials are absorbed through the roots, the higher the acidity of the soil (Johnson & Zhang, 2019). That were the reasons why Point A would become acidic.

Works by Mcfarland and Huggins (2019) have shown that soil pH strongly influences the availability of plant nutrients. Soil pH which is too peak from the neutral pH value, either low pH (acid) or high pH (alkaline), making plant nutrients less available (Mcfarland & Huggins, 2015). University of California (2019) stated that, the 'ideal' of soil pH is estimated to be in between slightly acidic pH of 6.5 and slightly alkaline pH of 7.5. Soil also need optimum pH value which is not too extreme for them to be fertile, so that the nutrient would be maximum available for plant. It has been determined that most of the available plant nutrients are optimum within the range of pH 6.5 to pH 7.5 (University of California, 2019). The optimum pH is usually very good for root growth of plant.

In addition, previous studies have shown various results of conflict over soil pH over time. The long-held belief that decomposition of dead biological specimens has led to the formation of ammonium (NH^{4+}) in the soil. This means that the pH of the soil will rise to a higher level such as either to slightly acidic, neutral or alkaline over time. Studied by researcher before had shown that the pH of the soil in the cemetery area was higher than in the area where it soil was used as a control soil (Benninger, Carter, & Forbes, 2008).

It can be concluded that at Point A, soil pH decreases from 6 to 5, making it more acidic over time. At Point B and Point C, soil pH remains unchanged at 7 and makes it the ideal soil pH for plants to grow well. Since pH 7 was the neutral soil pH or ideal soil pH, this suggested that the soil samples at Point B and Point C were not really affected by the formalin-treated human organs or biological specimen that was buried during the second to fifth sampling, even though formalin is acidic.

4.1.2 Soil Moisture Content

The in-situ moisture content measurement of soil samples has conducted at three sampling points. Referring to Table 4.2, the moisture content values for each sample were recorded. Moisture content for the sampling ranging from 1 to 6. The results show that the lowest moisture content value is 1. The majority of the soil moisture content were in optimum condition. From pie chart on Figure 4.2, it was clear that 67% of the soil sample showed a moisture content value reading of 1, 13% of the soil sample showed moisture content value of 4, only 7% of the soil sample showed moisture content value of 5 and 13% of the soil sample showed moisture content value of 6. Usually, the moisture content of the soil ranges from 10% to 50%, as according to the instrument used, it was equivalent to values of 0.8 to 4 respectively, but it may exceed that number during and after watering or raining (Pitts, 2016). Any soil moisture content below this level was belonging to the 'Stress Zone' while above this level was called as 'Excess Zone' (Pitts, 2016). As seen in Table 4.2, Point B and Point B showed optimal range of moisture content which was 1 for every sampling. Of the remaining, only Point A have excess soil moisture. Point A always show inconsistency of moisture content.

Table 4.2: Measurement of Moisture Content.

Sampling	Moisture Content Values		
	Point A (Loamy Soil)	**Point B (Sandy Soil)**	**Point C (Sandy Soil)**
First (Pre-Burial) 26/9/2018 2.00 pm	4	1	1
Second (Post-Burial) 27/12/2018 11.30 am	6	1	1
Third (Post-Burial) 3/4/2019 5.30 pm	4	1	1
Fourth (Post-Burial) 23/7/2019 9.00 am	6	1	1
Fifth (Post-Burial) 3/10/2019 7.42 am	5	1	1

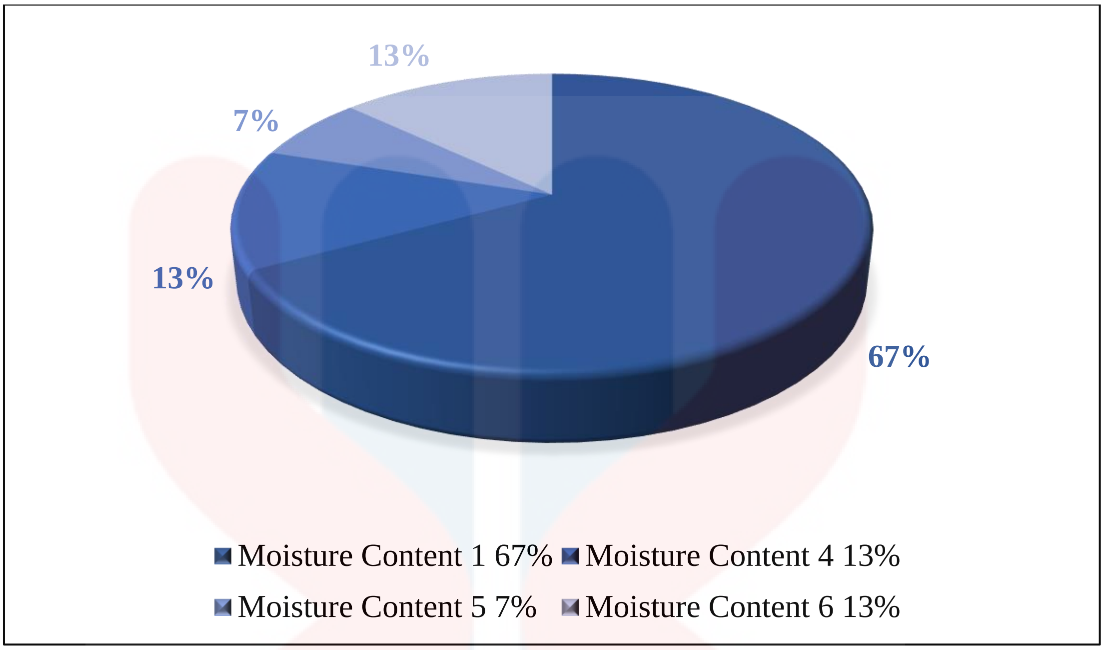

Figure 4.2: Pie Chart of The Percentage of Moisture Content Value.

Water in the soil dissolves mineral salts forming a soil solution which is an important mechanism for delivering nutrients for plants to grow well. It is the main factor of crop productivity lies on the soil as it helps fertilize the soil for the growth of the crop (Pitts, 2016). Poor soil moisture content has led to poor crop yields and may eventually lead to dead plants, while too high soil moisture content has led to root disease (Pitts, 2016). Similar situation on what happened to soil at Point A. No wonder why soil at Point A has mostly in wet condition compared to the other points. Plants that grow at Point A was the factor of that soil found in wet condition, while soils at Point B and Point C has no plant or vegetation at all.

Previous studied has shown that the other factors that influence the soil moisture content was soil texture itself (Pitts, 2016). Water holding capacity depends on the type of soil texture. Soil particles with smaller particles have a larger combined surface area or pore, where the surface tension can hold more water (Pitts, 2016). For example, clay and loam soil just like Point A. Easton and Bock (2016) stated that the most ideal soil texture and best suited to a wide variety of crops is loam soil. This is because the loam soil is made up of mix of various particle size which were sand, silt, clay and humus (Easton & Bock, 2016). Based on observation through naked eye, soil samples at Point A were likely loamy soil texture. It is a great soil structure where it not only holds large amounts of water but also drains the water well into the plant's root system and deep soil too and allows plants to easily extract both nutrient and water from soil particles for its healthy. The healthier the plant, the more fertile the soil.

On the other hand, what is happening on the sandy soil is different. When water enters the sandy soil such as soil samples at Point B and Point C, only a small volume of water attaches to the soil particles, while the rest rapidly dissolves into deep soil. The sandy soil has a large particle size. That is the reasons why the value of soil moisture content at Point B and Point C is very consistent with value 1. Works by Pitts (2016) stated that the sandy soil has a weak water holding capacity. Both water and nutrients can flow easily beyond the reach of plant roots system. Although, sandy soil is a weak water holding capacity, it is easily available to plant (Pitts, 2016). The larger the particle size of soil, the less pore space to hold water. The less pore space to hold water, the lower the surface tension of the soil. The lower the surface tension of the soil, the higher the availability nutrient of the soil for healthy plant growing. The healthier the plant, the more fertile the soil.

The other factor of moisture content become high might due to the climate of the sampling site itself which was generally its relative humidity become higher at early morning compare in evening (North Carolina State University, 2019). This would directly affect the soil moisture content. As stated in Table 4.2, first sampling and third sampling were done in the evening, while second sampling, fourth sampling and fifth sampling were done in the morning.

It can be concluded that at Point A, the moisture content of the soil remained unstable where initially it was 4 and then rose to 6, it drops dramatically to 4 again and back over to 6 again and finally fell to the lowest value among all the soil samples which was 5. At Point B and Point C, the soil moisture content remains unchanged at 1 which was an optimum range. Since the higher the pH value, the lower moisture content, this suggested that the soil samples at Point B and Point C were not really affected by the formalin-treated biological specimen that was buried during the second to fifth sampling, even though formalin is acidic.

4.1.3 Soil Organic Matter

The ex-situ organic matter measurement of soil samples were conducted at three sampling points. Referring to Table 4.3, the organic matter percentage for each sample were recorded. The results show that the highest organic matter percentage was 16.44% where it was sample at Point A on the fifth sampling, while the lowest recorded was 2.99% where it was sample at Point B on the third sampling. Followed by the second lowest was 3.72% where it was sample at Point C on the second sampling. The result indicate that majority of the soil sample has low and medium percentage of organic matter, while only a small monitory has high percentage of organic matter. Most of the highest percentage of organic matter located at Point A where the range in between 12.81% to 16.44% while the lowest percentage of organic matter located at both Point B where the range in between 2.99% to 8.83% and Point C were from 3.72% to 9.21%.

Table 4.3: Measurement of Organic Matter (%)

Sampling	Organic Matter (%)		
	Point A (Loamy Soil)	Point B (Sandy Soil)	Point C (Sandy Soil)
First (Pre-Burial) 26/9/2018 2.00 pm	12.81	8.83	8.64
Second (Post-Burial) 27/12/2018 11.30 am	12.99	7.58	3.72
Third (Post-Burial) 3/4/2019 5.30 pm	12.94	2.99	9.21
Fourth (Post-Burial) 23/7/2019 9.00 am	15.75	5.03	8.13
Fifth (Post-Burial) 3/10/2019 7.42 am	16.44	6.74	8.24

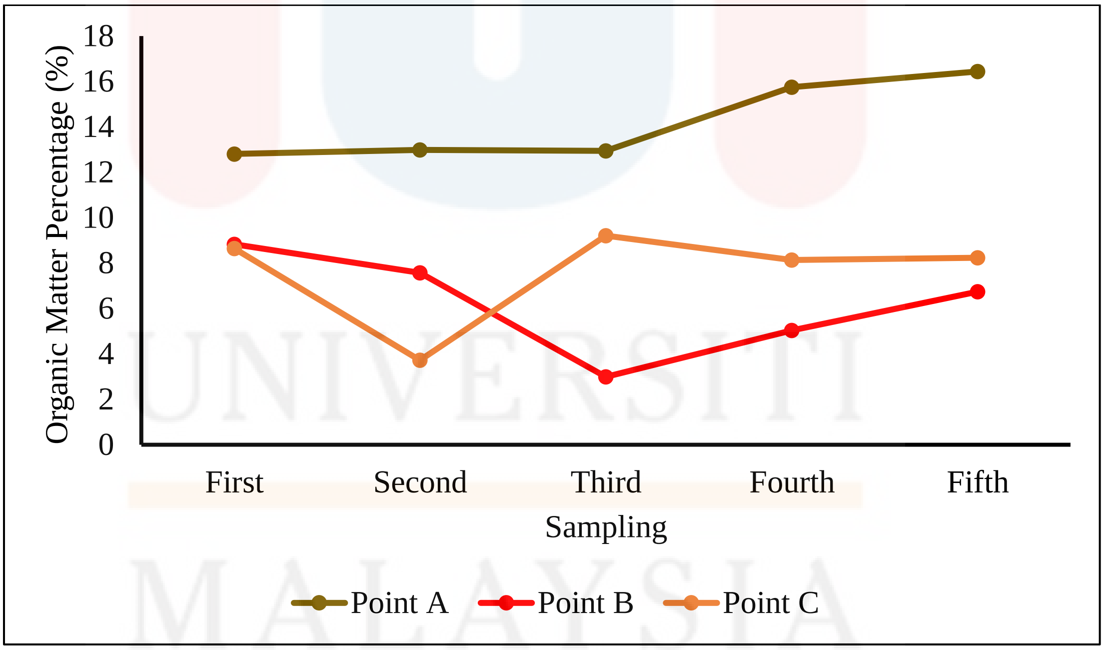

Figure 4.3: The Organic Matter Percentage (%).

There are several factors that influence in percentage of organic matter in soil. Fageria (2012), stated that soil has different organic matter content in each place, even it is located in a zone of similar weather or climate like Point A, B and C. Soil organic matter might be different according to their depth. Soil near the top layer normally have high amount of organic matter compared to lower soil depth. One of the reasons due to the addition of plant residues on the soil surface and their decomposition can increase the top layer organic matter content. Next, such differences in organic matter content of soils are usually due to the effects of temperature, moisture content, microbial population, vegetation and management practices adopted in plant production (Fageria, 2012).

All 10 soil samples at Point B and Point C shows the low and medium organic matter percentage, while only 5 soil samples at Point A show high organic matter percentage. Normally, soil organic matter percentage that should be found in the soil structure was 5% which is good for gardening (Whitman & DeJohn, 2009). It is no wonder why soil at Point A have higher organic matter percentage since the colour of the soil were darker compared to other soil at Point B and Point C. According to Connor (2014), soils rich in humus or organic matter tend to be dark with a good crumb structure and loam texture. Humus is a mixture of substances that act as a barrier agent against microbial attacks. Last but not least, soils with a smaller percentage of particle size such as loamy or 'heavy' soil, tend to have a higher percentage of organic matter than soils with larger particle size or 'lighter' soil such as sand (Barrera, 2018).

Organic matter is a solid substance derived from plant or animal residues. Adequate supply of organic matter in soil is essential for improving the physical, chemical and biological properties of soil. Good organic matter such at Point A can maintain the sustainability of the crop system. Both soils at Point B and Point C has low and medium of organic matter. So far there has been no critical level for organic matter in different plants, but the reduction in the value of organic matter in the soil remains a concern as it may have suppresses plant growth and soil fertility (Fageria, 2012). Several factors of these situation were due to the soil degradation through physically, chemically and biologically (Department of Planning, Industry and Environment, 2019). Specifically factor of declined organic matter were decrease presence of decaying organisms, or an increased rate of decay as a result of changes in natural or anthropogenic factors (European Commission Agriculture and Rural Development, 2009). Next, climatic conditions, such as rainfall, temperature, moisture, and soil aeration (oxygen levels) affect the rate of organic matter decomposition (United States Department of Agriculture, 2019). Generally, organic matter decomposes faster in climates that are warm and humid and slower in cool, dry climates (European Commission Agriculture and Rural Development, 2009). Then, organic matter also decomposes faster when soil is well aerated (higher oxygen levels) and much slower on saturated wet soils.

Regarding to Figure 4.3, soil samples at Point A which has smaller particles size (loamy soil), tend to have greater organic matter compared to Point B and C (sandy soil). It capable to hold nutrients and water better, thus providing good conditions for plant growth (European Commission Agriculture and Rural Development, 2009). It was no wonder why at Point A, there have a lot of plant growth or vegetation. On other hand, larger particles size of soil like sandy soil had better aerated and the presence of oxygen results in a more rapid decay of organic matter just like what happened to Point B and Point C.

It can be concluded that soil organic matter indicated the highest value at Point A while the lowest and medium value were at Point B and Point C. High soil organic content shows good characteristics for healthy plant production and increase the soil fertility. Mallorey (1921) stated that soil that rich in organic matter, generally possess greater acidity than the soils which are largely mineral in nature. That research finding in line to the soil pH result and organic matter in this study. From the result that obtained, since the higher the soil pH value, the lower the soil organic matter, this suggested that the soil samples at Point B and Point C were not really affected by the formalin-treated biological specimen that was buried during the second to fifth sampling, even though formalin is acidic. In fact, Point B and Point C were near to the buried biological specimen, but it did not affect too much.

4.2 Soil Elemental Distribution

The amount of soil element distribution were determined by using Energy Dispersive X-Ray Fluorescence (EDXRF) instrument.

4.2.1 X-Ray Fluorescence (XRF) Analysis

The ex-situ XRF analysis of soil samples were conducted only at one sampling points (Point C). As stated earlier, Point C was the closest point to the location of dead biological specimen that buried underground. Table 4.4 shows XRF analysis of the elemental distribution in soil samples Point C from first to fifth sampling. There were 20 types of soil element that obtained from XRF analysis include aluminium (Al), barium (Ba), calcium (Ca), chlorine (Cl), chromium (Cr), copper (Cu), iron (Fe), iodine (I), potassium (K), manganese (Mn), nickel (Ni), phosphorus (P), lead (Pb), rubidium (Rb), silicon (Si), strontium (Sr), tellurium (Te), titanium (Ti), zinc (Zn) and zirconium (Zr). From the result, mostly element has indicated significant increase amount of concentration ($mgkg^{-1}$) from first sampling (pre-burial) to fifth sampling (post-burial). Referring to Figure 4.4, soil elemental distribution of post-burial of biological specimen such as Al, Cr, Cu, Fe, K, Mn, Ni, Pb, Rb, Si, Sr, Ti, Zn and Zr have shown increase inconsistently in concentration compared to pre-burial of biological specimen. On other hand, elements of Ba, Ca, Cl, I, P and Te have shown decrease logarithmically in concentration over time from pre-burial to post-burial of biological specimen. Table 4.4 shows clearly about 25% of element distributed increase the highest in concentration on the second sampling (Fe, Mn, Rb, Ti, Zn), 35% of element distributed increase the highest in concentration on the third sampling (Cr, Cu, K, Ni, Pb, Rb, Sr), 10% of element distributed increase the highest in concentration on the fourth sampling (Si, Zr), 5% of element distributed increase the highest in concentration on the fifth sampling (Al) and 25% of element distributed decrease in concentration from pre to post burial (Ba, Ca, Cl, I, P, Te).

Table 4.4: XRF Analysis of The Elemental Distribution in Soil Samples Point C for Each Sampling.

Formula	Atomic Number	Element Concentration Each Sampling ($mgkg^{-1}$)					USEPA Regulatory
		1st (Pre)	2nd (Post)	3rd (Post)	4th (Post)	5th (Post)	
Al	13	5160	8000	7260	6730	8210	**300000**
Ba	56	670	459	640	105	97.9	**3000**
Ca	20	2110	1770	1670	835	761	**N.D.**
Cl	17	183	135	157	16.7	12.4	**N.D.**
Cr	24	34.5	31.4	47.5	12.3	10.7	**3000**
Cu	29	11.7	15.5	15.7	0	0	**4300**
Fe	26	6030	12100	7860	3100	3390	**550000**
I	53	46.9	15.3	24.9	0	0	**N.D.**
K	19	25400	26200	28800	16600	20000	**N.D.**
Mn	25	278	457	316	145	134	**3000**
Ni	28	13.3	13.3	17.1	0	0	**75**
P	15	581	340	425	0	38.9	**N.D.**
Pb	82	25.4	29.3	31.7	11.6	10.7	**420**
Rb	37	95.8	119	119	40.1	44.5	**N.D.**
Si	14	57400	48000	50700	71500	6530	**N.D.**
Sr	38	70.8	67.5	76.2	26.2	28.5	**N.D.**
Te	52	36.8	15.4	26.5	0	0	**N.D.**
Ti	22	1410	1820	1590	517	639	**N.D.**
Zn	30	19.3	39.5	26.9	10.4	0	**7500**
Zr	40	128	228	149	235	145	**N.D.**

Generally, the data demonstrated that the values of heavy metal detected in this study were within the safe limit recommended by USEPA Regulatory. Based on

Table 4.4, heavy metal such as Cr, Cu, Ni, Pb, Ti and Zn were still under acceptable limit but, it is still highly toxic even in minor quantity (United States Department of Agriculture, 2000). However, titanium is not considered as a toxic metal even though it is heavy metal and it does have serious negative health effects. The regulation is varied from country to another country and depends on soil types as well. Previous studies have shown an increase in the concentration of heavy metals in the cemetery. Examples are like Santa Candida municipal cemetery in Brazil. This study found that soil pollution was caused by the element of Cr, Pb and Ni. The increase in the content of this element is due to the material used for the interment of the deceased (Barros, Melo, Romanó, & Zanello, 2008).

In a previous study, Aphane (2018) has confirmed that the concentration of elements increased in the area of the cemetery compared to the non-cemetery area. Regarding to Table 4.4, all the elements did not show huge changes in concentration levels before (pre) and after (post) the burial of biological specimen even though the specimen has been treated by formalin. In this paper, it can be said that the area after burial of dead biological specimens having a higher elemental concentration compared to the area before burial of biological specimens even in the same climate and soil texture but, those concentration were still under control and follow the USEPA Regulatory. Element that increase after burial of dead biological specimen like Al, Cr, Cu, Fe, K, Mn, Ni, Pb, Rb, Si, Sr, Ti, Zn and Zr. Jonker and Olivier (2012) has analysed the distribution of trace metals in cemetery soils of the Zandfostein burial site at South Africa. The study had demonstrated that the concentration of trace elements in burial site is higher compared to offsite soil (Jonker & Olivier, 2012). The research findings were similar to research done before (Spongberg & Becks, 2000).

Sililo and Saayman (2001) has approved that the texture and surface area of the soil are interrelated. The sandy soil texture with large particle size as in Point C reduced its surface area per mass. This means that elements in the soil tend to be high mobile in large quantities (Sililo & Saayman, 2001). Sandy soil has facilitated the leaching of organic contamination which can be relate with the result of soil moisture content that have been mentioned before. This is contrary to what Allemann, Olivier, and Dippenaar (2018) have studied. Various environmental conditions such as rainfall distribution, soil texture, soil pH and temperature were found to have no effect on the amount of leachate dissolved or the rate of mobility through the soil, even though sand allowed for better leaching. (Allemann, Olivier, & Dippenaar, 2018). As the result show most of the elements recorded increase inconsistently in concentration from first sampling and only a few elements whose concentration in the soil decrease within 3 months. Overall, all the element obtained from XRF analysis has decrease in concentration over time on the last sampling except for aluminium.

There is no specific study on the influence of formalin-fixed in dead biological specimen toward the soil elemental distribution in past. It just simply states that decomposed carcasses are considered a high source of nutrients, and that nutrients are more beneficial than adding plant or fertilizer to soil which is good for subsequent decomposition (Niziolomski, Rickson, Marquez-Grant, & Pawlett, 2016).

Solubility of Si forms is low and biogeochemically immobile. The abundant concentration of Si elements among all the other elements was recorded. It might be due to the type of soil itself. Sandy soil at Point C contain silica substances which was one of the major components of silicon element. Si element is known to make the other types of elements available in many types of soil and directly affect nutrient uptake from nutrient solutions that resemble soil solution (Greger, Landberg, & Vaculík, 2018). Each of the other elements appears to have its own Si element relation. Therefore, no general conclusion can be drawn for all nutrients (Greger, Landberg, & Vaculík, 2018). Similar conclusion can also be drawn from the work by (Islam & Saha, 1969). Similar to this study, the net accumulation of Fe in plants slightly decrease in the presence of Si (Islam & Saha, 1969). According to Islam and Saha (1969), Si also reduced the K concentration which was equivalent in this study. Si element increased the P availability up to 50% in soils. The concentration of P element was found to decrease with time slowly. Similar to what Greger, Landberg, & Vaculík (2018) did before. The higher the concentration of Si element in the soil, the more noticeable affect other nutrients that were available for the plant. The high concentration of Si element does not adversely affect the environment especially soil pollution.

Potassium elements were believed to form naturally on the surface and underground. Its concentration can vary by several factors including geological conditions, climate and local geography (Aphane, 2018). The concentration of this element decreased with distance and time (Aruomero & Afolabi, 2014). The result gained from this study show the highest concentration of K was on third sampling which was 28800 $mgkg^{-1}$. As the USEPA Regulatory does not have an established limit for K concentration, it is difficult to determine whether the K concentration obtained from this study has pose negative impact to the environment or not.

The result gained from this study show the highest concentration of Fe was on second sampling which was 12100 $mgkg^{-1}$, but it was still under limit in soil given by USEPA Regulatory. Ezci et al., (2013) stated that the concentration of Fe was detected higher Fe levels in soil than in the bones. The possible source of Fe is unclear. Works by Cannell, Gustavsen, Kristiansen and Nau (2017) said that Fe elements can be found to be directly related to the burial ground. Fe is obtained in the soil due to natural process changes that occur in the affected soil (Cannell, Gustavsen, Kristiansen, & Nau, 2017). The decreasing concentration of Fe element in soil was believed by Cannell et al., (2017) due to the changing drainage caused by the nearby slope. Fe element is not one of the products but perhaps an effect caused by their presence. Depletion of Fe element due to the soil loaded by organic material.

The abundant concentration of Al elements cannot be scientifically proven. Although, Al element also one of the elements in human too. No wonder why its concentration quite higher at the burial soil. The result gained from this study show the highest concentration of Al was on second sampling which was 8210 $mgkg^{-1}$, but it was still under limit in soil given by USEPA Regulatory.

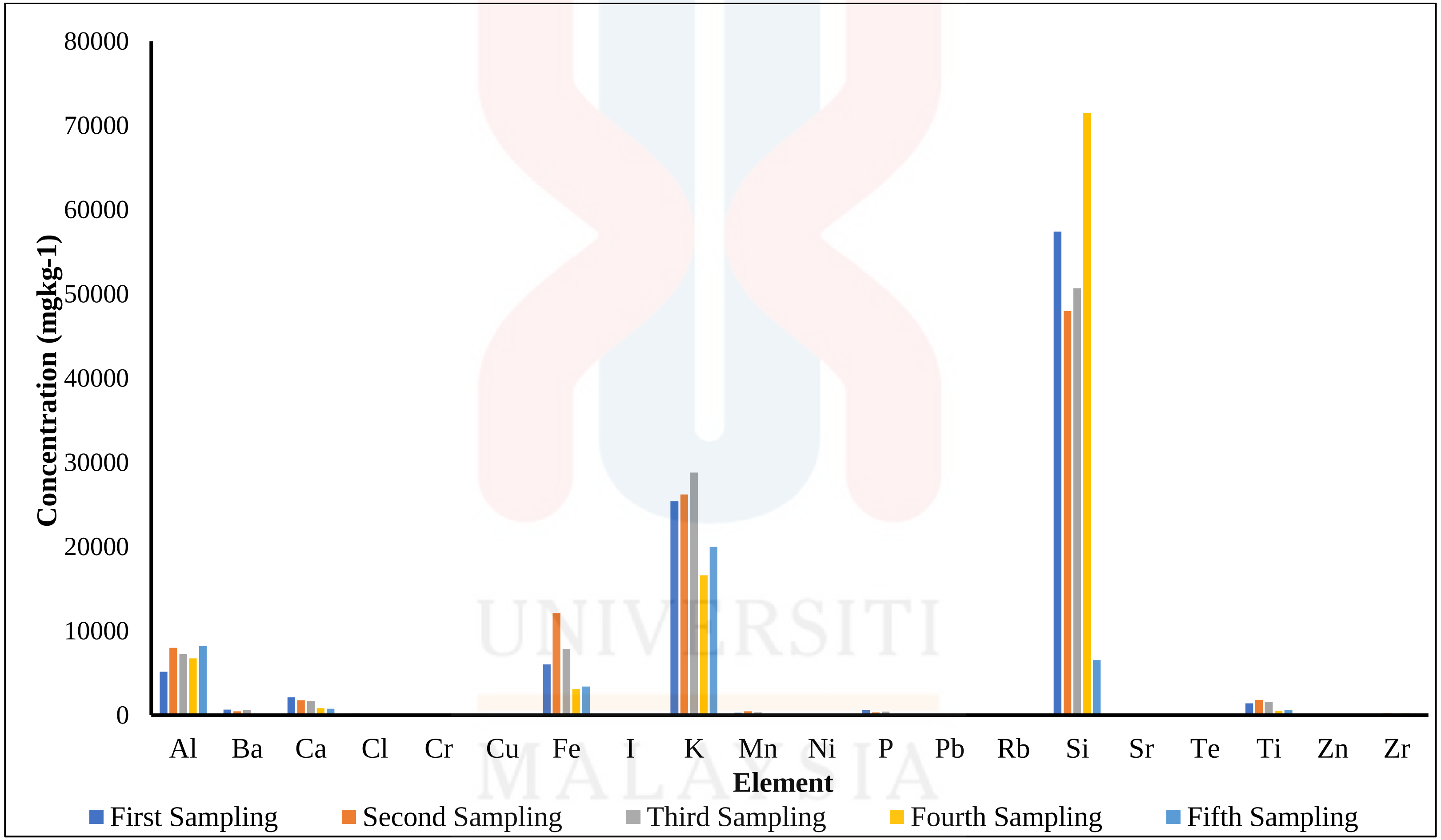

Figure 4.4: The Soil Elemental Distribution at Point C in Different Sampling Collections.

4.3 Formaldehyde Analysis

The presence of formalin has been tested by using Ultraviolet-Visible Spectrophotometer.

4.3.1 Calibration Curve

As shown in Figure 4.5, standard calibration curve of formaldehyde with concentrations of 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L were recorded. Those concentration have intensity or absorbance value such as 0.363 nm, 0.775 nm, 1.197 nm, 1.579 nm and 1.941 nm respectively through ultraviolet-visible spectrophotometer instrument.

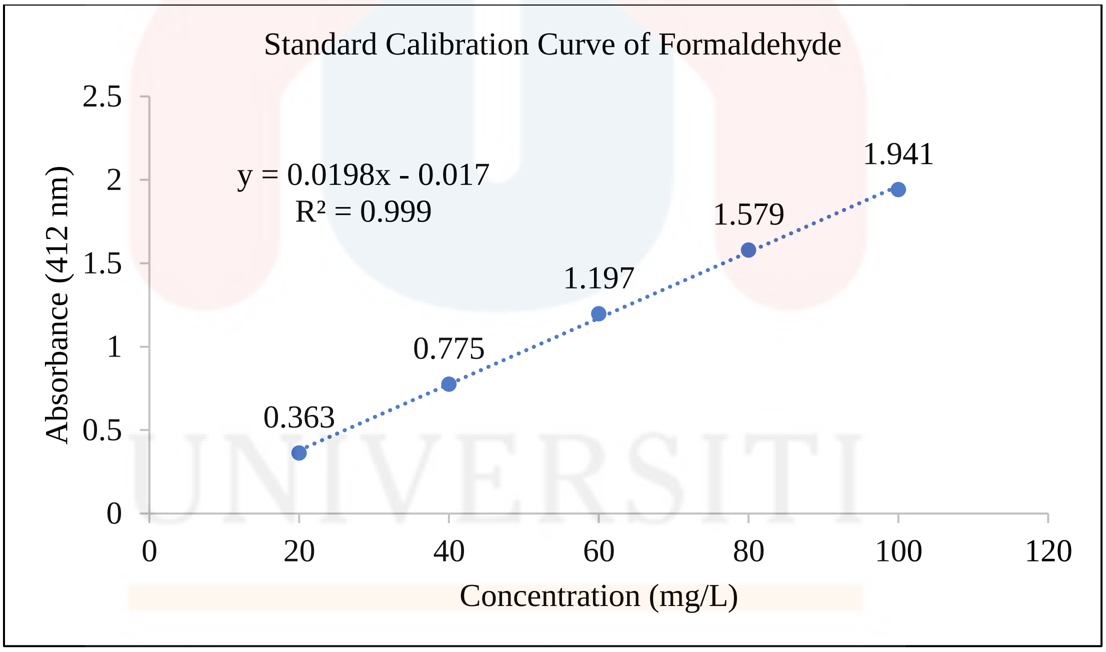

Figure 4.5: The Standard Calibration Curve of Formaldehyde.

4.3.2 Formalin Content Determination In Soil Samples

The absorbance of 15 soil sample solutions and the absorbance of standard solution that illustrated in Table 4.5 and Figure 4.5 were compared and linked together according to linear equation: Y = 0.0198X – 0.017 with correlation coefficient (R^2) =

0.999. Table 4.6 shows by comparing the similarity of the absorbance reading between sample solution and standard solution in calibration curve, formaldehyde identification can be determined.

Table 4.5: Absorbance Value of Each Soil Sample for Determination of Formalin Concentration.

Sampling	**Absorbance Value (nm)**		
	Point A (Loamy Soil)	**Point B (Sandy Soil)**	**Point C (Sandy Soil)**
First (Pre-Burial) 26/9/2018 2.00 pm	2.502	2.742	2.930
Second (Post-Burial) 27/12/2018 11.30 am	2.884	2.985	3.450
Third (Post-Burial) 3/4/2019 5.30 pm	2.672	2.963	3.010
Fourth (Post-Burial) 23/7/2019 9.00 am	2.612	2.926	3.004
Fifth (Post-Burial) 3/10/2019 7.42 am	2.582	2.895	2.938

The ex-situ formalin analysis of soil samples has conducted at three sampling points. Referring to Table 4.6, the results show that the highest concentration of formalin that obtained from this study was recorded on the second sampling (post burial) at Point C which was 175.10 mgL^{-1} while the lowest one was recorded on the first sampling (pre burial) at Point A which was 127.22 mgL^{-1}. It exceeds the tolerable concentration recommended by the World Health Organisation (WHO). The concentration of formalin for each sampling was increasing steadily from Point A to Point C (closest point). Figure 4.6 obviously shown that the concentration of formalin before burial of dead biological specimen seems lower than after burial of dead biological specimen for every sampling. Formalin concentration for each Point A, B and C appears to raise drastically on the second sampling and begins to drop gradually for subsequent sampling.

Table 4.6: Concentration of Formalin in Soil Samples (mgL^{-1}).

Sampling	Concentration of Formalin in Soil (mgL^{-1})		
	Point A (Loamy Soil)	Point B (Sandy Soil)	Point C (Sandy Soil)
First (Pre-Burial) 26/9/2018 2.00 pm	127.22	139.34	148.83
Second (Post-Burial) 27/12/2018 11.30 am	146.52	151.61	175.10
Third (Post-Burial) 3/4/2019 5.30 pm	135.81	150.51	152.88
Fourth (Post-Burial) 23/7/2019 9.00 am	132.78	148.63	152.58
Fifth (Post-Burial) 3/10/2019 7.42 am	131.26	147.07	149.24

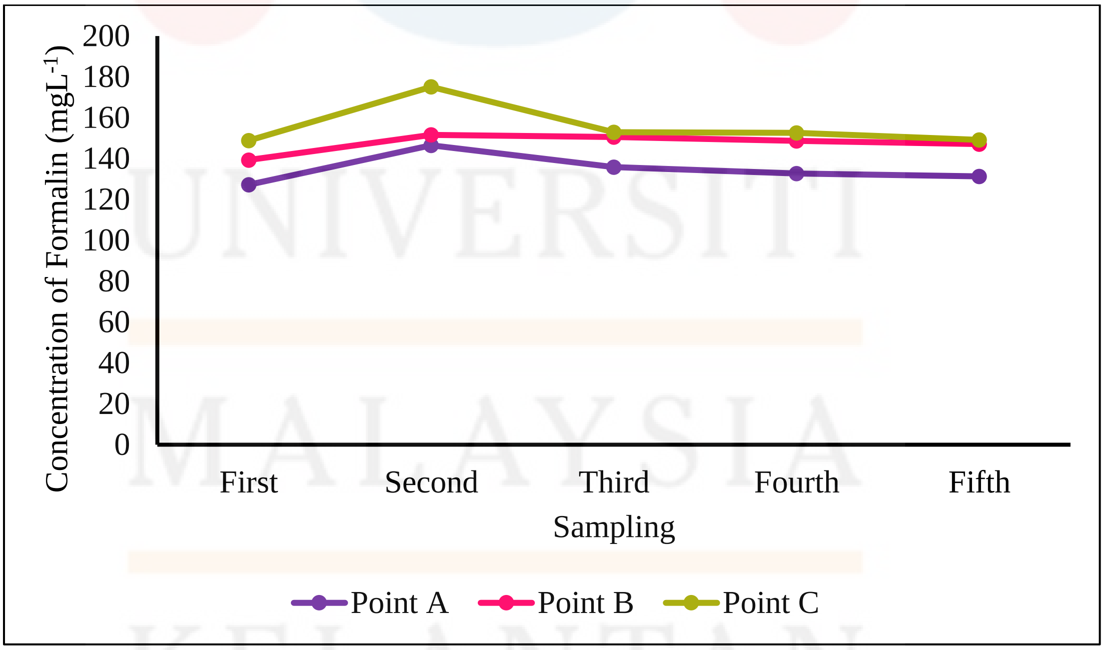

Figure 4.6: The Concentration of Formalin in Soil Samples (mgL^{-1}).

A study conducted by Allemann, Olivier, and Dippenaar (2018) has proven that the major sources of pollution in the cemetery area either water pollution or soil pollution may be due to the human body, embalming fluid and coffin materials which may contain potential toxic and hazardous metals that seeping down into the ground. The research findings were similar to what have been reported on this study. Similar to what happened in Point C. It can be said that almost all of the soil samples in Point C recorded higher formalin concentrations than the other points since it was the closest point to the dead biological specimen's disposal site.

The concentration of formalin varies with distance and time. Figure 4.6 clearly shows that at Point A, the formalin concentration recorded the lowest value, while Point B showed the medium value and Point C showed the highest value. The same concept is presented by Aruomero and Afolabi (2014). The concentration of this element decreased with distance and time (Aruomero & Afolabi, 2014). Therefore, the concentration of formalin was slightly higher at Point C than at other points, in fact, formalin concentration for each Point A, B and C appears to raise drastically on the second sampling and begins to drop gradually for subsequent sampling over time.

In the previous study, the highest amount of formaldehyde that was leached at the sandy soil in the 24 weeks was 140.2 mgL^{-1}, which was lower compared to the highest concentration of formalin that was found in this study (Allemann, Olivier, & Dippenaar, 2018). The highest concentration of formalin was recorded after 3 months or 12 weeks burial of dead biological specimen at Point C was 175.10 mgL^{-1}. The high concentration of formaldehyde in the soil at sampling site was believed came from the packaging materials of biological specimens such as plastics (Allemann, Olivier, & Dippenaar, 2018). The Material Safety Data Sheet of Polypropylene (Plastic) states that plastics are usually made of polypropylene and contain traces of formaldehyde

(Motlatsi, 2011). On other hand, World Health Organization (2002) also suggests that formaldehyde is a natural occurring substance in small concentrations.

The lowest concentration of formalin was determined at Point A which served as control soil on this study. The control soil as in Point A is however identified as the source of the formaldehyde itself, since there still have concentration of formalin too (Allemann, Olivier, & Dippenaar, 2018). Besides, previous study has proven that the total amount of formaldehyde leached from loamy soil much lower compared to sandy soil (Allemann, Olivier, & Dippenaar, 2018). That was the factor why concentration of formalin at Point A were lower compared to Point B and Point C. However, the concentration of formalin itself would remain longer in loamy soil. This may be due to the nature of the loamy soil itself which is has low permeability well-drained compared to sandy soil and this implying that formalin may be retained longer in finer-grained soils (loamy) compared to coarse-grained soil (sandy).

On other hand, according to a report released in 2002 by the World Health Organization (2002), it states that if formaldehyde interacts with water primarily in the soil, it can break down into methanol, amino acids and several other types of chemicals. This is why the formaldehyde is basically not always 'trapped' in the soil or in other environments such as flora, fauna and water (World Health Organization, 2002). Based on the result, formalin concentration starting to drop from second to third sampling and followed by fourth and fifth sampling. From another point of view, Allemann, Olivier and Dippenaar (2018) propose some ideas that some formalin itself will 'stick' to the soil particles and possibly slip out of the soil structure at a later stage. This will directly influence the decreasing of formalin concentration slowly in soil especially on third, fourth and fifth sampling where the concentration of formalin begins to drop over time. Of the 370000 mgL^{-1} of formalin concentration, which was placed on dead biological specimens at Point C, only 149.24 to 175.10 mgL^{-1} were released or leached into the soil. The rest of formaldehyde concentration can be exposed to the atmosphere in gas state and would photodegrade in sunlight only in a few hours (Hart & Casper, 2016). Hart and Casper (2016), report that formaldehyde is highly soluble and reactive. The solubility limit for formaldehyde is 550000 mgL^{-1}. Formaldehyde concentrations can be reduced by biological systems if their concentration is not high enough to be completely toxic to the degrading organism. The highest efficiency of formaldehyde removal from wastewater was reported in both aerobic and anaerobic conditions (Garrido, Méndez, & Lema, 2000). This means that burial area of the dead biological specimen where the availability of macronutrients and micronutrients is good and very high efficiency, then the rates of formaldehyde degradation in soil may be expected to happen (Hart & Casper, 2016).

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study focused on the soil fertility and variation of soil elemental distribution for pre-burial and post-burial of dead biological specimen at Kampung Demit Cemetery, Kubang Kerian, Kelantan. Additionally, this study also aims to quantify the concentration of formaldehyde in soil for pre and post burial.

As compared to three soil parameter of soil fertility, it can be concluded that the higher of soil pH level, would result in lower level of soil moisture content and soil organic matter.

Based on the results of the first objective, soil fertility status at Point C can be concluded that it is not adversely affected by formalin, although the formalin itself is acidic, the pH value there remains a neutral value. As known before, soil that is too acidic and too alkaline were not suitable for any kind of plant on this earth. The plant will die completely if exposed to that conditions.

The soil at Point C also always shows the soil moisture content value of 1, which indicate to optimum conditions. This is possible due to the texture of the soil itself as soil at Point C is sandy soil. Sandy soils are often associated with high permeability and have a weak water holding capacity. Water is essential for plant growth. Soils beyond the optimum linkage are classified as 'excess' or 'stress' of water.

In addition, the organic matter at Point C is at low and medium levels. The color of the soil at Point C also shows an early indication that the soil is infertile due to the light brown color. This means that the soil does not have humus that acts as a protective agent against bacterial attack. Organic matter can decompose faster when soil is well aerated. Organic matter is also important for maintaining the sustainability of the plant and soil systems especially in the sustainable agriculture industry.

For XRF analysis at Point C, the concentration of heavy metal in soil such as Cr, Cu, Ni, Pb, Ti and Zn were still under acceptable limit that set by USEPA Regulatory. In comparison with sampling time, generally all the elements did not show huge changes in concentration levels before (pre) and after (post) the burial of biological specimen even though the specimen has been treated by formalin. Elements in the soil tend to be high mobile in large quantities. To be more specific, sandy soil has facilitated the leaching of organic contamination. In fact, soil elemental distribution after burial of dead biological specimens having a higher compared to before burial of biological specimens even in the same climate and soil texture. This study just focused on the most abundant concentration of element such as Si, K, Fe and Al only. Overall, all the element obtained from XRF analysis has decrease in concentration over time on the last sampling except for aluminium.

Lastly, the concentration of formalin at Point C was higher after the burial of dead biological specimen compared to before burial and in fact, it exceeds the tolerable concentration recommended by the WHO. The high concentration of formaldehyde in the soil at sampling site also believed came from the source of packaging materials of biological specimens such as plastics. This is because plastics are usually made of polypropylene and contain traces of formaldehyde However, it is believed that those concentration would decrease with distance and time and might be leached out from the soil or exposed to the atmosphere due to its high removal rate characteristic, only if there is no subsequent burial of formalin-fixed specimen. This is why the formaldehyde is basically not always 'trapped' in the soil or in other environments in longer time. Based on the result, formalin concentration starting to rise from the first to second sampling where the dead biological specimen just buried during that time, then drop from second to third sampling and followed by fourth and fifth sampling.

5.2 Recommendations

In order to obtain more accurate results for soil fertility, additional soil parameters are needed such as cation exchange capacity (CEC) and electrical conductivity (EC). Soils with higher CECs are considered more fertile than soils with lower CECs, as they have the potential to preserve more nutrients for longer periods of time. Electrical conductivity is used to measure salinity in the soil and is one of the easiest ways to determine the level of fertilizer in the soil.

In this study, there are a few of recommendations need to be addressed. Establishment of legislation or guidelines for dead biological specimen treated by formalin in the cemetery area is required. More intensive studies are needed to determine the appropriate formalin concentration that does not cause any adverse effects on soil in future studies. This is to ensures that the effects of formalin on the dead body do not affect the quality of the environment such as air, water, soil, flora and even fauna.

Next, it is recommended that another way to dispose of dead bodies is to convert the dead biological specimens to organic waste through deep-freezing. After freezing, a small vibration is used to destroy the body so that it is 5 mm in size. Water from the body is then extracted as it promotes tissue decomposition. The remains of the dried corpse can be placed in a biodegradable casket and buried in a cemetery. Therefore, moisture from the surrounding soil can be absorbed into the coffin and nutrients are provided to stimulate plant growth and insect populations. From a scientific point of view, this method is better than preserving the body using formalin as it does not use any chemicals. In addition, it does not affect the quality of the environmental air such as incineration.

Hence, alternatives ways to practice are to put biological specimens in a coffin made of 100% biodegradable natural products before being buried in a cemetery. Natural products like willow and bamboo increase the availability and support of underground aerobic decomposition. Therefore, biological specimens can be degraded faster, rather than just using plastic to wrap it as before. Insects can also quickly access dead biological specimens, thus accelerating the process of decomposing dead biological specimens.

Since death is an inevitable stage in life, so that it is really recommended for religion and countries who believe in human embalming or preservation should try and continue with the practice (human embalming) so that proper plans can be made for funeral and other investigations like autopsy can be carried out when necessary in further research.

In addition, large-area disinfecting such as in laboratories at the hospital, should be scheduled for off-work times. Sufficient ventilation is mandatory. To prevent unacceptable risks, exposure to cytotoxic concentrations of formaldehyde should be avoided. It is recommended that the concentration of formalin especially in hospitals should be at a non-toxic concentration level of 1 ppm and below for 5 minutes. If it is above this concentration, then it cannot be exposed to more than 8 peaks in a working period of 8 hours. Exposed to concentrations above 1 ppm for the next 8 hours, it is possible but not recommended because the formalin itself is carcinogenic in spite of its low concentration. (up to 8 hours).

On other hand, one method that can be easily used in all cemeteries to reduce pollution is planting trees and shrubs throughout the cemetery, especially on the edges. Vegetables can take decomposition products through their roots and insert them into their tissues that prevent them from leaving the site. The soil system of the spider plant has the highest formaldehyde removal capacity compared to others.

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