IDENTIFICATION OF FORMALDEHYDE IN GROUNDWATER USING UV-VIS SPECTROMETER

By

NURSHAFIZA BINTI ROSLI

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FACULTY OF EARTH SCIENCE

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DECLARATION

I declare that this thesis entitled "Identification of Formaldehyde in Groundwater using UV-Vis Spectrometer" 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 : ________________________

Name : Nurshafiza binti Rosli

Date : 9 January 2020

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ABSTRACT

Identification of Formaldehyde in Groundwater by using UV-Vis Spectrometer

This research aims to identify the concentration of formaldehyde in groundwater by using UV-Vis Spectrometer and to assess the physical-chemical parameters of water quality in groundwater. The samples were collected from a cemetery of Kampung Darat Demit where the formalin-based specimen was buried. The unknown concentration of the formaldehyde was determined by using chromotropic acid method for the analysis of formaldehyde. A calibration curve was prepared with six different concentrations to determine the unknown concentration of formaldehyde in the samples. Seven parameters of water quality were analyzed using respective methods and the relationship with the presence of formaldehyde in water were discussed. By using the National Water Quality Standard (NWQS) of Malaysia 2008 the class of water for all samples were determined. A statistical study of one-way analysis of variance (ANOVA) was conducted using SPSS 20^{th} version software. The results showed the different concentration of formaldehyde found in all four samples for four consecutive weeks. The classes of water samples for each physical-chemical parameter vary and in range between Class II and III. The water quality parameters' results were measured and transferred into SPSS to determine the F test values. From the results, the presence of formaldehyde in water may have significant effect on the water quality parameters physically, biological and chemically.

ABSTRAK

Pengenalpastian Formaldehid dalam Air Bawah Tanah dengan menggunakan Spektrometer UV-Vis

Kajian ini bertujuan untuk mengenal pasti kepekatan formaldehid dalam air bawah tanah dengan menggunakan Spektrometer UV-Vis dan untuk menilai parameter fizikal-kimia kualiti air di dalam air bawah tanah. Sampel dikumpulkan dari tanah perkuburan Kampung Darat Demit di mana spesimen berasaskan formalin dikebumikan. Kepekatan formaldehid yang tidak diketahui ditentukan dengan menggunakan kaedah asid kromotropik untuk analisis formaldehid (Georghiou dan Chi, 1989). Kurva penentukuran telah disediakan dengan enam kepekatan yang berbeza untuk menentukan kepekatan formaldehid yang tidak diketahui dalam sampel. Tujuh parameter kualiti air dianalisis menggunakan kaedah masing-masing dan hubungan dengan kehadiran formaldehid dalam air dibincangkan. Dengan menggunakan Standard Kualiti Air Negara (NWQS) Malaysia 2008, kelas air untuk semua sampel ditentukan. Satu kajian statistik analisis varians satu arah (ANOVA) dijalankan menggunakan perisian versi SPSS ke-20. Keputusan menunjukkan kepekatan formaldehida yang berbeza yang terdapat dalam semua empat sampel selama empat minggu berturut-turut. Kelas sampel air bagi setiap parameter fizikal kimia berbeza dan berada di antara Kelas II dan III. Keputusan parameter kualiti air diukur dan dipindahkan ke SPSS untuk menentukan nilai ujian F. Dari hasilnya, kehadiran formaldehid dalam air mungkin mempunyai kesan yang signifikan terhadap parameter kualiti air secara fizikal, biologi dan kimia.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Formaldehyde is a highly reactive aldehyde gas formed by the oxidation or incomplete combustion of hydrocarbons. It is widely use in textiles manufacturing, disinfectants and as laboratory fixative or preservative or immunohistochemical. Formaldehyde also known as one of the hazardous and toxic compounds. The colourless and poisonous gas of formaldehyde are formed from the oxidation of methanol and commonly use as antiseptics, disinfectants and as a general-purpose chemical reagent in laboratory.

Modern medicinal use burial technique to dispose preserve organs that have been keep in formaldehyde solution where before dispose by incineration. The application of formaldehyde always on the skin surface and have strict steps during applying.

1.2 Problem Statement

In Kubang Kerian, Kota Bharu Kelantan, the Hospital Universiti Sains Malaysia already started the burial process for preserved organs that before were dispose by incineration. They suggested that incineration process is not a proper method to manage organs especially for Muslims as it is contradictory in ethics. Hence, the burial method was introduced. The issues come out when approximately 100 kg of organs will be buried on the site in once in two months and the possibility to affect the quality of groundwater at Kampung Darat Demit Muslim cemetery. Small amount of formaldehyde can biodegrade in the environment in air by the sunlight and by the microbes in the soil and water. However, the burial of organs in large amount might cause unseen effect to the environment. In this prospect, the effect of the formaldehyde to the water bodies become the main concern. Therefore, spectrometric method used to identify the concentration of formaldehyde in water.

1.3 Objectives

1. To identify the presence and concentration of formaldehyde in water source in burial site.
2. To assess the effect of formaldehyde to water quality parameters in groundwater.

1.4 Scope of Study

In this research the presence of formaldehyde in groundwater in Kampung Darat Demit Muslim cemetery will be analysed and identified by using spot test quantification and spectrometric method to determine the unknown concentration. Besides, the water quality will be assessed after burial process to identify any changes in water quality due to presence of formaldehyde in underground water.

CHAPTER 2

LITERATURE REVIEW

2.1 The Properties of Formaldehyde

Formaldehyde (CH_2O or H_2CO) is highly reactive aldehyde compound that produced from oxidation of methanol and incomplete combustion of hydrocarbons. Formaldehyde also have other synonyms or trade names such as methyl aldehyde, formic aldehyde, methanol, methylene oxide, oxomethane and oxymethane. The molecular weight is 30.03 with -19.5 °C of boiling point and -92°C of melting point (National Research Council, 2007).

It is considered as flammable and hazardous substance and appears in gases state in room temperature and produce pungent odour. Formaldehyde known to be denser than water where the vapours are heavier than the air which cause nose irritation. Formaldehyde are distributed as 37% in water while formalin distributed as 105 in water. Direct contact to formaldehyde lead to serious injury, starting from skin drying, crackling and scaling. Other than liquid form, formaldehyde also present in solid by name; paraformaldehyde. It is white in colour and have lighter pungent odour compared to liquid formaldehyde. Paraformaldehyde is harder to ignite due to higher flash point. The melting point for paraformaldehyde are higher than liquid formaldehyde which is 120°C. Other names for paraformaldehyde stated are polyoxymethylene, paraform, and formagene (Darvell, 2018). Formaldehyde can readily react with many substances and polymerizes.

2.2 Sources of Formaldehyde

The application of formaldehyde is no longer specific in one field. Starting from household sectors until the industrial sectors and even in human bodies, formaldehyde become a basic component in daily life. The application of formaldehyde is widely available in furniture manufacturing especially in resins making to act as adhesive for composite furniture that usually made up from wood fibre, board particles or plywood. New furniture emitted the highest concentration of formaldehyde based from the smell of the wood. In addition, they also can be found in paints and coating for wood furniture and some household products and cosmetics which have low concentration of formaldehyde (Gholami et al, 2016). The use of formaldehyde in manufacturing are due to the preservatives and anti-bacterial properties that made the products long lasting.

In household products, formaldehyde present in air fresheners and plug-in fragrance, cleaning products and paper towels. The air fresheners are considered as new second hand smoke because the sources come from chemicals. Synthetics fragrance can affect the immune system when breathed in absorbed by skin. Cleaning products contained terpenes to begin with, terpenes are substances that can react with ozone to produce formaldehyde and particulate matters.

For cosmetics products, the formaldehyde was used in tiny amount to prevent fungi and bacteria growth on and spoil the products and cause harm such as skin diseases, allergies and rashes. The ingredients are named Imidazolidinyl Urea DMDM Hydantoin. For example, when washing hair, the amount of formaldehyde release is equivalent to the amount in an apple or a pear. In nail hardener product, formaldehyde bonded with keratins that make nails become harder but continuously application of nail hardener makes nails brittle and have high potential to peel off. Contact of formaldehyde with skin can cause irritation of skin and allergic reaction.

Besides, formaldehyde also can naturally be found in living organisms, such as human, plants and animals as part of cell metabolism. A group of scientists from MRC Laboratory of Molecular Biology revealed a new research that stated that the reaction in human bodies can produce toxic formaldehyde as its by-product. They discovered that the formaldehyde formed from the 'one carbon cycle' process which require a vitamin, folate to create DNA and amino acids. The discovery become the target for developing new cancer therapies.

In the environment, the presence of formaldehyde contributed from smokes of fire, vehicles' exhaust and cigarette smoke. In addition, the formaldehyde has the ability to form in atmosphere by photochemical oxidation with reactive organic gases at polluted region in presence of ozone and nitrogen oxides. In California, photochemical oxidation processes are the main factors that induced 88% of formaldehyde in the air. Formaldehyde can be categorized as one of the environmental components. In small in amount, formaldehyde is readily biodegraded in water, air and soil whether aerobically or anaerobically.

2.3 Use of Formaldehyde in Medical Field

In medical field, formaldehyde use as antiperspirant to treat sweat and unpleasant smell and drying agent for certain situation. The application of formaldehyde in medical always on skin surface with no wound or injury and following the strict instruction of how to use the solution. Improper handling of formaldehyde may cause irritation or redness to skin, eyes or any areas that have exposure to the solution. Some cases may lead to respiratory problems due to long exposure. A research on the respiratory effect due to occupational exposure to formaldehyde were done where the main target were individuals involve are from industrial workers. Most of the workers shown the symptoms of respiratory problems such as cough, asthma, eyes and nose irritations, bronchitis symptoms, phlegm and dyspnoea (Mathur & Rastogi, 2007).

Embalming process which known in funeral planning use formaldehyde-based chemicals to replace the liquid in the bodies through surgical. The purpose of the embalming is to preserve and disinfect the bodies which then make them last longer for visitation and viewing for funeral or crime cases. Embalming is one of the alternatives for funeral if the family members of the deceased refuse to use casket.

In immunohistochemistry (IHC), formaldehyde is use in aiding immunophenotyping and prognosticating neoplastic disease, detecting or identifying pathogens related to histologic lesions and characterize the cellular infiltrates. The primary advantage in using formaldehyde in IHC due to ability to detect antigen in tissue and cellular morphology that include relationship of antigen with specific cell types or histologic lesions. Formaldehyde is not directly use in IHC but need to be transform into formalin by using 4% of formaldehyde and water. Formalin help in inhibiting cellular processes to prevent tissues degradation, preserve tissues and kills pathogen. Therefore, the need for fresh tissues will be reduced and allows the use for diagnostic cases and retrospectives studies (Webster et al, 2009).

2.4 Effect of Formaldehyde to Human Health

Skin, nose and eyes irritations seems to be the basic effect when exposed to formaldehyde. The benchmark for exposure to formaldehyde is 1.13 ppm. However, long – term exposure may increase chances of receiving cancer and other chronic diseases especially in respiratory tract due to inhalation of gases. Studies in United States found that short – term exposure to formaldehyde cause watery eyes, nose and throat. This occur when the presence of formaldehyde in the air exceeds 0.1 ppm. However, the effect of the exposure seems to differ to different individuals. As for long – term exposure, a laboratory studies conducted in 1980s discovered that formaldehyde could cause nasal cancer in the specimen used those times; rats. Not until 1987s, the U.S. Environmental Protection Agency (US EPA) announced that formaldehyde was classified as probable carcinogen to human (Thetkathuek, Yingratanasuk, & Ekburanawat, 2016).

Besides, epidemiologic studies have been done to a group of people to identify the long-term effect of formaldehyde exposure. Two types of studies were carried out. The first study; the cohort study, where a group of people have different exposure due to some factor and the time require to develop the disease. Next, the second study; the case-control study. In case control study, the community that already diagnosed with the disease are compared with groups that did not receive the disease.

These people will act as control variable to identify the factors that catalyst the development of the disease (National Cancer Institute, 2011). For example, anatomists and embalmers have the highest possibilities in getting cancer than general community because they dealt with bodies that were injected with formaldehyde. Most of them were diagnosed with myeloid leukaemia. Other than that, nasopharyngeal cancer also has been reported to be associated with exposure to formaldehyde in 10 different locations (Facts, 2019). The workers with high exposure, have higher potential in gaining cancer especially in upper respiratory tract cancers.

The exposure to formaldehyde can cause both chronic and acute illnesses such as irritation and cancer. National Cancer Institute (NCI) in United States undergo studies and they discovered the funeral services staffs have an increase in risk of developing lung and nasopharyngeal cancer and leukemia.

2.5 Effect of Formaldehyde on Animals

The impact of formaldehyde exposure in animals appears to be the same as on human if ingested or inhaled. In addition, it said to influence the reproductive system of animals which result to low fertility. A research has been conducted to identify the effect of formaldehyde on sperms and testicular tissues on mouse. 36 adult male mice were divided into three groups, one control group and the other two groups act as experimental groups with different concentration. The first group contained low concentration of formaldehyde; 10 ppm while the second groups contained higher concentration; 20 ppm. Those two groups were leave for ten days, eight hours per day.

The short term and long-term time exposure effects examined by dissecting half of the male mice from both groups after 24 hours and the rest of the mice after 35 days. The result shown the mice exposed to higher concentration have substantial decrease in sperm cells numbers and sperm viability. Total percentage of abnormal sperm increase when compared to control group because of reduction in progressive motility (Vosoughi, Khavanin, & Salehnia, 2013). Low in fertility cause the depletion in offspring especially animals that are categorized as endangered such as Orangutan, Malayan Tiger and Malayan Sun bear and lead to extinction. Despite the effect on animals, in the atmosphere, formaldehyde will quickly break down into formic acid and carbon monoxide.

2.6 The Significance of Water for Living Organisms

Water is the most essential compound needed by all living things because its function in cells metabolism in animals and plants cells. Water acts medium for most biochemical reactions because the reaction tale place in aqueous condition. Besides, water also influence structure of molecules such as proteins, nucleic acids and polysaccharides (Fagerstedt, 2017). Plants take up water from the soil together with nutrients which then transporting those nutrients and organic materials throughout plants areas such as from roots to leaves, stem and fruits. During hot weather, water will keep the herbaceous plants turgor because herbaceous plants do not have woody stem as support. Water will fill the vacuole with water and maintain the pressure maintain the shape.

In human body, 70% of total body mass are from water. Lack of water in body result in dehydration. Dehydration can cause blood viscosity to increase and influence the efficiency of blood to transport oxygen and nutrient into cells inside the body (Ahmad, 2017). There will be high concentration of toxins and decomposed products from cell activities. The high concentration of carbon dioxide in body can cause breathing failure which lead to drowsiness and faint. In some critical cases, can cause death. Other than that, water function in removing waste from human body through perspiration, urination and defecation with the aid from kidneys and liver to flush the waste out.

Drinking enough water can prevent constipation by softening the and ease the mobility of food in intestinal tract. In food digestion, water plays crucial role in delivery food and digestion. Saliva contain enzymes to break down the food eaten into smaller molecules to increase nutrient absorption intestinal tract (Laskey, 2015).

In addition, in regulating body temperature, water have high heat capacity which act very fast in realising excess heat from body by sweating. The evaporation of sweat bring along the calories and release huge amount of energy to stabilize the body temperature especially during hot weather and fever (Nestle, 2019).

2.7 The Physical-Chemical Parameters of Water

Normal range of groundwater pH are between 6 to 8.5 while for surface water it is in range of 6.5 to 8.5. The pH is sensitive to several factors that lead into cause water unsuitable to be use. First factor is the present of carbon dioxide. Carbon dioxide linked to the pH level as the concentration of carbon dioxide in water increases, the pH level will automatically decrease due to formation of carbonic acid. Similar with Carbon dioxide, acid rain contains acids that present in the air which enter water bodies together during precipitation. The acids in acid rain not only carbon dioxide but also from industrial activities such as sulphur dioxide and nitrogen oxide. Besides, the presence of dissolve minerals in underground waterway influence the pH level. Are with limestone bedrock usually will have higher pH level due to the alkalinity of the limestone.

For groundwater temperature, the changes cause by direct and indirect influences. The direct influence commonly from the heat input into the water through sewage system, piping, underground railway and any constructions. Indirect influences mostly come from urbanization and heat from transportation. High degree of surface imperviousness disturbs water balance because of the changes in thermic surface properties such as surface conductivity and heat capacity.

Dissolved oxygen is free non-compound oxygen that could be found in water or other liquid without bonding with any element. The dissolved oxygen of groundwater only can be available in the water surface that exposed to the atmosphere in range of 1.0mg/L to 20 mg/L. Aside from being one of most crucial parameters in identifying the water quality, dissolved oxygen plays important role to the organisms for respiration. However, under specific condition, extreme dissolved oxygen level is dangerous that harm aquatic life and affect the quality of water.

The water in Class II is divided into two; Class IIA and IIB. The Class II which in range of 76.5-92.5. The IIA acts as secondary water supply that require conventional treatment before being distributed to the public and habitat to very sensitive fisheries while IIB acts as recreational body contact. The water in Class III is in range of 51.9-76.5 functioned as tertiary water supply but requires more extensive treatment as it is more polluted than Class I and II but water in Class III is widely used for locals in economic generation in fisheries and for livestock's usage. Class IV was classified for irrigation purpose where to allow water to flow but for water supply purposes, it requires extensive water treatment to remove the impurities in the water. The Class IV water is categorized as polluted that are not safe to be use by human or animals. For water in Class V, the rivers, lakes or basin categorized under this class indicates that the pollutants content in the water already exceeded the threshold levels allowed by the Department of Environment (DOE) Malaysia.

2. 8 Formation of Formaldehyde during Ozonation Process

Ozonation process in water also one of the sources of formaldehyde in drinking water. Before ozonation was used, water was disinfected by using disinfectant. Chlorine is the common disinfectant because it is effective in low concentration and cheaper compared to other disinfectants. Chlorine has high oxidizing properties which able to oxidize the enzymes in microbial cells. When chlorine dissolved in water, it will react with organic compound and forming two possible compounds; chlorophenols and trihalomethanes. Chlorophenols produce unpleasant smell and taste to water and the concentration of phenols can be identify from chlorophenols. However, the ozonation process using ozone, O_3 which is a strong oxidant than chlorine (Bidhendi, Hoveidi, Jafari, Karbassi, & Nasrabadi, 2006).

Ozonation did well in disinfecting and eliminating synthetic chemicals remove the taste, odour and colour of water and dissolve both manganese and iron metal. The concentration of trihalomethanes decrease as well as other disinfectant by products and increase flocculation. However, during ozonation process, dissolved organic material only partially oxidized. A research in 2010 about the formation of formaldehyde during ozonation was conducted. In the research it was explained that those with higher molecular weight will change into less molecular weight compounds and the dissolved organic carbon concentration (DOC) left unaffected. The reaction of ozone with natural organic materials also resulted in few by products like epoxides, organic peroxides and aldehydes (Can & Gurol, 2010). When there is no accurate method in identify the present of epoxides and organic peroxides in water, scientists were able to detect aldehyde in drinking water after ozonation process.

The formation of aldehydes during ozonation was not clear as scientists conclude that maybe due to reaction of ozone between unsaturated side chains or the aromatic functionalities from natural organic materials to produce ozonides and then converted into aldehydes. Several variables were proposed to induce the aldehydes formation such as ozone dosage, temperature, pH, alkalinity and nature of natural organic materials. There are observations that stated the formation of aldehydes increase when there was increase in ozone dosage and the initial organic carbon concentration. The result obtained from the paper shown formaldehyde was formed directly all samples and the accumulation process gradually increase before degraded throughout the process. The formation of peak indicate that formation rate of formaldehyde is initially higher than the oxidizing rate. It is not after the ozonation the formation rate shown a decline and increase in oxidation rate (Can & Gurol, 2010).

2.9 The Determination of Formaldehyde in Cigarette Smoke

In cigarette smoke the formaldehyde can be found in mainstream, side stream and in environmental tobacco smoke. The three mediums have different formaldehyde concentration recorded from the previous studies. The range of concentration of the mainstream started from 10 µg/cigarette to more than 100 µg/cigarette which then influence by the type of tobacco used and type of brand. However, environmental tobacco smoke associated with room formaldehyde levels that showed high concentration. A researcher named Howlett already carried out and experiment in a room by smoking a cigarette and resulted in increase of formaldehyde in the air to 0.21 ppm in thirty minutes duration (Godish, 1989). Hence, the difference of result obtained from the three mediums were due to the different methods used in identifying the rates of formaldehyde production in cigarette smoke. Both mainstream and side stream use the same methodologies, the 2,4 dinitrophenylhydrazine-HPLC method to identify the free formaldehyde presence. While for environmental tobacco smoke, the method used is chromotropic acid method as it is widely used to detect the presence of formaldehyde in air. Formaldehyde will form a stable addition product in sodium bisulphite solution and when the product being destroyed it will transform into free formaldehyde.

Several factors are important in determining the presence of formaldehyde in cigarette smoke listed in a paper by Spincer and Chard in 1971. The first factor suggested was using sulphur dioxide. Sulphur dioxide have sensitivity in detecting formaldehyde when added into standard sodium hydrogen sulphite solution to form reaction mixture (Spincer & Chard, 1971). The maximum level of colour development occurred between 700 µg and 1100 µg with volume sulphur dioxide at 25 mL. If the volume of sulphur dioxide exceeding the said volume, it will completely destroy the colour development.

The second factor is the time needed for the colour development to take place. At fixed temperature of 18° to 25° C, the optimum time required was 100 minutes for colour to form and stabilize for the next three hours. However, at temperature of 39° colour fully developed less than one hour and at 0° took eighteen hours to develop (Spincer & Chard, 1971).

2.10 The Presence of Formaldehyde in Rain Water

In Heraklion, City of Crete in Greece 66 rain samples have been measured to detect the presence of formaldehyde from September 1999 – May 2000. The range of the concentration found worldwide was in between 0.42 µM to 11.14 µM. The result obtained showed the concentration of 3.05 µM which approximately about 3 % of dissolved organic carbon present in air where the formaldehyde levels are influence by air mass origin. Air mass from NW Europe and Turkish are twice higher from the air masses originated from Africa due to anthropogenic sources that accumulate in the air (Economou & Mihaloupoulos, 2002).

In Wilmington, North Carolina, total of 116 rainwater samples. By using the volume weighted average of formaldehyde concentration at 1.4 M, the annual deposition was determined at 4.6 mmol m^{-2} yr^{-1}. Rainwater also main source of formaldehyde in surface waters up to 30 times of the total amount of natural waters south eastern North Carolina.

In Nigeria, rain water was used for drinking and domestic purpose. The reason why the research conducted to determine whether the concentration of formaldehyde present in the rain water will be affected the people in Nigeria. Using the chromotropic method, they discovered the highest concentration of formaldehyde was harvested in June, while the lowest concentration harvested in September. The samples were collected once a week because Nigeria did not have rain on daily basis thus, the results were duplicated every week to get average for a month. The low concentration of formaldehyde in rain water because it was absorbed during the cloud droplets formation and also as it was readily soluble to form methylene glycol and oligomers. There was experimental report that stated formaldehyde was readily absorbed in gastro-intestinal tract and oxidized into formic acid, carbon dioxide and water in rats and monkeys. Those metabolic will be discarded in form of urine, faeces and air. However, a research clarified that event when the concentration of formaldehyde consumed was low, but the exposure to it will resulted in human health if ingested (Erhabor, Uwumarongie-ilori, Agho, & Obahiagbon, 2013).

2.11 The Presence of Formaldehyde in Food

Formaldehyde known to be naturally present in common food such as vegetables, meats, fish and fruits at different range levels. In Bangladesh, formaldehyde was mixed with methanol to form formalin that use to extent the shelf life of food. The factors that lead to problem are the lack of strong regulatory controls, weak infrastructure in transportation and increase in consumer demand. Yet, food adulteration can cause developmental defects, chronic disease or worse death. The most vulnerable target group was children which resulted in increase in child mortality.

The research was done by using four type of samples; mango, shrimps, milk and fish. These sample then compared with freshly caught and from market. The results have showed no significant different but after the marketed fish being dipped into the formaldehyde solution, it gave out pungent smell while the other fish showed lower level, 2.83-2.95 mg/kg. Milk also have no significant different after compared from farm milk and marketed milk. Instead, in shrimp product both fresh and marketed have significant difference and higher compared to previous research. However, the cause of high concentration was doubt whether it was from the environment or adulteration process.

CHAPTER 3

MATERIALS AND METHODS

3.1 Identification of Water Quality

The water samples were collected from one point, a well that is in Kampung Darat Demit Muslim cemetery site. Samples were taken at one point for four consecutive weeks at the same the time range to ensure the data accuracy for all samples. In-situ data were collected by using a YSI multiparameter such as pH and temperature. The samples were then let chill in an icebox filled with ice to slow down the microbial activities in the water that might affected the result accuracy.

3.2 Laboratory Method

Firstly, for Biochemical Oxygen Demand (BOD) identification, 100 ml of the sample was placed in a 300 mL BOD bottle by using a measuring cylinder and filled with prepared dilution water and then being poured slowly by the bottle wall surface to prevent the formation of bubbles. Then, slowly the bottle was closed with a stopper and was be inverted the a few times to mix well. A HACH LBOD Intellical™ probe was used to read the initial BOD concentration from the bottle. The bottle was closed again carefully, and a bottle cap was put on top to prevent evaporation of water sample in the bottle. After that, the sample was kept in incubator at 20° C temperature for five days. After five days, a probe was used to measure the final reading of the BOD in the water sample.

Secondly, for Chemical Oxygen Demand (COD) identification, 100 ml of water sample was blended in a mixer for 30 seconds and 2 ml was transferred into a COD vial and inverted for several times. Then, the vial was inserted into a reactor for 2 hours at 150° C temperature and then let cool in room temperature. Next, the vial was wiped with tissue paper and inserted into HACH UV VIS for reading.

Thirdly, for Total Suspended Solid (SS), the water sample was blended for 2 minutes and 10 ml of sample was transferred into a sample cell and distilled water into another sample cell as blank and inserted into HACH UV VIS after wipe.

Fourthly, the identification for Ammonia Nitrate by transferred 25 ml of water sample into a volumetric flask and 25 ml distilled water in to another volumetric flask as blank. Two drops of mineral stabilizer and three drops of Polyvinyl Alcohol Dispersing Agent for both volumetric flasks were added using a dropper. After that, both volumetric flasks were inverted to well mix the solution. One drop of Nester Reagent was added using a pipette and inverted to mix well. The flasks were left to react for a minute and transferred 10 ml sample from each flask into two different sample cells. Sample cells were inserted for reading in HACH UV VIS.

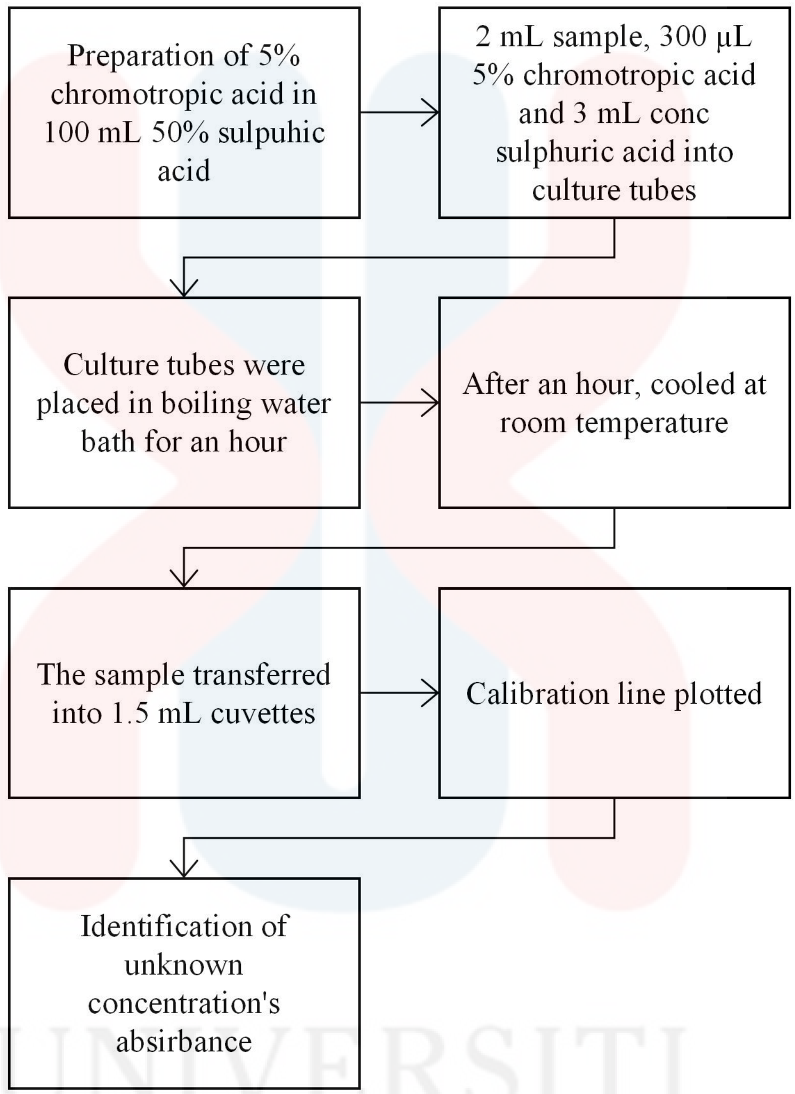

Figure 3.1 Flowchart of formaldehyde detection

A stock solution of formaldehyde was prepared from 1000 mg/L into six different concentrations; 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm. The chromotropic acid disodium salt dihydrate and sulphuric acid of 95-98% were used to for the preparation of 5% chromotropic acid by dissolving 5g of chromotropic acid disodium salt dihydrate in 100 mL 50% sulphuric acid. The solution was shaken until the salt dissolved and until a purple colour solution formed. The chromotropic acid was prepared several times due to expiratory date only for two days of storage.

For spectrometric method, 2 mL of prepared solution, 300 μL of prepared 5% chromotropic acid and thoroughly mixed by inverting and then 3 mL of conc sulphuric acid added by using a pipette and inverted. The tubes then sealed with parafilm and closed using the cap and left in the boiling water bath for one hour. After one hour, the tubes were cooled at room temperature and mixed thoroughly by inverting. Brown coloured solution produced while blank solution maintains purple. The sample then transferred into 1.5 mL cuvettes for absorbance reading using UV-Vis reading at 412 nm wavelength. The blank was prepared pipetted 2 mL of distilled water, 300 μL of prepared 5% chromotropic acid and 3 mL conc sulphuric acid. The calibration line was plotted to identify the concentration of formaldehyde in the samples. The method was repeated by substituting the prepared solution to water samples collected.

3.2 Statistical Analysis

A statistical analysis was done to reduce the range of uncertainty in water. Statistical test used analysis of variance (ANOVA) to assess the water quality and the significant different in the physical-chemical parameters. A correlation was constructed to determine the relation between variables. From one-way ANOVA it was identified whether the significant was achieved ($P<0.005$). The statistical method was carried out using SPSS 20th software version. The F test was applied to investigate the null hypothesis.

CHAPTER 4

RESULTS AND DISCUSSION

Among the water quality assessment, the measurements of physical-chemical conditions have been regarded as one of the common practices that address the water status. In this study, the physical-chemical parameters comprise temperature, conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), and pH measurement collected at each sampling sites summarized in Table 4.1. Each parameter has its own role to play, moreover, the aggregate effect is the summation of the interaction of all parameters by referring to National Water Quality Standard (NWQS).

Table 4.1 Water Quality result of four samples

Sample	Water Quality Parameter						
	pH	Temperature (°C)	BOD (mg/L)	DO (mg/L)	NH_3-N (mg/L)	TS (mg/L)	COD (mg/L)
1	7.11	27.57	1.97	1.15	0.05	7.00	87.00
2	8.13	27.86	4.21	1.36	0.03	7.00	17.00
3	4.22	27.39	3.00	1.00	0.03	8.00	43.00
4	4.87	27.45	2.48	1.08	0.08	6.00	182.00

Under NWQS, there are five main classes of water that can be obtained by calculating all the parameters into a formula constructed by the Department of

Environment (DOE). Each class has different function based on the index calculated. The best water quality is in Class I where the index value is < 92.7. The water belong to this class is used as conservation point for natural environment, act as direct freshwater supply and protected fishery areas with usually become the habitat of very sensitive species.

4.1 Water pH

pH of water can affect both chemical and biological processes in the water body. The concentration of hydrogen ion (H^+) and hydroxide ion (OH^-) will determine the acidity and alkalinity of water. In biological process, low pH in water will affect the aquatic environment by altering the balance of chloride and sodium in aquatic organisms. High intake H+ in cell from the loss of sodium caused respiratory failure and loss of regulation in osmotic pressure then death to aquatic life (Stutsman, 2017).

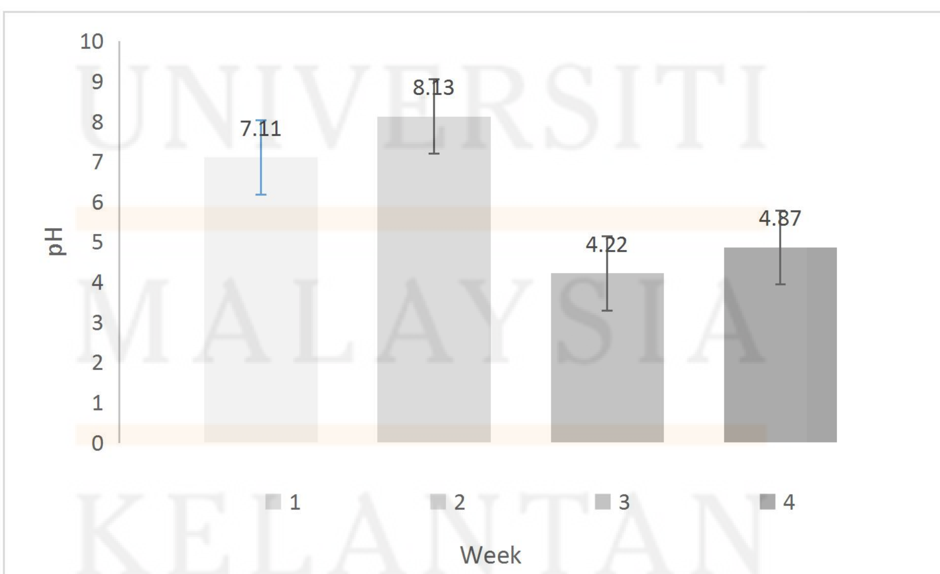

Figure 4.1 pH value for four samples

Based on Figure 4.1, the pH for four weeks was different. Sample 2 had the highest pH 8.13 while the lowest is Sample 3; 4.22 with mean value is 6.08±0.92 for all four samples. Based on NWQS, the class of water for Sample 1 and 2 were classified in Class I but Sample 3 and 4 showed the lowest that indicate water was in the Class V. However, the pH of formaldehyde is lies between 2.8 to 4 and after oxidation of formaldehyde into formic acid the pH is in range of 2.38 to 3.47 in different concentrations. The interaction of water and formalin alter the original pH to made it acidic. Under another circumstances, the pH would change when formaldehyde dissolved in water by solvation process. In this process, the water molecules will attract the molecules from formaldehyde that lead to attraction of ion-dipoles and hydrogen bonds, which are the main forces that made the solutes to dissolve in solvents (Ernest, 2014). The heat was generated from bond breaking which caused an increase in temperature that become one of factor that affected the pH of water (Ashida & Iwasaki, 1995). Since the solvation process cannot be control, the temperature of water will continuously change as long as they are presence of other solutes in the water and difficult to get the accurate reading.

4.2 Water Temperature

Water temperature is a crucial factor in water quality assessment as all water chemistry, biochemical processes are functions of temperature. Figure 4.2 shows a deviation of about ± 0.21° C in all temperatures measured. The variations in water temperature are related to the temporal variability during sampling period (7.30 -8.30 am).

Temperature and influenced by the its environment. Therefore, to ensure the accuracy the water and data collected at the same time range from 7.30 am to 8.30 am. The temperature of water is the highest temperature in Sample 2 and the lowest at Sample 3 with mean value of 27.57±0.1.

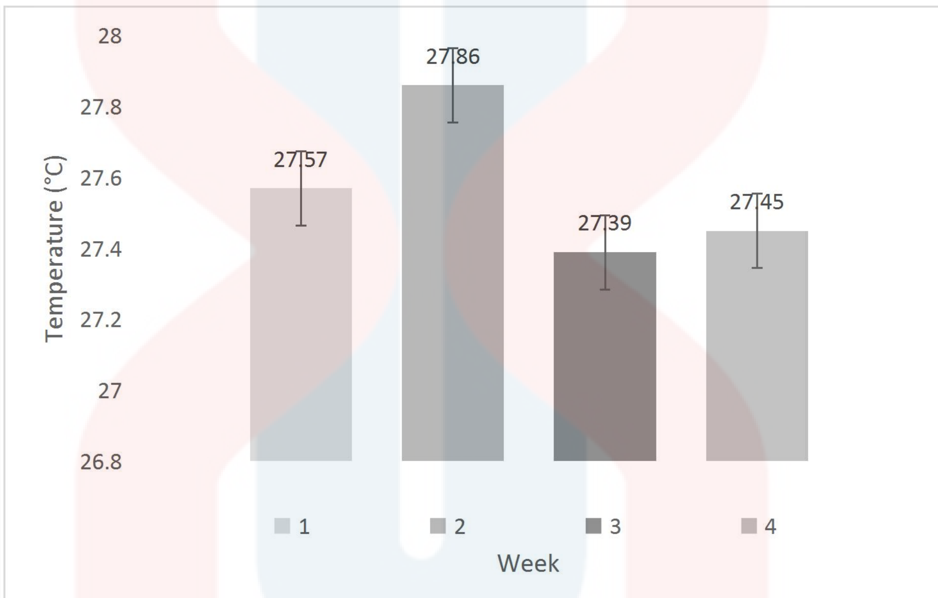

Figure 4.2 Temperature of water samples

The possibility of solvation process occurring in the area contribute to the changes in temperature before and after burial process due to interactions of solute and solvents (Ben-Amotz et. al, 2005). The increase in temperature raise the thermal energy present in the water to increase the kinetics energy that enable the bond breaking. This due to the high specific heat capacity of water to supply heat for hydrogen bonds to absorb during bond breaking process.

4.3 Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand is important it is representing the amount of oxygen used microbes in the water when decomposition of organic matter occur in water with the presence of oxygen. The value of BOD in the highest in Sample 2 and the lowest at Sample 1 with mean value of 2.92±0.48 mg/L for all four samples. The high concentration of BOD indicates how polluted the water is. The high amount of organic matters found in the water increase the demand of oxygen for decomposition and the number will decline after all the organic matters in the water are consumed.

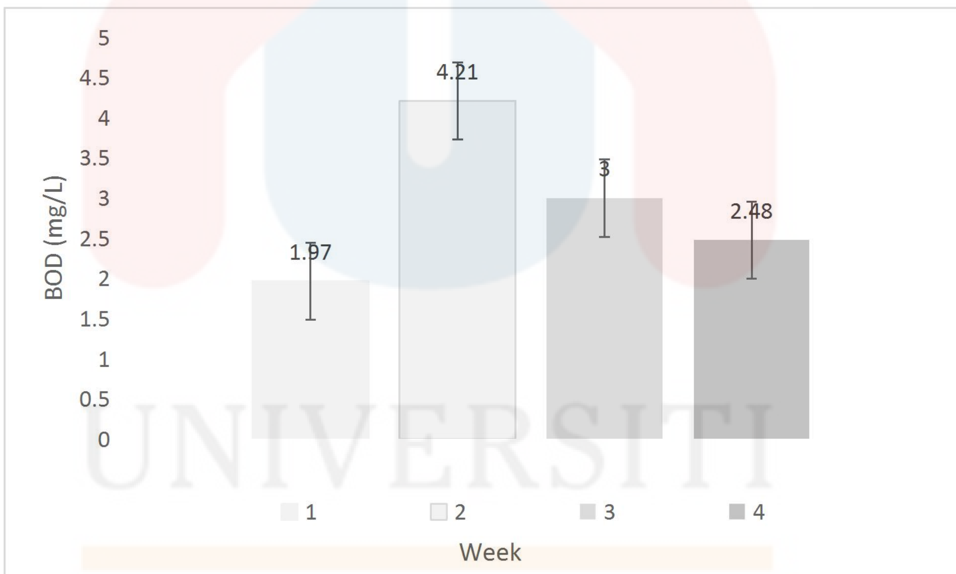

Figure 4.3 BOD of water samples

From Figure 4.3, the BOD values are relatively low which means less microbial activities occur in the water. Based on the result, the class of water for Sample 1, 3 and 4 were classified in Class II while Sample 2 in Class III. The low value of BOD indicates less microbial activities in the water which means the demand of oxygen from microbes for decomposition process is low. The influence of temperature also contributes to BOD value in water as the warmer water has higher BOD. Looking back at figure 4.2, Sample 2 was higher in temperature than others which resulted in higher BOD. Another factor is that in this case, the presence of formalin could have inhibited the microbial activities. Formalin has the ability to accumulate protein that caused heat shock in cell. The activation of heat-shock responses in cell inhibits the protein responses in cell and mitochondria become inactive (Ortega-Atienza et. al, 2016). Due to the inactivating the nucleic acids and enzymes of the microorganisms diminished the viability made them cannot digest the organic matter that presence in the water.

4.4 Dissolved Oxygen (DO)

DO is an important environmental parameter which indicates the ecological health status of the aquatic ecosystem. Higher percentage of DO was recorded in the sample 1 with mean value 1.15±0.08 mg/L for all four samples.

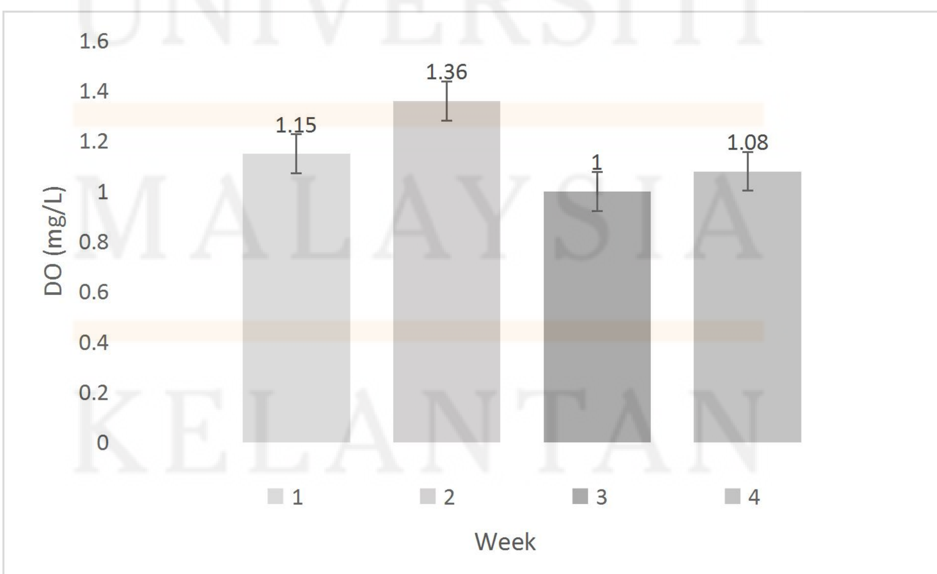

Figure 4.4 DO concentration of samples

In Figure 4.4, all four water samples from the same point were categorized as Class IV based NWQS. The DO in water is influence by the atmospheric pressure, water temperature and number of dissolved substances in water. Warmer temperature decreases the oxygen solubility in water, but from the result the increases in temperature does not showed decreases in DO. There is possibility the DO was affected by another external factor that contribute to oxygen depletion such as salinity or pressure. Lower DO will influence other processes like as BOD decay, sediments oxygen demand, nitrification, respiration and photosynthesis (Minnesota Pollution Control Agency, 2009) and also process called photochemical between formaldehyde and water molecules that induce the formation in OH radical and O_3 (Wang, Zhu, He, & Han, 2017).

4.5 Ammoniacal Nitrogen (NH_3-N)

NH_3-N is one of the parameters used in identifying the amount of ammonia, toxic pollutant that can be found in leachate on landfill and the health of a water body.

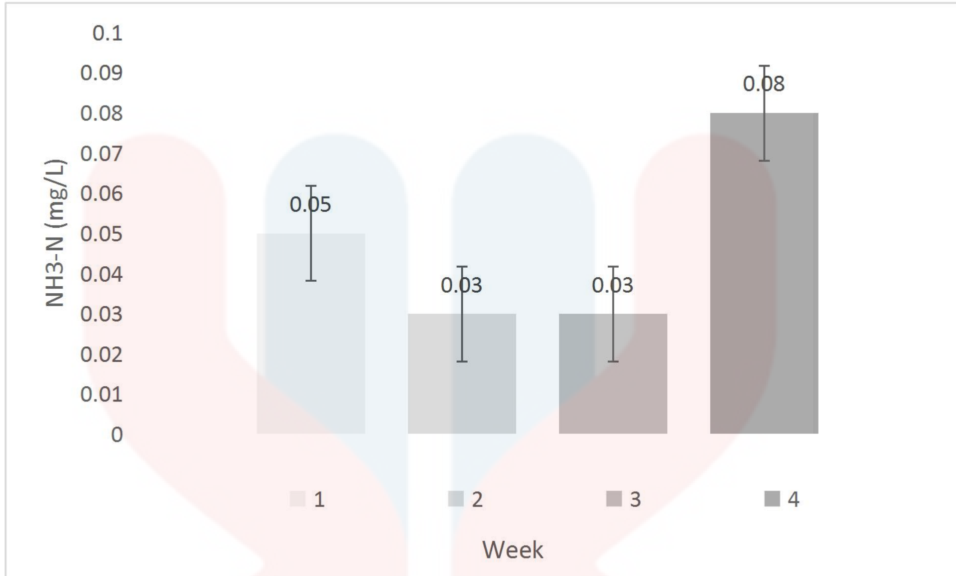

Figure 4.5 NH_3-N value in water sample

The highest NH_3-N was recorded at Sample 4; 0.08 mg/L and the lowest were Sample 2 and 3; 0.03 mg/L. All water samples were classified in Class 1 in NQWS because all values were less than 0.1 mg/L. The reaction of ammonia with formalin produced aldehyde ammonia under neutral or slightly alkali condition to form amino acid compounds such as amino ethyl alcohol. This parameter once used to detect formaldehyde by forming hexamethylenetetramine forcefully in aqueous solution at room temperature and atmospheric pressure through condensation process (Lazzaroni et al, 2015). The result shown that the NH_3-N concentration for all samples were within the limit by World Health Organization (WHO).

4.6 Total Suspended Solids (TSS)

Total Suspended Solids is water quality parameter that determine the number of solids present in the water in form of suspension as colloid due to water motion. The highest Total Suspended Solids (TSS) recorded on Sample 3; 8 mg/L while the lowest is Sample 4 which is 6 mg/L with mean value 7±0.41 mg/L for all samples. The quality of water in Sample 1, 2 and 4 were classified in Class II and Sample 3 was in Class I.

The different between TSS to Total Dissolved Solid (TDS) can be explain in terms of size where the size of the solids in the water with less than 2 microns, the solids will fall into TDS type.

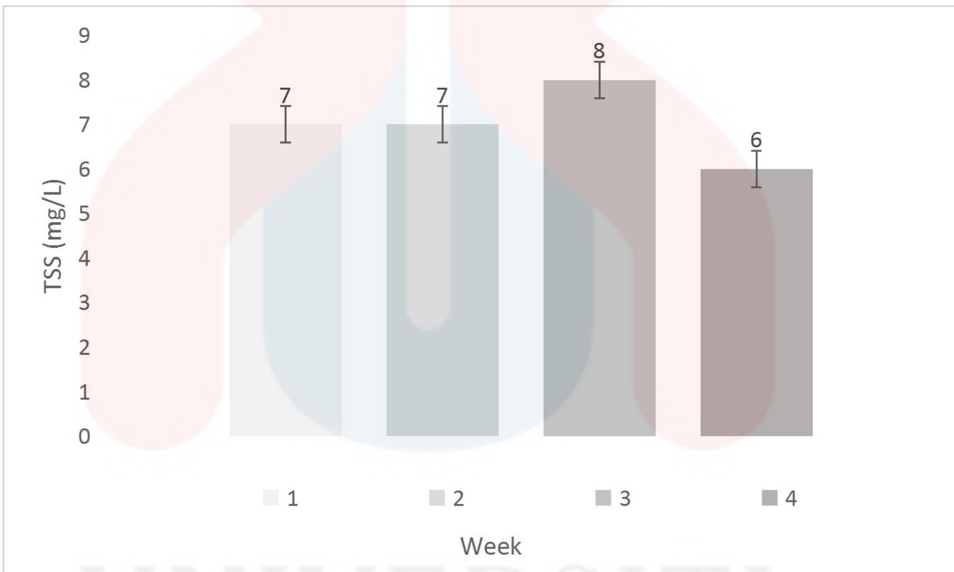

Figure 4.6 TSS concentration in the water

Most of solids made up from inorganic matter, silt, sediments, and some algae or bacteria (Fondriest, 2017). High concentration of TSS block light from reaching water base that reduce the photosynthesis of aquatic plants and stop producing oxygen in the water for respiration aquatic animals. In water the presence of TSS can disturb biological processes where affected the amount of oxygen in water. The concentration of oxygen in water will become lesser and no longer sufficient for aquatic organism.

4.7 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is used to determine the amount of oxygen that can be used in reactions or chemical process occur in a solution. The highest COD concentration recorded was 182 mg/L in Sample 4 and the lowest was Sample 2, 17 mg/L with mean value is 82.25±36.25 mg/L for all samples.

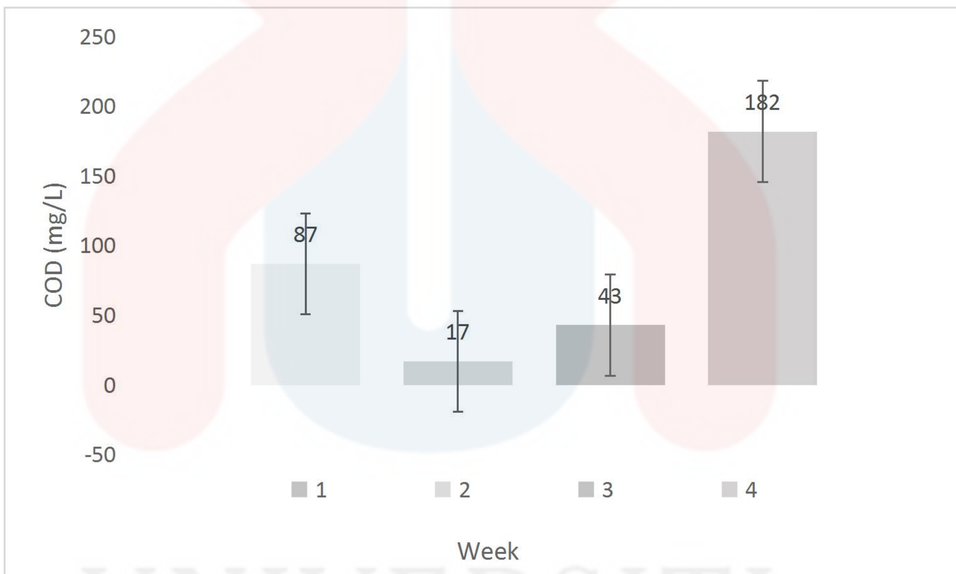

Figure 4.7 COD value of water

The quality of water from Sample 1 was categorized as Class IV, Sample 2 as Class II, Sample 3 as Class III and Sample 4 as Class V based on NWQS. Higher COD mean the presence of oxidizable organic matters are higher in water that contribute depletion of DO in water. The organic matter might oxidized by dichromate and interfere the actual value of water (Merck, 2019). The depletion of DO trigger anaerobic conditions that are threatening to the aquatic life (RealTech, 2017). Several factors can alter the value to high falsely COD reading such as the presence of foreign chlorine in distilled water and microorganism in deionized water used in the laboratory.

However, the concentration of formaldehyde seemed to not correlate with precipitation volume by means it may be due to continuous supply of during raining by direct photochemical production in aqueous phase. Besides, another parameter like pH, nitrate and ammonia also contribute to the increase in photochemical activities together with biogenic and anthropogenic activities in summer that gave the highest reading for formaldehyde on summer. Formaldehyde was correlated with hydrogen peroxide and no-sea-salt-sulfate at troposphere to form a relationship between both formaldehyde and hydrogen peroxide (Kieber, Rhines, & Willey, 1999).

Since the location of sampling is a cemetery area, the decomposition process occurred frequently. All parameters are affected hence alter the actual values for all parameters. Decomposition of bodies by decomposer especially by microbes increases the microbial rate of metabolism. Temperature plays crucial roles in determining the metabolism rate of decomposers, because when the metabolism increase, the temperature increase due to heat release as byproduct when decomposers digested the organic matters. Besides, due to abundance of organic matters, the decomposition process can actively occur that will influence BOD, COD and DO in the water. In some seafoods, the formaldehyde is the product of breaking down of trimethylamine oxide existed in their bodies. Both formaldehyde and dimethylamine are formed equally after the seafood dies and, in some fish, it can accumulate during frozen storage up to 400mg/kg. In 2006 to 2010, total of 250 food samples were analysed to identify the formaldehyde and the results obtained were reliable and no worries about formaldehyde ingestion occurred (Yau, 2017).

Table 4.2 One Way ANOVA analysis of variance of water samples

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	10.187	3	3.396	.	.
	Within Groups	.000	0	.		
	Total	10.187	3			
Temperature	Between Groups	.131	3	.044	.	.
	Within Groups	.000	0	.		
	Total	.131	3			
BOD	Between Groups	2.766	3	.922	.	.
	Within Groups	.000	0	.		
	Total	2.766	3			
DO	Between Groups	.071	3	.024	.	.
	Within Groups	.000	0	.		
	Total	.071	3			
AN	Between Groups	.002	3	.001	.	.
	Within Groups	.000	0	.		
	Total	.002	3			
TSS	Between Groups	2.000	3	.667	.	.
	Within Groups	.000	0	.		
	Total	2.000	3			
COD	Between Groups	15770.750	3	5256.917	.	.

Table 4.2 (Continued)

ANOVA						
		Sum of Squares	dF	Mean Square	F	Sig.
	Within Groups	.000	0	.		
	Total	15770.750	3			

From Table 4.2, the ANOVA analysis were done to identify the statistically significant difference between group mean. The significance value for all parameters showed in the table is 0, which is below 0.05 which means there is a statistically a significant difference in mean for all parameters of water quality.

Table 4.3 Correlation matrix for water quality

Correlations								
		pH	Temp	BOD	DO	NH_3-N	TSS	COD
pH	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	4						
Temp	Pearson Correlation	.931	1					
	Sig. (2-tailed)	.069						
	N	4	4					
BOD	Pearson Correlation	.386	.685	1				
	Sig. (2-tailed)	.614	.315					
	N	4	4	4				
DO	Pearson Correlation	.931	.995**	.651	1			
	Sig. (2-tailed)	.069	.005	.349				
	N	4	4	4	4			
NH_3-N	Pearson Correlation	-.307	-.393	-.597	-.304	1		
	Sig. (2-tailed)	.693	.607	.403	.696			
	N	4	4	4	4	4		
TSS	Pearson Correlation	-.144	-.117	.221	-.212	-.864	1	
	Sig. (2-tailed)	.856	.883	.779	.788	.136		
	N	4	4	4	4	4	4	
COD	Pearson Correlation	-.441	-.524	-.650	-.441	.989*	-.783	
	Sig. (2-tailed)	.559	.476	.350	.559	.011	.217	
	N	4	4	4	4	4	4	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

From Table 4.3, the strongest correlation is between NH_3-N and COD with r = -0.117 where the correlation closer to 0. It was based on N= 4 samples and its 2-tailed significance was, p = 0.883. This mean that there are lower chances in finding the samples to correlate since the correlation is significant at 0.05 level when the population correlation is zero.

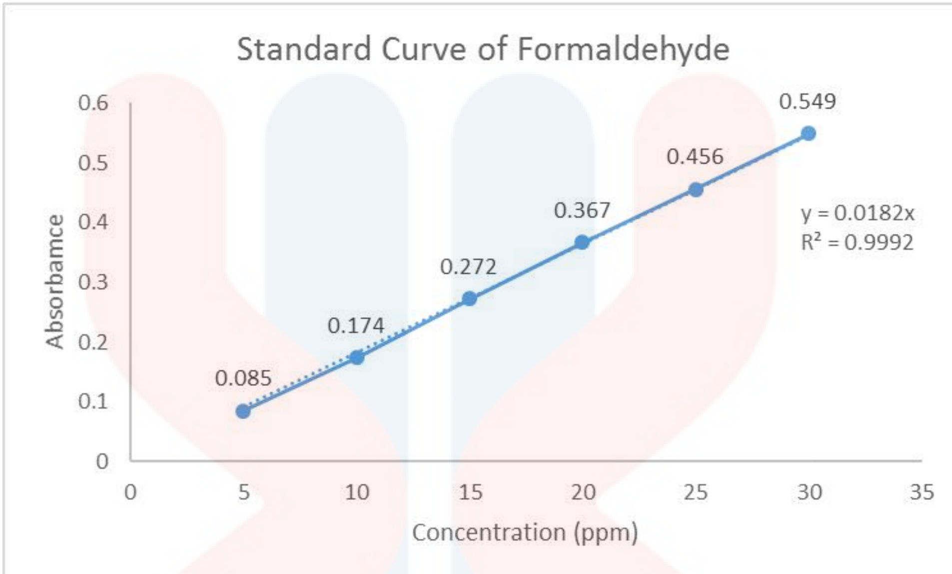

Figure 4.8 Calibration line of spectrometer method

Six standard solution were prepared with different concentration; 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm respectively to plot calibration curve to identify the absorbance value for each concentration. The transformation of colour started once the samples were added to the culture tubes together with concentrated sulphuric acid (H_2SO_4) and prepared CA 5% left heated in the water bath for one hour and cooled in room temperature.

Table 4.4 Absorbance and concentration of formaldehyde found in water samples

Sample	Absorbance at 412 nm	Concentration (ppm)
Sample 1	0.81	44.50
Sample 2	1.03	56.60
Sample 3	1.15	63.19
Sample 4	1.29	70.88

The standard curve was prepared to aid the identification of unknown concentration of formalin in samples collected as showed in Table 4.4. The R^2 showed the value of 0.9992% for the accuracy for the unknown samples. By substituting the obtained absorbance at fixed wavelength, the concentration in unit of ppm were calculated for four samples. H_2SO_4 used in the methods acted as oxidizing agent due to its sensitivity and independence to the present of oxygen (Fagnani, Pezza, & Pezza, 2003).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Findings of study

The objectives of this study to identify the presence of formaldehyde in groundwater from Kampung Darat Demit, Kubang Kerian. The reaction of formaldehyde with chromotropic acid in acid solution; sulphuric acid, formed an adduct consist of methylene bridged that produce from the joining of a single formaldehyde molecule with two molecules of chromotropic acid. The adduct then showed the same chromogen that have same result in spectroscopy. From the result, the concentration of formaldehyde detected were varies from all four samples from 44 ppm to 70 ppm which are not safe because the values already exceeded the threshold amount allowed for exposure which is 1.13 ppm. Therefore, direct exposure will cause immediate flaming to body parts and increase the risk of getting chronic illness due prolong expose to the sources.

For the water quality parameters, the values obtained for all parameters showed an average water quality within Class II to III. It was difficult to determine whether the water was polluted or not since each parameter have different range of index and some results were not interrelated with each other. The BOD, COD and DO supposed to have interconnected result as they are related but the high values of BOD from the same sample had higher concentration COD and DO which were unrealistic. Therefore, there were possibilities errors during storage or parallax errors during practical. In order to control the error, it is necessary to take precaution steps each time carrying out the sampling process and lab work. Besides, the influence of external factors such as sudden change in temperature or unstandardized samples collection time also affected the water condition. Samples can be sensitive to the changes in the environment like temperature because a slight difference can disturb the biological and chemical processes that happened on the water.

5.2 Limitation of study

In this study, there was a difficulty in determining the suitable method for formaldehyde identification. Most all of the existed methods were written from the researchers from the last century that still haven't been revised or improved. Due to the limited access to some journals and the insufficient equipment and chemicals to carry out the study, the accuracy of the results obtained were uncertain. There is possibility that the methods were no longer valid and suitable to be adapted.

5.3 Recommendation

For the recommendation, the need of better methodology conducting research in formaldehyde. As the technology have been developed, there should be more methods introduced to increase the efficiency and produce faster and more accurate result for the detection of formaldehyde.

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