Identification of formaldehyde in groundwater using UV-Vis spectrometer

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Abstract. This research aims to identify the concentration of formaldehyde in groundwater by using UV-Vis spectrophotometer and to assess the physico-chemical parameters of water quality in groundwater. The samples were collected from a cemetery of Kampung Darat Demit, Kubang Kerian, Kota Bharu where the formalin-based specimen/pathological waste 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 20th 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 presence of formaldehyde in water may has significant effect on the water quality parameters physically, biological and chemically.

1. Introduction

The Hospital Universiti Sains Malaysia, Kubang Kerian Kelantan has conducted a new protocol for pathological waste management through the burial method. The conventional method which is the incineration is not a proper method to manage organs especially for Muslims as it is contradictory in ethics. Although it has been hailed as an effective method and adopted in Malaysia, incineration procedure carries with it potential threat to the environment as it emits toxic by products such as dioxins, furans and others. Long term exposure poses various health complications to humans and animal such as liver failure and cancer. Hence, the burial method was introduced. Recent years have seen increasing demands from Hospital USM’s patients and their relatives to choose for deep burial procedure. The measurement for the deep opening in the ground is 7 feet (length) x 3 feet (width) x 7 feet (depth), which the specification of the dimension has been hypothesized to prevent unpleasant odors that may encourage animals to access to the burial site. The issues come out when approximately 100 kg of organs will be buried on the site once in two months and the possibility to affect the quality of groundwater at Kampung Darat Demit Muslim cemetery, Kubang Kerian, Kelantan (beside Hospital USM Kubang Kerian). 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, spectrophotometry method was used to identify the concentration of formaldehyde in water.
2. Materials and Methods

The water samples were collected from one point, which is a well (represents underground water) that was located in Kampung Darat Demit Muslim cemetery site, near Hospital USM, Kubang Kerian. Samples were taken at one point for four consecutive weeks at the same time range to ensure the data accuracy for all samples. In-situ data such as pH, conductivity, salinity, dissolved oxygen (DO) and temperature were collected by using a YSI multiparameter. The samples were then chilled in an icebox filled with ice to slow down the microbial activities in the water that might affected the result accuracy.

2.1. Physico-chemical analysis.

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. A HACH LBOD Intellical™ probe was used to read the initial BOD concentration from 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.

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 cooled in room temperature. Next, the vial was inserted into HACH UV VIS for reading.

For Total Suspended Solid (TSS), the water sample was mixed 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.

The identification for ammonia nitrate was conducted by transferring 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 [1].

2.2 Formaldehyde Determination Analysis

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 5 g 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. 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 concentrated sulphuric acid was added and inverted. The tubes were 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 and blank solution were then transferred into 1.5 mL cuvettes for absorbance reading using UV-Vis reading at 412 nm wavelength. 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.

2.3 Statistical Analysis

A statistical analysis was done to reduce the range of uncertainty in water samples. 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 20\textsuperscript{th} software version.

3. Results and Discussion
Among the water quality assessment, the measurements of physico-chemical conditions have been regarded as one of the common practices that address the water status. 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 1). 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. In this study, they comprise of temperature, conductivity, total dissolved solid (TSS), ammonia nitrate, salinity, dissolved oxygen (DO), COD, BOD and pH measurement collected at each sampling sites were summarized in Table 2.

| Parameter                  | Unit    | Class          |
|----------------------------|---------|----------------|
| Ammoniacal Nitrogen        | mg/l    | I 0.1 IIA 0.3 IIB 0.3 III 0.9 IV 2.7 V > 2.7 |
| Biochemical Oxygen Demand  | mg/l    | I 1 IIA 3 IIB 3 III 6 IV 12 V > 12 |
| Chemical Oxygen Demand     | mg/l    | I 10 IIA 25 IIB 25 III 50 IV 100 V > 100 |
| Dissolved Oxygen           | mg/l    | I 7 IIA 5-7 IIB 5-7 III 3-5 IV < 3 V < 1 |
| pH                         | -       | 6.5 - 8.5 IIA 6 - 9 IIB 6 - 9 III 5 - 9 IV 5 - 9 V - |
| Electrical Conductivity*   | uS/cm   | 1000 IIA 1000 IV 6000 V - |
| Salinity                   | ppt     | 0.5 IIA 1 IV 2 V - |
| Total Dissolved Solid      | mg/l    | 500 IIA 1000 IV 4000 V - |
| Total Suspended Solid      | mg/l    | 25 IIA 50 IIB 50 III 150 IV 300 V 300 |
| Temperature                | ºC      | Normal + IIA Normal + IIB Normal + IV - V - |
| Turbidity                  | NTU     | 5 IIA 50 IIB 50 IV - V - |
Table 2. Water Quality result of four samples

| Sample | pH | Temperature (°C) | BOD (mg/L) | DO (mg/L) | NH₃-N (mg/L) | TSS (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     |

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. The heat was generated from bond breaking which caused an increase in temperature that become one of factor that affected the pH of water [3]. Since the solvation process cannot be controlled, 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. Water temperature is a crucial factor in water quality assessment as all water chemistry, biochemical processes are functions of temperature. 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 [4]. 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.

Biochemical Oxygen Demand is important in representing the amount of oxygen used by 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. 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. 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 [5]. 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.
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, all four water samples from the same point were categorized as Class IV based NWQS. The DO in water is influenced 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 and also process called photochemical between formaldehyde and water molecules that induce the formation in OH radical and O3 [6].

NH₃-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. The highest NH₃-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 I 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 [7]. The result shown that the NH₃-N concentration for all samples were within the limit by World Health Organization (WHO).

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 explained 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. Most of solids made up from inorganic matter, silt, sediments, and some algae or bacteria. 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.

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 82.25±36.25 mg/L for all samples. 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. The depletion of DO trigger anaerobic conditions that are threatening to the aquatic life. Several factors can alter the value to high falsely COD reading such as the presence of foreign chloride in distilled water and microorganism in deionized water used in the laboratory.

The standard curve was prepared to aid the identification of unknown concentration of formalin in samples collected as showed in Table 3. H₂SO₄ used in the methods acted as oxidizing agent due to its sensitivity and independence to the present of oxygen [8]. 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 [9]. 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 seafood, 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.

### Table 3. 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               |

From Table 4, the ANOVA analysis was done to identify the statistically significant difference between group mean. The significance value for all parameters showed in the table is below than 0.05 which means there is a statistically significant difference in mean for all parameters of water quality. From Table 5, 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.

### Table 4. 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 | . |
|             | Within Groups        | .000  | 0 | .     |      |
|             | Total                | 15770.750 | 3 |       |      |
Table 5. Correlation matrix for water quality

|       | Correlations       | pH     | Temp | BOD   | DO    | NH$_3$.N | TSS  | COD   |
|-------|--------------------|--------|------|-------|-------|----------|------|-------|
| pH    | Pearson Correlation| 1      |      |       |       |          |      |       |
|       | Sig. (2-tailed)    | 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| 1     |
|       | Sig. (2-tailed)    | .559   | .476 | .350  | .559  | .011     | .217 |       |
|       | N                  | 4      | 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).*

4. Conclusion

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 presence of formaldehyde in water samples may have significant effect on the water quality parameters physically, biological and chemically.

References

[1] Matta G, Srivastava S and Pandey R R 2017. Assessment of physicochemical characteristics of Ganga Canal water quality in Uttarakhand. Environ Dev Sustain, 19, 419–31.

[2] Fagnani E, Pezza L and Pezza H R 2003. Chromotropic acid?formaldehyde reaction in strongly acidic media. The role of dissolved oxygen and replacement of concentrated sulphuric acid. Talanta, 60(1), 171–76.

[3] Ashida K and Iwasaki K 1995 Handbook of Plastic Foams Types, Properties, Manufacture and Applications. (A. Landrock, Ed.). New Jersey.

[4] Ben-Amotz D, Raineri F and Stell G 2005 Solvation Thermodynamics: Theory and Applications. The J. Physic. Chem. B, 109(14), 6866–78.

[5] Ortega-Atienza S, Rubis B and McCarthy C 2016 Formaldehyde Is a Potent Proteotoxic Stressor Causing Rapid Heat Shock Transcription Factor 1 Activation and Lys48-Linked Polyubiquitination of Proteins. The J. America Pathol., 186(11), 2857–68.

[6] Wang C, Zhu B, He L Y and Han Y 2017 Sources and Potential Photochemical Roles of Formaldehyde in an Urban Atmosphere in South China. J. Geophysic. Res. Atmospheres, 122(21), 11934–47.
[7] Lazzaroni S, Profumo A, Buttafava A and Serpone N 2015 Formation of Hexamethylenetetramine (HMT) from HCHO and NH₃ – Relevance to Prebiotic Chemistry and B3LYP Consideration Formation of Hexamethylenetetramine (HMT) from HCHO and NH₃ – Relevance to Prebiotic Chemistry and B3LYP Consideration. Origins of Life and Evol. of Biospheres, (46), 223–31.

[8] Fagnani E, Pezza L and Pezza H R 2003 Chromotropic acid-formaldehyde reaction in strongly acidic media. The role of dissolved oxygen and replacement of concentrated sulphuric acid. Talanta, 60(1), 171–76.

[9] Kieber R J, Rhines M F and Willey J D 1999 Rainwater formaldehyde: concentration, deposition and photochemical formation. Atm. Environ., 33(22), 3659–67.