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Determination of Formaldehyde from Disposal of Formaldehyde Fixed Biological Specimen Buried in Soil

(Penentuan Formaldehid daripada Spesimen Biologi Formaldehid Kekal yang Tertanam dalam Tanah)

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ABSTRACT

Biomedical waste specifically anatomical specimens and body parts will be incinerated by a local incineration facility. However, the incineration of formaldehyde fixed specimen from hospitals poses hazardous effect to human and environment due to an exposure of highly toxic gases such as dioxins and furans. In addition, this practise is considered as non-shariah compliance by Muslim community. Thus, a safer and shariah-compliance option to dispose anatomical specimens through deep burial has been introduced. The concern has been raised on the side effect of the formaldehyde treated specimen to the environment. Formaldehyde is used widely for preservation of surgical and anatomical specimens. The formaldehyde toxicity specifically on the soil, soil water, soil animals and plants should be considered after the burial of the anotamical specimens. Thus, the aim of this study was to investigate the side effect of formaldehyde on soil after the burial of formalin fixed specimen on the environment. In this study, the amount of soil elemental distribution and formaldehyde concentration of pre-burial and post-burial of biological specimen were evaluated by using Energy Dispersive X-Ray Fluorescence (EDXRF) and Ultraviolet-Visible Spectrophotometer instrument, respectively. For EDXRF analysis at Point C, soil elemental distribution after burial of dead biological specimens has higher concentration compared to before the burial. The concentration of formaldehyde at Point C was higher after the burial of dead biological specimen compared to before burial, which exceeds the tolerable concentration recommended by the World Health Organisation (WHO).

Keywords: Burial; formaldehyde; formalin; soil

ABSTRAK

Sisa bioperubatan terutamanya spesimen anatomi dan bahagian tubuh akan dibakar oleh kemudahan pembakaran setempat. Walau bagaimanapun, pembakaran spesimen yang dirawat dengan formaldehid daripada hospital boleh menimbulkan kesan berbahaya kepada manusia dan persekitaran disebabkan oleh pendedahan gas yang sangat toksik seperti dioksin dan furan. Tambahan pula, amalan ini dianggap sebagai tidak patuh syariah bagi komuniti Muslim. Oleh itu, pilihan yang lebih selamat dan patuh syariah untuk membuang spesimen anatomi melalui penanaman telah dicadangkan. Isu yang membimbangkan adalah kesan formaldehid pada spesimen yang dirawat kepada alam sekitar. Formaldehid digunakan secara meluas bagi pengawetan spesimen anatomi dan bedah. Ketoksikan formaldehid terutamanya ke atas tanah, air tanah, organisma dan tumbuhan pada tanah harus dipertimbangkan selepas penanaman spesimen anatomi telah dilakukan. Tujuan kajian ini adalah untuk mengkaji kesan sampingan formaldehid kepada tanah selepas penanaman spesimen anatomi. Dalam kajian ini, jumlah pengagihan unsur dalam tanah dan kepekatan formaldehid sebelum dan selepas penanaman spesimen telah ditentukan menggunakan Serakan Tenaga Pendarfluor Sinar-X (EDXRF) dan Sinar Ultra-ungu Boleh Nampak Spektrofotometer (UV-Vis). Dalam analisis EDXRF pada titik yang terdekat dengan penanaman (Titik C), didapati pengagihan unsur tanah selepas penanaman spesimen anatomi lebih tinggi berbanding sebelum penanaman. Kepekatan formaldehid pada Titik C juga lebih tinggi selepas penanaman spesimen anatomi berbanding sebelum penanaman, iaitu melebihi kepekatan yang dicadangkan oleh Organisasi Kesihatan Sedunia (WHO).

Kata kunci: Formaldehid; formalin; penanaman; tanah

INTRODUCTION

In general, biological specimens are referred to as anatomical specimens and they were burnt by local incineration facilities (Human Tissue Authority 2015). This specimen has been preserved using formaldehyde. However, the process of incineration of biological specimens treated with formaldehyde in most hospitals gave harmful effects to humans, animals, plants and the environment. 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 (Emmanuel 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 occurred. 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 and contribute further to the air pollution (Emmanuel et al. 2004).

The methods by which biological specimens can be respectfully disposed are also very limited. Therefore, the economic way to remove specimen remains is required. In this study, environmentally friendly burial method into the soil was introduced. Incineration method was found to be non-sharia and contrary to the way of life of the Muslim community (Kandoli et al. 2019; Laurent et al. 2013; Scalenge & Pantani 2020). Therefore, the study of the effects of formaldehyde 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.

MATERIALS AND METHODS

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

Two soil samples were taken near the biological specimen disposal site containing formaldehyde at different point, while one sample was taken 2 km away from the biological specimen disposal area containing the formaldehyde material. Each of the soil samples was taken at 20 cm depth. The soil sample was collected before and after the disposal of the biological specimen. Then, this soil sample was dug by using auger with a depth of 20 cm. After that, the soil samples were put into a zip lock bag and labelled as point A, B, and C, where Point C was the closest point to the burial site. The pH and moisture content of the soil samples were also measured by using pH and moisture meter. These soil samples were taken to the laboratory to maintain or preserve its composition in the refrigerator at cool temperature (4 °C).

35 g of soil sample was transferred into an aluminium foil. The soil sample was dried in air-oven at 80 °C within a day of 24 h. 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. To prepare the finer particle size of soil sample, it was placed in grinder machine. Soil elemental distribution of all soil samples before and after the burial of the biological specimen treated by formaldehyde was analysed by using XRF instrument.

15 soil samples were crushed into small particles by using mortar and pestle. 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. About 3.0 mL of 96% concentrated sulphuric acid solution and 300 µL of 5% chromotropic acid solution were mixed together with soil samples that were weighed earlier and warmed-over boiling water bath for 1 h at 100 °C (Georghiou & Ho 1989). Then, it was centrifuged to get the supernatant. 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 (Georghiou & Ho 1989).

Stock solution of formaldehyde (1000 mg/L) was prepared by diluting 2.5 mL of 37% formaldehyde solution with distilled water. Standard series solution (20, 40, 60, 80, and 100 ppm) was also prepared. 412 nm reflectance was used as the wavelength for formaldehyde. 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.

RESULTS AND DISCUSSION

The *in-situ* pH measurement of soil samples has been conducted at three sampling points. Referring to Table 1, the results show that the highest pH value is 7, where almost every samples taken, gave the reading of pH 7. The lowest recorded pH value was 5, which located at Point A sample on the fifth sampling. 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 in the fourth and fifth sampling which showed acidic reading of pH 5 (Point A). Point A which is loamy soil recorded moisture content ranging from 4-6, meanwhile Point B and C recorded moisture content reading 1 for all samplings. It indicated that both point B and C were dry compared to point A. Loamy soil (Point A) has the highest content of organic matter with the reading of 16.44% and the lowest is recorded at Point B (sandy soil) with 2.99% organic matter.

TABLE 1. pH/moisture content/organic matter of soil samples

Sampling	pH Values/Moisture content/organic matter (%)		
	Point A (Loamy soil)	Point B (Sandy soil)	Point C (Sandy soil)
First (Pre-Burial) 26/9/2018 2.00 pm	6/4/12.81	7/1/8.83	7/1/8.64
Second (Post-Burial) 27/12/2018 11.30 am	6/6/12.99	7/1/7.58	7/1/3.72
Third (Post-Burial) 3/4/2019 5.30 pm	6/4/12.94	7/1/2.99	7/1/9.21
Fourth (Post-Burial) 23/7/2019 9.00 am	5/6/15.75	7/1/5.03	7/1/8.13
Fifth (Post-Burial) 3/10/2019 7.42 am	5/5/16.44	7/1/6.74	7/1/8.24

The *ex-situ* XRF analysis of soil samples were conducted at Point C. The interaction of the organic substances and mineral in the soil are very complex. Elemental distribution of the soil was characterized at the burial plot pre- and post-burial. As stated earlier, Point C was the closest point to the buried specimens. Table 2 shows XRF analysis of the elemental distribution in soil samples Point C from first to fifth sampling. From the result, most elements showed significant increase in concentration (mgkg^{-1}) from first sampling (pre-burial) to fifth sampling (post-burial). Soil elemental distribution of post-burial of biological specimen namely Al, Cr, Cu, Fe, K, Mn, Ni, Pb, Rb, Si, Sr, Ti, Zn, and Zr showed increment in concentration compared to pre-burial of biological specimen. On the other hand, elements of Ba, Ca, Cl, I, P, and Te decreased in concentration over time from pre-burial to post-burial of biological specimen. Generally, the data demonstrated that the values of heavy metal detected in this study were within the safe limit recommended by US EPA Regulatory (Environmental Research Laboratory. Solid and Hazardous Waste Research Division 1983). Based on Table 2, heavy metal such as Cr, Cu, Ni, Pb, and Zn were still under acceptable limit. These metals are highly toxic even in trace quantity. However, titanium is not considered as a toxic metal even though it is heavy metal, and it does have serious negative health effects. Previous studies have shown an increase in the concentration of heavy metals in the cemetery (Barros et al. 2008). For instance, Santa Candida municipal cemetery in Brazil was found to be polluted by 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 et al. 2008). In the previous study, Aphane (2018) has confirmed that the concentration of elements increased in the area of the cemetery compared to the non-cemetery area. 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.

TABLE 2. Elemental distribution by using XRF in soil sample collected from Point C (the nearest sampling point with the burial location)

Formula	Atomic Number	Element Concentration in Each Sampling (mgkg^{-1})					US EPA Regulatory Source: Environmental Research Laboratory. Solid and Hazardous Waste Research Division (1983)
		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.

N.D: not detected

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 concentrations were acceptable by the US EPA Regulatory. Jonker and Olivier (2012) has analysed the distribution of trace metals in cemetery soils of the Zandfostein burial site in South Africa. The study had demonstrated that the concentration of trace elements (Li, Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Sn, Sb, Te, Cs, Ba, La, W, Pt, Hg, Tl, Pb, Bi, U) 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 et al. (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 highly mobile in large quantities. Overall, all the elements obtained from XRF analysis decreased in concentration over time from the last sampling except for aluminium.

The presence of formalin was tested by using Ultraviolet-Visible (UV-Vis) Spectrophotometer. The absorbance of 15 soil sample solutions and the absorbance of standard solution are illustrated in Table 3 and Figure 1. The *ex-situ* formalin analysis of soil samples was conducted at three sampling points. Referring to Table 3, 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 1 shows that the concentration of formalin before burial seems lower than after burial for every sampling. Formalin concentration for each Point A, B, and C appears to rise on the second sampling and begins to drop gradually for subsequent sampling.

TABLE 3. 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

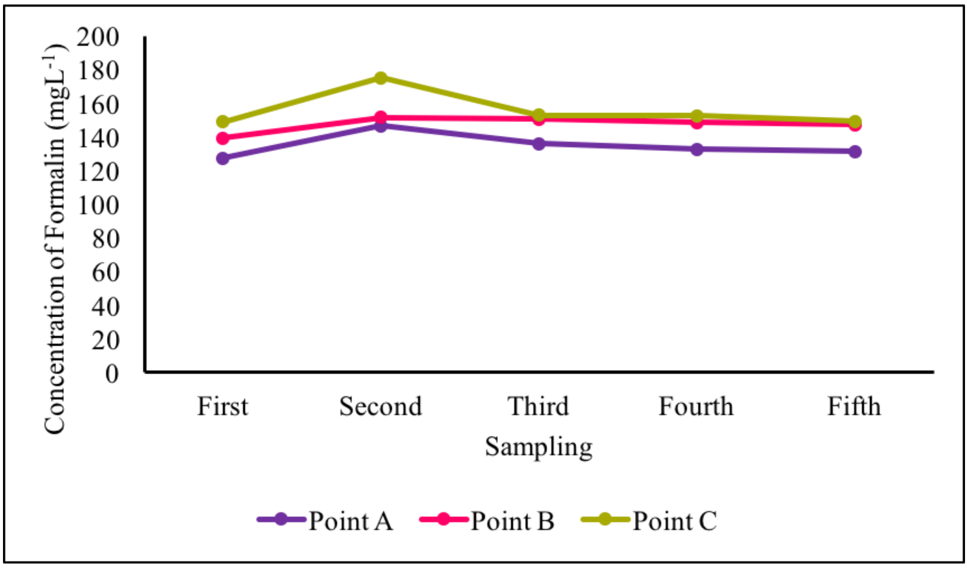

FIGURE 1. The concentration of formalin in soil samples (mgL^{-1})

Table 4 shows a statistical difference analysis among soil samplings and absorbance values for formaldehyde concentration. The significant difference ($p<0.05$) was found between sample A and B, and also between A and C. A study conducted by van Allemann et al. (2018) has proven that the major sources of soil or water pollution in the cemetery area 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 formaldehyde 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 1 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. 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 was leached in the sandy soil at the 24 weeks was 140.2 mgL^{-1}, which was lower compared to the highest concentration of formalin that was found in this study (van Allemann et al. 2018). The highest concentration of formalin was recorded after 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 (van Allemann et al. 2018). The Material Safety Data Sheet of Polypropylene (Plastic) states that plastics are usually made of polypropylene and contain traces of formaldehyde. On the other hand, World Health Organization (2002) also suggests that formaldehyde is a natural occurring substance in small concentrations.

TABLE 4. Statistical difference analysis among soil sampling and absorbance value for formaldehyde concentration

	A	*B*
Mean	2.6875	2.94225
Variance	0.018561	0.001585
Observations	4	4
Hypothesized Mean Difference	0	
df	4	
t Stat	-3.58964	
P(T<=t) one-tail	0.011485	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.022969	
t Critical two-tail	2.776445	

	B	*C*
Mean	2.94225	3.1005
Variance	0.001585	0.055353
Observations	4	4
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.3264	
P(T<=t) one-tail	0.138322	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.276644	
t Critical two-tail	3.182446	

	A	*C*
Mean	2.6875	3.1005
Variance	0.018561	0.055353
Observations	4	4
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.0382	
P(T<=t) one-tail	0.014405	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.02881	
t Critical two-tail	2.570582	

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 has concentration of formalin too. Besides, previous study has proven that the total amount of formaldehyde leached from loamy soil much lower compared to sandy soil (van Allemann et al. 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 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 the other hand, according to a report released in 2002 by the World Health Organization, 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. Based on the result, formalin concentration was starting to drop from second to third sampling and followed by fourth and fifth sampling. From another point of view, van Allemann et al. (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 formaldehyde 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 2004). Hart and Casper (2004) reported 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 et al. 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 2004).

Conclusion

In this study, the amount of soil elemental distribution and formaldehyde concentration of pre-burial and post-burial of biological specimen were evaluated by using Energy Dispersive X-Ray Fluorescence (EDXRF) and Ultraviolet-Visible Spectrophotometer instrument, respectively. The results showed that the soil fertility in terms of pH at Point C (closest point to burial plot) indicates neutral pH 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 (XRF) 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 (US EPA) 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 and 148.83 mgL^{-1}, respectively, which exceeds the tolerable concentration recommended by the World Health Organisation (WHO). However, the formalin concentrations were found to be decreasing with time due to its high mobility in soil and water.

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