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NEUTRALIZE BACTERIAL ACTIVITY WITH ANATOMICAL EMBALMING SOLUTIONS

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Abstract

Formaldehyde had some degree of toxicity in the human body as a cadaver preservation solution. Recent studies showed that moderate formaldehyde levels in cadavers could neutralize SARS-CoV-2. However, other effects of formaldehyde levels in the air are not yet known on bacteria. This study aims to determine the optimal level of formaldehyde that can be used to neutralize bacteria and is safe for humans. This study used a post-test control group design with formaldehyde level as the independent variable and bacterial colonization as the dependent variable. The results showed that the mean levels of formaldehyde with the bacterial colony respectively were P1 (1.378 ± 0.716; 0.40 ± 0.10), P2 (0.347 ± 0.038; 1.40 ± 0.10), P3 (0.137 ± 0.006; 2.40 ± 0.10), P4 (0.042 ± 0.005; 3.32 ± 0.09), and P5 (0.009 ± 0016; 4.40 ± 0.10). The statistical analysis results revealed a value of $p<0.05$, which indicated that the higher the formaldehyde level, the higher the ability to neutralize bacterial activity. The study concluded the optimum level for neutralizing bacterial activity safe for humans is about 0347 ± 0038 mg/m3.

Keywords: *anti-bacterial; formaldehyde level; environmental health; health risk*

1. Introduction

Anatomy in the context of medicine or health sciences often involves dissection (Boscolo-Berto et al., 2021). The use of donated human cadavers in laboratory learning activities in anatomy education departments is often described as a gold standard resource to support students understanding of anatomy (Thompson, Green, Scotcher, & Keenan, 2022). Dissection anatomy is the procedural and energetic study of human tissues and organs by cutting them into body parts and organs in a manner that facilitates structural identification and examination (Owolabi, Tijani, & Ihunwo, 2022). The use of formaldehyde for the fixation of cadavers in the dissection laboratory is an integral part of absorbent fluids (Aung et al., 2021). The benefits of using formalin in terms of cost-effectiveness, adequate immobilization, and efficient maintenance, should outweigh the adverse health effects of the user. Provide the use of personal protective equipment and raise awareness of the harmful effects (Bhat, Chittoor, Murugesh, Basavanna, & Doddaiah, 2019). Formaldehyde is a highly reactive toxic colorless alkaline environmental pollutant used in a variety of industries and products. Inhalation of formaldehyde in humans causes genotoxic effects such as the formation of reactive oxygen species and DNA damage (Kang et al., 2022). From formaldehyde to respiratory cancer

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exposure sensory irritation can have serious health consequences (Adamović et al., 2021). In a 2-year inhalation study conducted nearly 40 years ago, formaldehyde increased the incidence of frontal squamous cell carcinoma in mice by 50-fold after lifetime exposure of 6 hours/day and 5 days/week (Andersen et al., 2019). Formaldehyde produced in nature is essential for life and essential for carbon transfer reactions. However, formaldehyde from external sources (exogenous formaldehyde) can take the form of methanol and in vivo concentrations exceed normal physiological levels (Albertini & Kaden, 2017). The World Health Organization (WHO) has established air quality guidelines of approximately 0.1 mg/m3 for a 30-minute exposure to formaldehyde (Nielsen, Larsen, & Wolkoff, 2017). According to previous studies, the level of exposure to formaldehyde should be less than 0.614 mg/m3 (Lin et al., 2021). The amount of formaldehyde in cut labs can be higher than normal. It is proof based on the feedback of students that they always face eye irritation while studying.

In addition to the negative effects of using formaldehyde, there is a positive effect namely formaldehyde can neutralize microorganisms. Previous studies have reported that formaldehyde can destroy the SARS-CoV-2 virus-validated cytotoxicity test for cadaveric donation. A representative solution in this study was generated using a series of 1:2 to 1:256 dilution ratios from 1 to 20. Interestingly the result is a low concentration of formaldehyde due to its low virus-neutralizing ability but the main objective of this study was to identify a solution that is non-toxic to humans (Quondamatteo et al., 2021). Note that in almost all scenarios/activities studied the known carcinogen formaldehyde was found at higher levels in the air than outside. Therefore abatement or targeted reduction strategies should be implemented when formaldehyde cannot be eliminated or replaced (Cammalleri et al., 2022). The effects of formaldehyde on airborne microbes are yet known that shown the indoor bacterial population (using glass containers as mimic) potentially creating bacterial communities that pose a greater risk to human health over time during formaldehyde exposure (Guo et al., 2021). However, it is still unknown how formaldehyde exposure affects airborne bacteria predominance and whether specific formaldehyde levels are safe for people whilst decreasing bacterial presence. The author then researched to determine the effect of formaldehyde levels on bacterial activity. The author assumes that the level of formalin in the air affects the activity of bacteria so the higher the concentration of formaldehyde the greater the ability to neutralize bacteria.

2. Method

This study was a true experimental design with a post-test control group. The independent variable in this study was the concentration of formaldehyde in the air and the number of bacterial colonies. The investigation was carried out by measuring the number of samples of formaldehyde and bacteria at the same place and time. The sampling area is divided into five sites (S) according to the distance from the formaldehyde pool which determines the continuous exposure level with simple random sampling technique. S1 is the closest position and S5 is the farthest position to the formaldehyde source (cadaveric's tubs). Each site were separated from the next by a distance of 7 meters. Measure formaldehyde levels using a digital air quality monitor resulting in mg/mm3 and sample airborne bacteria using nutrient agarous plate colony. In order to collect air samples, a petri dish must first be filled with nutrient agarous, a pure bacterial culture medium, and then the media must be placed in one of five locations (S1–S5) with open conditions. Following a 10-minute sampling period, samples were aseptically kept in an incubator. Total repetitions that we used each site were five, so there were 25 data in total.

The instruments and supplies used in this study were broken down into the following categories: (1) formaldehyde levels were measured using auto-detector formaldehyde tools, (2) airborne bacteria were captured using nutrient agarous plates in a petri dish, (3) the captured bacteria were then incubated in an incubator set to 37 degrees, and (4) bacterial colonies were counted using colony counter tools.

Data collection was done from January to April 2020. This research was conducted in the Anatomy Laboratory at the Faculty of Medicine

Universitas Surabaya. Statistical analysis was done used the non-parametric Kruskal-Wallis test. The software used for analysis was SPSS version 25.0.

3. Result and Discussion

Levels of Formaldehyde

Descriptive analysis showed that formaldehyde was proportional to the sites. S1 has the highest formaldehyde level and then gradually decreases to S5 which has the lowest formaldehyde level, as shown in Figure 1.

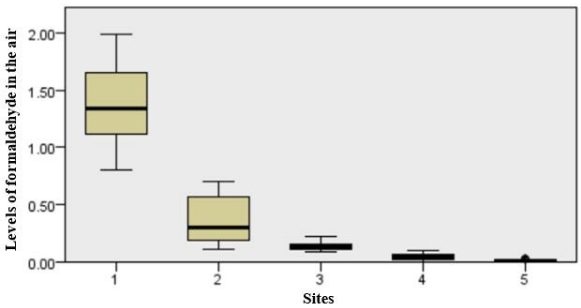

Figure 1. *Boxplots of Formaldehyde Levels and Sites*

The mean formaldehyde levels in S1 were 1.378±0.071, S2 0.347±0.038, S3 0.137±0.006, S4 0.042±0.005, and S5 0.009±0016, respectively. The results of the descriptive analysis of formaldehyde content are shown in Table 1.

Table 1. Descriptives Analysis of Formaldehyde Content

Sites (S)	Descriptives				
	Mean±SE	Median	SD	Min.	Max.
1	1.378±0.07	1.342	0.35	0.809	1.994
2	0.347±0.03	0.295	0.19	0.109	0.708
3	0.137±0.00	0.133	0.03	0.089	0.216
4	0.042±0.00	0.038	0.02	0.002	0.098
5	0.009±0.00	0.006	0.00	0.000	0.034

The normality test showed that there were significant values of $p<0.05$ for many groups implying that the assumption of normality for the data was not met. Therefore, we performed statistical tests using the non-parametric test Krukal-Wallis. Statistical test results showed a significance value of $p<0.05$ ($p=0.000$). This indicates that the hypothesis of research of an effect on formaldehyde levels is accepted, as shown in Table 2.

Table 2. Statistical Analysis of Formaldehyde Content

Sites (S)	Normality Shapiro-Wilk	P value Kruskal-Wallis
1	0.374	
2	0.015*	
3	0.400	0.000*
4	0.222	
5	0.001*	

**The significance level is 0.05*

All groups except S1 ~ S2 and S4 ~ S5 showed significant values following additional tests with Bonferroni correction for multiple tests. Bonferroni test showed adjusted mean values between treatment groups S1 ~ S3, S1 ~ S4, S1 ~ S5, S2 ~ S4, S2 ~ S5, S3 ~ S5 were $p=0.000$, respectively, S3 - S4 was $p=0.027$. This indicates that close-range exposure classification has no significant effect on formaldehyde levels, the further away from the source of formaldehyde, the lower the concentration of formaldehyde, as shown in Table 3.

Table 3. Bonferroni Test of Formaldehyde Content

Sites (S)	1	2	3	4	5
1					
2	0.065				
3	0.000*	0.583			
4	0.000*	0.000*	0.027*		
5	0.000*	0.000*	0.000*	0.665	

**The significance level is 0.05*

Levels of Formaldehyde vs. Colony Bacteria

Detailed analysis showed that the levels of formaldehyde were directly proportional to the ranks of the bacterial colonies. The highest formaldehyde content rate has the lowest colony and vice versa, as shown in Figure 2.

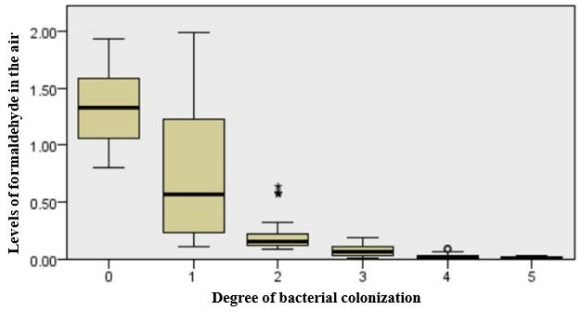

Figure 2. *Boxplot for Formaldehyde Levels and Prevalence of Colonizing Bacteria*

The mean of bacterial colonies were: 0.40±0.10 for S1, 1.40±0.10 for S2, 2.40±0.10 for S3, 3.32±0.09 for S4, and 4.40±0.10 for S5. The results of the descriptive analysis of bacterial colonization are shown in Table 4.

Table 4. Descriptives Analysis of Bacterial Colonization

Sites (S)	Descriptives				
	Mean±SE	Median	SD	Min.	Max.
1	1.40±0.10	0.00	0.50	0.00	1.00
2	1.40±0.10	1.00	0.50	1.00	2.00
3	2.40±0.10	2.00	0.50	2.00	3.00
4	3.32±0.09	3.00	0.47	3.00	4.00
5	4.40±0.10	4.00	0.50	4.00	5.00

The normality test showed a significant value of $p<0.05$ for multiple groups that did not meet the assumption of normality of the data. Therefore, performed statistical tests using the non-parametric Kruskal-Wallis test. The results of the statistical test showed a significant value of $p<0.05$ ($p=0.000$) which indicates that the research hypothesis is accepted, as shown in Table 5.

Table 5. Statistical Analysis of Bacterial Colonization

Sites (S)	Normality Shapiro-Wilk	P value Kruskal-Wallis
1	0.000*	
2	0.000*	
3	0.000*	0.000*
4	0.000*	
5	0.000*	

**The significance level is 0.05*

According to further Bonferroni test results there are significant values in all groups except S1 ~ S2, S2 ~ S3, S3 ~ S4, S3 ~ S5, and S4 ~ S5. Bonferroni test showed adjusted significance between treatment groups S1 ~ S3, S1 ~ S4, S1 ~ S5, S2 ~ S4, and S2 ~ S5 were $p=0.000$, respectively. This indicates that the higher the formaldehyde level, the lower the amount of bacterial colonization, as shown in Table 6.

Table 6. Bonferroni Test of Bacterial Colonization

Sites (S)	1	2	3	4	5
1					
2	0.503				
3	0.000*	0.078			
4	0.000*	0.000*	0.321		
5	0.000*	0.000*	0.384	1.000	

**The significance level is 0.05*

There is research bias based on the results of the study. That is the results between S3 and S5 were not significantly different. Looking at the pattern of significance values S3 - S5 should show a difference but in this case, it did not. Overall however this did not affect the findings of studies that showed that formaldehyde levels affected the extent of bacterial colonization.

Studies have shown that airborne levels of formaldehyde are known to neutralize bacterial activity. Further studies on appropriate levels of bactericidal activity and anti-toxin effects in humans are needed. The recommended level of formaldehyde in the air for humans is less than 0.614 mg/m^3 according to WHO recommendations (Nielsen et al., 2017). A study showing the presence of formaldehyde levels below 0.614 mg/m^3 is S2 with an average of 0.347 ± 0.038 and an average degree of the bacterial colony of 1.40 ± 0.10. This result is the most ideal value for determining the range of formaldehyde levels that are beneficial in neutralizing bacteria but safe for humans. Depending on the data, it showed represent the aggregation results that moving to a slightly larger value the situation is not favorable for the people, but if it moves a little the number of bacterial colonies is high. Formaldehyde is responsible for the observed antimicrobial effect. The results presented here suggest that formaldehyde's strong antimicrobial activity

makes it difficult for bacteria to develop adequate resistance to formaldehyde and that low concentrations of formaldehyde may be safe for use in humans (Nikolic, Mudgil, & Whitehall, 2020). This is consistent with previous studies that showed formaldehyde levels affect SARS-CoV-2 activity in the cadaver (Quondamatteo et al., 2021). Additionally, other studies have reported that formaldehyde concentration and exposure time affected the bacterial community. At 0.054 mg/m^3 the bacterial community composition changed significantly over time (Guo et al., 2021).

Formaldehyde gas significantly reduced the number of viable spores on rare and non-porous materials with two alternatives shown to be comparable. These results provide new comparative information on the use of formaldehyde gas to clean Bacillus anthracis with alternatives on covered surfaces (Stuart, Chewins, & Tearle, 2020). Findings from other studies have shown that the effects of formalin contamination on microbes are also affected by environmental conditions one of which is air temperature. Previous research has shown that low temperatures (approximately 10^0C) reduce the formaldehyde concentration in the air while the formaldehyde concentration at ambient temperature (approximately 22^0C) is still higher than at low temperatures (Choi et al., 2021). A similar study also reported the efficacy of formaldehyde in laboratory decontamination with type BSL-3 by formaldehyde fumigation. Optimizing microbial decontamination in the laboratory is usually performed at a temperature of 48–50^0C. However, there is no human activity during the process for the next 48 hours. Despite the microbial decontamination process will be completed in 2 - 10 minutes (Häcker et al., 2020).

Formaldehyde is a ubiquitous environmental toxin and a key intermediate in one-carbon metabolism (Woolston, Roth, Kohale, Liu, & Stephanopoulos, 2018). Formaldehyde is a product of various metabolic pathways and participates in the carbon cycle providing the synthesis and modification of biological compounds such as DNA, RNA, and amino acids. It plays a role in epigenetic regulation, especially in DNA and RNA methylation and histone demethylation (Li et al., 2021). Cells obtain the energy and molecules they need to sustain life through interconnected biochemical reactions called metabolic pathways. This reaction produces additional reactive metabolites that damage important biomolecules such as DNA and proteins. One of these toxic metabolites is formaldehyde one of the simplest and most reactive aldehydes (Umansky, Morellato, & Pontel, 2021). Formaldehyde reacts with the reduced flavin coenzyme to form a methanolamine intermediate that is used by ThyX for DUMP methylation. Therefore amino acids such as purine and thymidylate do not have a single carbon unit that can be used in different oxidation states for the de novo synthesis of essential DNA nucleotides (Bou-Nader et al., 2021). Maintaining genome stability requires coordination among various subcellular compartments that provide cells with DNA repair systems that protect them from environmental and endogenous stressors. The effect of formaldehyde on genotoxicity due to various parameters such as oxidative stress inflammation and inhalation exposure was evaluated. The main results showed that inhalation exposure resulted in increased NO and inflammatory cell levels. Oxidative stress is evident and indicated by elevated TBARS levels and decreased NPSH levels (Bernardini et al., 2021). Endogenously produced formaldehyde is essential for life and is essential for carbon transfer reactions. However, formaldehyde from external sources (exogenous formaldehyde) which can take the form of methanol can increase in vivo concentrations above normal physiological levels. Both endogenous and exogenous formaldehyde stimulate DNA mono-channels and protein-DNA cross-links (DDX and DPX) while exposure to exogenously generated free formaldehyde causes the DDX and DPX mono channel interaction. Formaldehyde also induces oxidative stress/lipid peroxidations and the resulting reactive aldehydes can cause distal DNA damage (Albertini & Kaden, 2017). Hematopoietic stem cells are highly sensitive to aldehyde-induced DNA damage. Formaldehyde is caused by a defect in the FA repair pathway that removes DNA crosslinking (ICL). The combined loss of FANCD2 (a key protein in this pathway) and ALDH2 (which detoxifies endogenous aldehydes) results in loss of HSC-H2AX (a marker of DNA damage) and high genomic instability (Jung & Smogorzewska,

2021). Formaldehyde imbalances have been linked to many diseases including cancer and neurodegenerative diseases (Nadalutti, Prasad, & Wilson, 2021). Formaldehyde monitoring in living things can lead to biochemical findings of pathological conditions characterized by elevated formaldehyde levels such as cancer levels and neurodegenerative disorders (Lipskerov, Sheshukova, & Komarova, 2022).

Exposure to formaldehyde has an impact on bacterial activity as well. The findings of earlier research demonstrated that exposure to formaldehyde at concentrations of 0.1 and 0.25 resulted in the identification of several species of bacteria, including Clostridia, Bacilli, Sphingobacteria, etc (Guo et al., 2021). The findings of this investigation are consistent with, the greatest formaldehyde concentration measured in the anatomy lab while the covered cadaver was present was higher than the USA-NIOSH suggested ceiling standard of 0.1 ppm but lower than the 0.3 ppm ceiling standard set by the American Conference of Governmental Industrial Hygienists (Ahmed, 2011). So, in order to be considered sanitary, there must be no germs present and a formaldehyde level of at least 0.3 ppm. These circumstances indicate that bacteria are extremely unlikely to be present at a level of 0.3, indicating that bacteria cannot be present at higher levels and vice versa. The study's findings also showed that, at these levels, it is a situation that is relatively safe and appropriate for human respiration in order to prevent it from having too many hazardous consequences.

A limitation of this study is that the independent variable does not directly control the level of formaldehyde required ($x \leq 0.614 \leq y$) but is modified by assuming the distance from the localization point to the source of exposure. However, with the help of advanced technology in the form of formalin detectors, it is still something that can be explained for further measurements.

4. Conclusion and Suggestion

The study concluded that the higher the level of formalin in the air the greater the ability to neutralize bacterial activity and vice versa. In this case for human safety, the optimum level to neutralize bacterial activity and keep it safe for humans is about 0.347 ± 0.038 mg/m3.

One idea for further research development is that researchers could directly monitor formaldehyde levels to effectively determine the optimal average level. Using variations in the dilution solution will produce more valuable data for analyzing formaldehyde levels in the air.

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