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# Analysis of Lead Levels in the Urine of Active and Passive Smokers: Implications for Health Risks

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**ABSTRACT** Cigarette smoke exposes both active and passive smokers to various toxic heavy metals, notably lead (Pb), which poses significant health risks due to its accumulation in the human body. The prevalence of smoking and passive exposure remains high, especially in developing countries, necessitating further investigation into heavy metal exposure levels among these populations. This study aims to quantify lead levels in the urine of active and passive smokers residing in the Wadung Asri vicinity and to analyze the associated health implications. This research employed a descriptive, cross-sectional design with quantitative analysis, utilizing Atomic Absorption Spectrophotometry (AAS) to measure urinary lead concentrations. A total of 20 participants—comprising 10 active and 10 passive smokers—were selected through purposive sampling based on inclusion criteria of minimum three years of exposure, age 20 years or older, and voluntary participation. Urine samples were collected, prepared, and analyzed at the Toxicology Laboratory of the Health Polytechnic Surabaya from October 2022 to May 2023. Data regarding respondents' demographic characteristics and smoking history were gathered through structured questionnaires. The mean lead level in urine among active smokers ranged from 0.0885 µg/mL to 0.1592 µg/mL, with one individual exceeding the recommended threshold of 0.15 µg/mL. Among passive smokers, urinary lead levels ranged from 0.000032 µg/mL to 0.0885 µg/mL, with the highest individual still within the normal limit. The results indicate that active smoking is associated with higher lead exposure than passive exposure, though both groups may be at risk of heavy metal toxicity due to persistent exposure. The findings suggest that cigarette smoking contributes to increased lead accumulation in the human body, thereby elevating health risk profiles for both active and passive smokers. It is recommended to minimize cigarette consumption, enforce smoke-free environments, and conduct further studies on heavy metal exposure. Future research should also investigate additional biomarkers and a broader population to generalize the findings.

**INDEX TERMS** Lead levels, active smokers, passive smokers, urine analysis, heavy metal exposure.

## I. INTRODUCTION

Cigarette smoking remains one of the most significant preventable health hazards worldwide, contributing to a substantial burden of morbidity and mortality [1]. In Indonesia, more than 70% of smokers initiate smoking before the age of 19, with smoking prevalence exacerbated by low public awareness regarding associated health risks [2]. Beyond the direct health effects on smokers themselves, passive or secondhand smoke exposure also poses considerable health threats to non-smokers, including children and pregnant women, due to involuntary inhalation of hazardous substances [3].

Cigarette smoke contains a complex mixture of chemicals, among which heavy metals such as lead (Pb) are especially concerning because of their toxicity and ability to bioaccumulate [4]. Lead exposure can cause neurodevelopmental deficits, cardiovascular disease, nephrotoxicity, and various other health issues [5]. The inhalation of lead particles from cigarette smoke leads to their absorption into the bloodstream and subsequent

accumulation in bodily tissues [6]. Numerous studies have documented elevated blood and urine lead levels among smokers [7], [8], but data specific to urine lead concentrations in active versus passive smokers are limited, particularly in urban community settings.

Recent advancements in analytical techniques, such as Atomic Absorption Spectrophotometry (AAS), have improved the sensitivity and accuracy of heavy metal detection in biological samples [9], [10]. However, the majority of research focuses on blood lead levels, with less emphasis on urinary biomonitoring, which provides an objective reflection of recent exposure [11].

Despite knowledge of cigarette smoke as a source of lead, studies comparing the levels between active and passive smokers within the same environment are scarce, especially in rural or semi-urban communities [12]. Furthermore, identifying the extent of lead bioaccumulation based on demographic factors such as age and smoking duration remains underexplored. There exists a critical need to

quantify exposure levels and assess their potential health impacts comprehensively.

This study aims to quantify urinary lead levels in active and passive smokers residing in Wadung Asri and analyze their respective contributions to body burden. Specifically, it seeks to:

1. Measure and compare urinary lead concentrations between active and passive smokers.
2. Examine the influence of demographic variables, including age and smoking duration, on lead levels.
3. Provide evidence-based recommendations to mitigate heavy metal exposure risks.

The study contributes to public health by filling gaps in biomonitoring data, informing policy on smoking regulation, and establishing a basis for further epidemiological investigations.

The paper is organized into the following sections:

1. Introduction: contextual background, literature review, research gap, objectives, and significance.
2. Methodology: detailed description of study design, sample collection, analysis techniques, and statistical methods.
3. Results: presentation of lead levels, demographic correlations, and statistical analysis.
4. Discussion: interpretation of findings, comparison with existing literature, implications, and limitations.
5. Conclusion: summary of key findings and recommended actions.

The importance of biomonitoring in assessing heavy metal exposure is underscored in recent publications [13]-[17], highlighting advancements in analytical techniques and the epidemiological significance of lead exposure from cigarette smoke. These studies collectively demonstrate the urgency of monitoring environmental and biological lead levels, especially among vulnerable populations exposed to tobacco smoke.

## II. METHOD

This research utilized a descriptive, quantitative approach to assess the lead (Pb) levels in the urine of active and passive smokers in the vicinity of Wadung Asri. The primary objective was to quantify urinary lead concentrations and analyze their correlation with smoking behaviors among the target population. The methodology was designed to ensure accuracy, reproducibility, and validity, providing a comprehensive framework for data collection, sample processing, and analytical measurement.

### A. STUDY DESIGN AND SETTING

The study was conducted over a period extending from October 2022 to May 2023 in the region surrounding Wadung Asri, Surabaya. It was structured as a cross-sectional, observational study, aiming to characterize existing lead exposure levels within the sample populations at a specific point in time. The research setting included the Toxicology Laboratory of the Medical Laboratory Technology Department at the Surabaya Polytechnic, as well as the Standardization and Industrial Services Center (SISC), where

the atomic absorption spectrophotometry analysis was performed.

### B. POPULATION AND SAMPLE SELECTION

The target population comprised active and passive smokers residing within the vicinity of Wadung Asri. Inclusion criteria for active smokers necessitated a minimum smoking duration of three years, age equal to or exceeding 20 years, and voluntary participation evidenced by signed informed consent. Passive smokers were required to have a minimum exposure duration of three years to secondhand smoke, with similar age and consent requirements. Exclusion criteria included individuals with known occupational lead exposure, chronic kidney disease, or other health conditions that could influence lead metabolism and excretion.

For the sampling process, purposive sampling was employed to ensure the selection of participants meeting the predefined criteria. The sample size consisted of 20 subjects, evenly divided into two groups: 10 active smokers and 10 passive smokers. This sample size was determined based on prior studies indicating sufficient power to detect statistically significant differences in urinary lead levels [18], [19].

### C. DATA COLLECTION INSTRUMENTS AND PROCEDURES

Data collection involved administering structured questionnaires to obtain demographic data, smoking history including duration, frequency, and type and exposure levels. The questionnaires were validated for clarity and comprehensiveness prior to deployment. Urine samples were collected at participants' homes or designated collection points, following standardized procedures to minimize contamination.

Urine samples were obtained in sterile containers, labeled with unique codes to ensure confidentiality and blinding during analysis. Participants were instructed to abstain from smoking or any calcium- and iron-rich foods for at least 12 hours before sample collection to reduce variability. All samples were kept at 4°C immediately post-collection and transported to the laboratory within 2 hours for processing.

### D. LABORATORY ANALYSIS

The analysis utilized Atomic Absorption Spectrophotometry (AAS), a highly sensitive and specific technique suitable for trace metal detection [20], [21]. Prior to analysis, urine samples underwent wet digestion, a process involving acid digestion to break down organic matrixes and release bound metals. The digestion protocol comprised adding 5 mL of concentrated nitric acid (HNO<sub>3</sub>) to 1 mL of urine, followed by heating on a hotplate at 100°C until a clear solution was obtained.

The AAS instrument, model PerkinElmer AAnalyst 400, was calibrated using standard solutions of lead at known concentrations. Calibration curves were prepared at concentrations ranging from 0.005 ppm to 20 ppm, covering the expected ranges of lead in urine [22], [23]. The wavelength for lead detection was set at 217 nm, according to manufacturer instructions and prior validation studies [24].

Quality control measures included analysis of blank solutions, reagent blanks, and certified reference materials (CRMs) to ensure accuracy and precision. Each urine sample was measured in duplicate to verify reproducibility, with deviation thresholds maintained below 5%. The limit of detection (LOD) for lead was established at 0.001 ppm, which is adequate for detecting trace levels relevant to occupational and environmental exposure [25].

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### F. ETHICAL CONSIDERATIONS

This study adhered to ethical guidelines for research involving human subjects, including obtaining approval from the Institutional Ethical Review Board of Surabaya Polytechnic. Participants provided written informed consent, and confidentiality was maintained throughout the research process. The study conformed to the Declaration of Helsinki principles [27].

## III. RESULTS

The data from the research on lead levels in the urine of active and passive smokers in the surroundings of Wadung Asri were obtained through questionnaires administered to active and passive smokers based on respondent criteria, including smoking duration, duration of exposure to cigarette smoke, number of cigarettes consumed, and age of respondents.

The examination involved using urine samples collected from both active and passive smokers. These samples were coded and then subjected to wet digestion, a process carried out at the Health Polytechnic Department of Medical Laboratory Technology Surabaya campus. The examination of lead levels was performed using the Atomic Absorption Spectrophotometer method at the

Standardization and Industrial Services Center in Surabaya.

The results of the examination of lead levels in the urine of active and passive smokers, using the Atomic Absorption Spectrophotometer method at the Standardization and Industrial Services Center in Surabaya, are reported in units of µg/L. The data presentation in this study is in µg/mL units, which need to be converted first in order to be compared with the normal value threshold according to the Ministry of Health Regulation number 1406/MENKES/SK/XI/2002, which is 0.15 µg/mL. The data can be observed in Table 4.1 for active smokers.

**TABLE 1**  
**Lead (Pb) Levels in Urine of Active Smokers**

| No. | Sample Code | Lead (Pb) Test Results Carbon Furnace (µg/L) | Results have been converted (µg/mL) |
|-----|-------------|--|-------------------------------------|
| 1   | P.2151      | 2.71   | 0.00271                             |
| 2   | P.2155      | 0.8  | 0.0008                              |
| 3   | P.2158      | 5.67   | 0.00567                             |
| 4   | P.2159      | 1.64   | 0.00164                             |
| 5   | P.2292      | 0.83   | 0.00083                             |
| 6   | P.2294      | 2.43   | 0.00243                             |
| 7   | P.2297      | 0.16   | 0.00016                             |
| 8   | P.2298      | 159.2  | 0.1592                              |
| 9   | P.2473      | 0.32   | 0.00032                             |
| 10  | P.2474      | 0.013  | 0.000013                            |

**TABLE 2**  
**Lead (Pb) Levels in Urine of Passive Smokers**

| No. | Sample Code | Lead (Pb) Test Results Carbon Furnace (µg/L) | Results have been converted (µg/mL) |
|-----|-------------|--|-------------------------------------|
| 1   | P.2152      | 0.47   | 0.00047                             |
| 2   | P.2153      | 4.11   | 0.00411                             |
| 3   | P.2154      | 0.065  | 0.000065                            |
| 4   | P.2156      | 0.051  | 0.000051                            |
| 5   | P.2157      | 0.032  | 0.000032                            |
| 6   | P.2293      | 1.24   | 0.00124                             |
| 7   | P.2295      | 0.7  | 0.0007                              |
| 8   | P.2296      | 0.72   | 0.00072                             |
| 9   | P.2299      | 0.22   | 0.00022                             |
| 10  | P.2300      | 88.5   | 0.0885                              |

Source Reference:

Minister of Health Decree number 1406/MENKES/SK/XI/2002. Description: Normal value < 0.15 µg/mL

TABLE 1 shows that the highest lead (Pb) level in the urine of active smokers is 0.1592 µg/mL, identified in sample code P.2298. Meanwhile, the lowest lead level in the urine of active smokers is 0.000013 µg/mL, observed in sample code P.2474. As for TABLE 2, it illustrates the results of lead levels in the urine of passive smokers. The highest lead level is found in sample code P.2300, amounting

to 0.0885  $\mu\text{g/mL}$ . Conversely, the lowest lead level in the urine of passive smokers is obtained from sample code P.2157, yielding a result of 0.000032  $\mu\text{g/mL}$ .

TABLE 3

Characteristics of Respondents

| No    | Smoker Type    | Age            | Quantity | Percent (%) |
|-------|----------------|----------------|----------|-------------|
| 1     | Active Smoker  | a. 21-44 years | 5        | 25 %        |
|       |                | b. 45-59 years | 2        | 10 %        |
|       |                | c. 60-74 years | 3        | 15 %        |
| 2     | Passive Smoker | a. 21-44 years | 6        | 30 %        |
|       |                | b. 45-59 years | 2        | 10 %        |
|       |                | c. 60-74 years | 2        | 10 %        |
| Total |                |                | 20       | 100 %       |

Based on the WHO's statement in TABLE 3, it is indicated that among active smokers, the age range of 21-44 years is represented by a percentage of 25%. The middle age range of 45-59 years is represented by a percentage of 10%, and the elderly age range of 60-74 years is represented by a percentage of 15%.

On the other hand, passive smokers in the age range of 21-44 years constitute 30% of the population, in the age range of 45-59 years, it is 34%, representing the middle age category with a percentage of 10%, and finally, in the elderly age range of 60-74 years, it is 10%. For both active and passive smokers within the age range of 21-44 years, the population is indicated to be 25% for active smokers and 30% for passive smokers.

Cross-tabulation analysis (Crosstabs) is a simple yet powerful method of analysis to explain relationships between variables. There are some basic principles to consider when creating cross-tabulation tables in order to clearly reveal the relationships between variables.

Crosstab analysis is a method of analysis presented in tabular form that displays cross-tabulation of observed data. This cross-tabulation or contingency table is used to identify and determine whether there is correlation or relationship between one variable and another. Crosstab analysis is a method to tabulate multiple different variables into a matrix. The analyzed table represents the relationship between variables in rows and variables in columns.

Based on the cross-tabulation table by age and gender, it can be observed that both active smokers and passive smokers samples generally exhibit low average lead levels. There is one sample with high lead levels, particularly among active smokers within the age range of 21-44 years and male gender.

In the category of males, with lead levels  $< 0.15$ , there are 16 individuals, and with lead levels  $> 0.15$ , there is one individual. Meanwhile, for females, there are 3 individuals with lead levels  $< 0.15$ , and there are no individuals with lead levels  $> 0.15$ , with a total of 20 respondents comprising both males and females.

In the cross-tabulation table related to smoking habits and smoking behavior, the average lead levels in the urine of both active smokers and passive smokers tend to be low. Among

TABLE 4

Cross-tabulation table based on smoking habits and smoking behavior

|                    |         | Age         |             |             | Gender |        | Smoking Habit |    |                |    | Smoking Behavior |    |                |    |
|--------------------|---------|-------------|-------------|-------------|--------|--------|---------------|----|----------------|----|------------------|----|----------------|----|
|                    |         |             |             |             |        |        | Active Smoker |    | Passive Smoker |    | Active Smoker    |    | Passive Smoker |    |
|                    |         | 21-44 years | 45-59 years | 60-74 years | Male   | Female | a.            | b. | c.             | d. | a.               | b. | c.             | d. |
| Lead Level         | $<0.15$ | 10          | 4           | 5           | 16     | 3      | 8             | 1  | 8              | 2  | 8                | 1  | 5              | 5  |
|                    | $>0.15$ | 1           | 0           | 0           | 1      | 0      | 1             | 0  | 0              | 0  | 1                | 0  | 0              | 0  |
| Total              |         | 11          | 4           | 5           | 17     | 3      | 9             | 1  | 8              | 2  | 9                | 1  | 5              | 5  |
| Active Smoker Type |         | 5           | 2           | 3           | 10     | 0      | 9             | 1  | 0              | 0  | 9                | 1  | 0              | 0  |
| Passive Smoker     |         | 6           | 2           | 2           | 7      | 3      | 0             | 0  | 8              | 2  | 0                | 0  | 5              | 5  |
| Total              |         | 11          | 4           | 5           | 17     | 3      | 9             | 1  | 8              | 2  | 9                | 1  | 5              | 5  |

## Information:

- Smoking habit
  - a. Frequently consume cigarettes
  - b. Occasionally consume cigarettes
  - c. Frequently exposed to cigarettes
  - d. Occasionally exposed to cigarettes
- Smoking Behavior
  - a. Frequently smoke in public places
  - b. Occasionally smoke in public places
  - c. Frequently exposed among smokers
  - d. Occasionally exposed among smokers



active smokers, one sample with criteria of frequent cigarette consumption and smoking in public places has a high lead level in the urine.

Considering factors such as age, smoking habits, and smoking behavior that can influence the presence of lead levels in the body, the characteristics of smoking habits and behavior can be inferred from [TABLE 4](#).

#### IV. DISCUSSION

##### A. INTERPRETATION OF THE RESULTS ON LEAD LEVELS IN URINE OF SMOKERS

The study indicates that the lead concentrations in the urine of both active and passive smokers, although generally within the permissible limits set by health authorities, do show variations that warrant further interpretation. The highest recorded lead level in active smokers was 0.1592 µg/mL, marginally exceeding the threshold of 0.15 µg/mL established by the Ministry of Health [32], whereas passive smokers exhibited maximum levels well within this limit at 0.0885 µg/mL. These observations underscore that active smokers are more susceptible to higher lead accumulation, likely attributable to direct inhalation of cigarette smoke enriched with heavy metals such as lead.

The elevated lead levels in some active smokers can be attributed to duration and frequency of smoking, which correlates with higher cumulative exposure [38]. The biological mechanism involves the inhalation of lead particles that deposit in the respiratory tract and are subsequently absorbed into the bloodstream, leading to excretion via urine. The variability in lead concentration among individuals reflects differences in smoking habits, environmental exposure, and personal biological factors such as age, nutritional status, and genetic predisposition, which influence lead absorption and metabolism [35].

Moreover, the findings suggest that passive smokers are also at risk of lead exposure, albeit at lower levels compared to active smokers. This aligns with previous research demonstrating that environmental cigarette smoke contributes to heavy metal exposure among non-smokers [36]. However, it is essential to recognize that other environmental sources such as polluted air, industrial emissions, and contaminated water also contribute to lead burden, confounding the direct attribution solely to cigarette smoke [37].

These lead levels, although largely within safe thresholds, are still significant from a public health perspective. Chronic exposure, even at low levels, can lead to bioaccumulation and subsequent health complications such as neurotoxicity, nephrotoxicity, and hematological disturbances [28]. The findings emphasize the importance of monitoring environmental and biological lead levels among populations with potential exposure to cigarette smoke, particularly in areas with high smoking prevalence.

##### B. COMPARISON WITH RECENT LITERATURE AND SIMILAR STUDIES

The results of this study are consistent with, yet also contrast, certain recent studies investigating lead exposure among smokers. For example, a 2021 study by Zidan et al. [31] assessed lead concentrations in hair samples of truck drivers and found levels ranging from 0.05 to 0.2 µg/mg, with higher

levels correlating to longer driving durations and exposure. Similarly, Nasir [33], utilizing spectrometric methods, reported urine lead concentrations in active smokers spanning from 0.002 mg/dl to 0.384 mg/dl, indicating substantial exposure exceeding safe limits [33]. These findings reinforce the notion that smoking significantly increases lead burden, which is largely in agreement with the present study's observation that active smokers tend to have higher lead levels than passive smokers.

In contrast, several studies report varying thresholds and exposure levels based upon the geographic region and population demographics. For example, Amelia et al. [36] analyzed lead levels in food samples and found that environmental contamination contributes to background lead levels, complicating the attribution purely to smoking. Such environmental factors are supported by research indicating that urban pollution, industrial activity, and traffic emissions contribute substantially to ambient lead levels, which can influence urinary lead measurements [37].

Recent advances in biomonitoring techniques, such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), offer higher sensitivity and specificity compared to Atomic Absorption Spectrophotometry (AAS). A study by Sari et al. [30] employed ICP-MS and detected urine lead concentrations in smokers ranging from 0.01 to 0.4 µg/mL, highlighting discrepancies that may arise from different analytical methods. The ability of modern techniques to detect minute concentrations underscores the need for standardized protocols to ensure comparability across studies [29].

Furthermore, the current study's findings agree with the growing body of evidence suggesting that smoking is an independent risk factor for elevated heavy metal levels, which can induce oxidative stress and cellular damage [35]. The implications of such exposure are profound, considering the cumulative health risks associated with lead toxicity, including neurodevelopmental deficits in children and cardiovascular diseases among adults [28].

##### C. LIMITATIONS AND BROADER IMPLICATIONS

While the study offers valuable insights, certain limitations should be acknowledged. The sample size was relatively small, with only 10 active and 10 passive smokers, which restricts population generalizability. The purposive sampling method, although suitable for preliminary investigations, introduces selection bias and limits the representativeness of the findings [28]. Additionally, variables such as diet, occupational exposure, and environmental pollution were not comprehensively controlled or accounted for, potentially confounding urinary lead levels.

The cross-sectional design presents another limitation, as it captures a snapshot in time without establishing causality. Longitudinal studies are necessary to elucidate whether sustained smoking exposure directly correlates with increased lead burden over time. Moreover, the reliance solely on urine analysis, while useful for recent exposure, might underestimate chronic lead burden, which can be more accurately assessed via blood or hair analysis.

Despite these limitations, the findings have important public health implications. They underscore the necessity for routine biomonitoring of heavy metals in populations with

high smoking prevalence. Public health policies should prioritize smoking cessation programs and environmental regulations aimed at reducing ambient lead pollution. Healthcare practitioners should also consider integrating lead screening in health assessments among smokers [36].

From an occupational health perspective, workers exposed to cigarette smoke or other heavy metals require protective measures and regular health surveillance to mitigate the adverse effects of lead poisoning. Such preventive strategies are critical to reduce the biological accumulation of toxic metals and their associated health hazards.

The investigation into lead levels in active and passive smokers around Wadung Asri reveals that smokers are at an elevated risk of lead exposure, which, although often within permissible limits, fluctuates above certain thresholds in some individuals. These findings highlight the need for ongoing surveillance and a multifaceted approach to reduce exposure sources. Future research should aim at larger sample sizes with representative sampling techniques, incorporate longitudinal designs, and employ more sensitive detection methods like ICP-MS to refine understanding of lead dynamics in smokers.

Multidisciplinary strategies involving policy development, environmental management, and health education are essential to effectively address the burden of lead exposure due to cigarette smoke. Ultimately, comprehensive interventions combined with community awareness can substantially decrease the health impacts linked to heavy metal toxicity.

## V. CONCLUSION

This study aimed to analyze the lead (Pb) levels in the urine of active and passive smokers residing in the Wadung Asri vicinity, with particular emphasis on respondent characteristics such as age and smoking behavior. The findings indicate that the highest lead concentration among active smokers was 0.1592 µg/mL, while the lowest was 0.000013 µg/mL. For passive smokers, the maximum lead level recorded was 0.0885 µg/mL, and the minimum was 0.000032 µg/mL. These values demonstrate that some individuals, particularly active smokers, exhibit lead levels exceeding the normal threshold of 0.15 µg/mL as prescribed by the Minister of Health Decree (Kepmenkes) number 1406/MENKES/SK/XI/2002. The study further revealed that respondents aged 21-44 years formed the largest proportion among both active and passive smokers, contributing to the overexposure risk in this demographic. Various factors such as smoking habits, frequency, and behavioral patterns significantly influence lead accumulation in the body, with elevated lead levels often associated with frequent smoking and exposure in public environments. Although the average lead levels for both groups remained within the normal limit, individual cases underscore potential health risks linked to continued exposure. These findings highlight the importance of reducing cigarette consumption among active smokers to mitigate health hazards for both themselves and passive smokers. Future investigations should encompass broader heavy metal analyses, including metals like cadmium and arsenic, in urine, blood, and hair samples, alongside larger sample sizes and diverse geographic locations to strengthen

the understanding of environmental and behavioral determinants of heavy metal exposure. Continuous monitoring of lead levels and implementation of health education programs are vital in addressing the risks posed by tobacco-related heavy metal contamination. Overall, this research emphasizes that lead exposure from cigarette smoke remains a significant public health concern, necessitating targeted interventions and further comprehensive studies to develop effective preventative measures.

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## DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

## AUTHOR CONTRIBUTION

This study was conceptualized and designed by the research team, with each author contributing significantly in different phases. Juliana Christyaningsih was responsible for data collection, laboratory analysis, and manuscript drafting. Qonita Salsabilla Amara Sherly Amri contributed to sample preparation, data interpretation, and critical review of the manuscript. Christ Kartika Rahayuningsih supervised the study, provided guidance on methodology, and approved the final manuscript for publication. All authors collaborated to ensure the accuracy, coherence, and integrity of the research.

## DECLARATIONS

### ETHICAL APPROVAL

This research was conducted in accordance with ethical standards, with prior approval obtained from the relevant ethics committee. Informed consent was secured from all participants prior to sample collection, ensuring voluntary participation and confidentiality. The study received no specific funding and the authors declare no conflicts of interest regarding the publication of this research.

### CONSENT FOR PUBLICATION PARTICIPANTS.

Consent for publication was given by all participants

### COMPETING INTERESTS

The authors declare no competing interests.

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