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Optimization of Cryogenic Grinding as a Hair Sample Preparation Technique for Heavy Metal Concentration Analysis

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Abstract: This study aimed to evaluate the effectiveness of cryogenic grinding as a hair sample preparation technique and to compare heavy metal analysis results obtained using Energy Dispersive X-ray (EDX) and Atomic Absorption Spectrophotometry (AAS). Hair samples were collected from five residents of Sekotong and analyzed for Cd, Hg, and Pb content. Data were analyzed descriptively and compared using the Friedman test. The results indicated that AAS consistently yielded higher metal concentrations than EDX. Cd concentrations measured by AAS, EDX of powdered hair, and EDX of intact hair were 0.00656 mg/kg, 0.00130 mg/kg, and 0.00010 mg/kg, respectively. For Hg, the corresponding measurements were 0.00380 mg/kg, 0.00212 mg/kg, and 0.00362 mg/kg, whereas Pb exhibited the largest disparity, with 0.12930 mg/kg for AAS, 0.00224 mg/kg for EDX of powdered hair, and 0.06978 mg/kg for EDX of intact hair. The high variability, particularly in Pb measurements, suggests that heterogeneity in metal distribution and surface contamination affect EDX readings of intact hair. Cryogenic grinding yielded a more homogeneous particle distribution, resulting in EDX measurements of powdered hair that were more representative and numerically closer to AAS results than measurements of intact hair. Although differences were not statistically significant ($p > 0.05$), these findings support the use of cryogenic grinding to enhance the accuracy of hair-based heavy metal analysis. Furthermore, this method has the potential to reduce the use of destructive chemicals and hazardous waste, making it a safer and more sustainable alternative for community biomonitoring in areas affected by heavy metal contamination.

Keywords: Cryogenic grinding; hair; biomonitoring; small-scale gold mining

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INTRODUCTION

Heavy metals such as mercury (Hg), lead (Pb), and cadmium (Cd) have long been recognized as hazardous contaminants capable of polluting the environment and accumulating in humans and other organisms. These metals can enter the environment through diverse sources, including industrial activities, domestic waste, agricultural practices, natural geological factors, and small-scale gold mining (ASGM) (Molina-Mesa et al., 2022; Masruroh & Purnomo, 2023). Globally, heavy metal contamination is a major concern due to their persistence, resistance to biodegradation, and potential for bioaccumulation and biomagnification in food chains. In aquatic ecosystems, the presence of heavy metals can degrade habitat quality, disrupt microbial community structure, inhibit the growth of aquatic biota, and reduce biodiversity through chronic toxicity to sensitive organisms (Oginawati et al., 2023; Velda et al., 2023).

In humans, chronic exposure to Hg, Pb, and Cd has been linked to a range of systemic health disorders. Mercury, particularly in the form of methylmercury (MeHg), is highly neurotoxic, capable of crossing the blood–brain barrier and placenta, and can

impair neurological development in fetuses and children (Hong et al., 2012; Nuraini et al., 2023). Lead exposure has been associated with reduced cognitive function, neurodevelopmental deficits, and decreased intellectual capacity in children, even at low exposure levels (Stepanyan et al., 2023; Kerna et al., 2024). Cadmium exhibits nephrotoxic, hepatotoxic, and carcinogenic effects, and can induce chronic oxidative stress in multiple organs (Ijomone et al., 2020). Long-term exposure to mixtures of heavy metals has also been correlated with anemia, endocrine disruption, cancer, and other degenerative diseases (Santos-Lima et al., 2020; Vianna et al., 2022; Purbayanti et al., 2024).

One Indonesian region significantly affected by mercury contamination from ASGM is Sekotong, West Lombok. For over a decade, intensive amalgam-based small-scale gold mining has been conducted with limited waste management. Practices such as open amalgam burning, ore processing near residential areas, and direct disposal of tailings into the environment have led to mercury release into soil, sediments, water bodies, and aquatic organisms. Studies have documented mercury accumulation across multiple ecological compartments, including sediments, water, and fish, indicating persistent exposure that has entered the local food chain. Consequently, the local population is at risk not only through inhalation of mercury vapor during amalgamation processes but also via consumption of bioaccumulated marine and freshwater fish (The ASEAN Daily, 2023).

Recent biomonitoring findings by Nexus3 Foundation (2024) reinforce this situation, showing chronic mercury accumulation in hair and acute blood exposure in children aged 8–14 years in Sekotong. These results indicate that mercury exposure from ASGM has extended beyond miners to include vulnerable non-mining populations such as children, highlighting the critical need for accurate and sensitive biomonitoring methods that can reflect long-term heavy metal exposure in affected communities.

In this context, hair serves as a relevant biomarker for assessing chronic heavy metal exposure, as it records the accumulation of toxic elements non-invasively and remains relatively stable over hair growth periods. Hair is an easily obtainable, storable biological matrix capable of providing chronological exposure information (Runkel et al., 2026). Metals such as methylmercury are incorporated into the keratin structure during hair formation, offering a more stable indicator of chronic exposure compared to fluid biomarkers like blood or urine (de Sousa Parreira et al., 2022; Yamakawa et al., 2025). Hair-based biomonitoring has been widely applied in gold mining regions worldwide, providing chronological accumulation profiles that support sustainable public health surveillance.

For example, in Ghana, Mozhgon Rajaee et al. (2015) conducted mercury biomonitoring in ASGM communities using hair samples and reported higher hair mercury levels in miners compared to non-miners and control communities, indicating elevated Hg exposure in mining areas. In Colombia, studies have documented variations in Pb, Cd, and Se concentrations in children's hair across mining regions, reflecting environmental and spatial influences on heavy metal accumulation (Palomares et al., 2025). Mierzyńska et al. (2024) employed energy-dispersive X-ray fluorescence (ED-XRF), validated with ICP-MS, for multi-element analysis in hair and nails, demonstrating that non-destructive methods can facilitate rapid screening but are highly dependent on sample preparation quality. These findings emphasize that although hair is a promising biomarker, analytical accuracy critically depends on homogenization and preparation techniques prior to instrumental measurement.

Despite various preparation methods, technical and operational limitations remain. Wet digestion with HNO_3 and H_2O_2 achieves good recovery but poses risks of

volatile mercury loss during heating and contamination in open systems (Hong et al., 2012). Manual cutting prior to digestion introduces particle size heterogeneity, reducing sample homogeneity and analytical reproducibility. These issues are particularly critical for hair samples from high-exposure regions like Sekotong, where measurement accuracy is essential for health risk assessment and policy interventions.

As an alternative, cryogenic grinding offers homogenization without heating by using liquid nitrogen at –196°C. This technique allows uniform fragmentation of hair into smaller particles with minimal risk of losing volatile elements such as mercury, potentially improving sample homogeneity and representativeness (Brummer-Holder et al., 2020). While cryogenic grinding has been applied to various biological matrices, studies on its use for human hair in heavy metal biomonitoring—particularly in tropical ASGM contexts—remain limited. Therefore, this study aims to optimize cryogenic grinding as a hair sample preparation method and evaluate its impact on heavy metal analysis quality through comparison of Energy-Dispersive X-Ray (EDX) and Atomic Absorption Spectrophotometry (AAS) performance. The evaluation focuses on Cd, Hg, and Pb due to their high toxicological significance. AAS serves as the quantitative destructive reference method, while EDX is utilized as a rapid screening tool providing surface morphology information (Mierzyńska et al., 2024). The results are expected to strengthen the methodological basis for hair-based heavy metal biomonitoring and support the development of more accurate analytical protocols for high-risk regions.

METHOD

Study Design

This study employed a laboratory experimental design to evaluate the application of cryogenic grinding as a sample preparation technique for hair prior to heavy metal analysis. The evaluation was conducted by comparing two analytical methods—Energy Dispersive X-Ray (EDX) and Atomic Absorption Spectrophotometry (AAS)—based on measurements of cadmium (Cd), mercury (Hg), and lead (Pb) concentrations. Sample preparation was carried out at the Integrated Research Laboratory, Faculty of Medicine and Health Sciences, University of Mataram, whereas instrumental analyses were performed at the Integrated Laboratory, State Islamic University of Mataram, and the Environmental Laboratory of Mataram City.

The study procedure included hair washing, cryogenic grinding, elemental composition analysis using SEM–EDX, and wet digestion for AAS analysis. The design aimed to evaluate the potential of cryogenic grinding to improve sample homogeneity, as indicated by more uniform distribution patterns in powdered samples compared to intact hair, while also assessing its ability to minimize the loss of volatile elements during preparation.

Study Samples/Subjects

The study samples consisted of hair from five residents of Sekotong, West Lombok, an area with potential mercury exposure due to small-scale gold mining activities. Participants were selected purposively based on willingness to participate and residence in a high-risk area. This study was not intended to generalize population exposure levels but rather to provide a preliminary proof-of-concept evaluation of cryogenic grinding for hair sample preparation in heavy metal analysis. In this context, five subjects were considered sufficient for the initial methodological optimization. Each participant provided approximately 1–2 grams of hair from the posterior scalp region. Subjects who had undergone chemical hair treatments in the past three months were excluded to minimize analytical interference. The study protocol was approved by the

Health Research Ethics Committee, Faculty of Medicine and Health Sciences, University of Mataram (Approval No. 216/UN18.F8/ETIK/2025).

Instruments and Procedures

The main instruments used in this study included a cryogenic mill/mortar, a JEOL JCM-7000 SEM–EDX, and a Thermo Scientific 3300 AAS. All instruments were operated according to manufacturer standard operating procedures and established heavy metal analysis protocols for biological matrices.

a. Sample preparation

Hair samples were sequentially washed with 70% ethanol to remove lipids, followed by non-ionic detergent Triton X-100 and deionized water to eliminate surface contaminants. The samples were air-dried at room temperature for 24 hours and cut into small pieces.

b. Cryogenic grinding

Hair samples were placed in cryogenic tubes and cooled using liquid nitrogen at –196°C for approximately 24 hours. The frozen hair was then ground into fine powder using a cryogenic mill/mortar. The resulting powder was stored in sterile vials for subsequent analysis. This procedure aimed to enhance sample homogeneity and minimize metal volatility, particularly for mercury.

c. EDX analysis

Both intact hair and powdered hair were analyzed using EDX for the detection of Cd, Hg, and Pb through two observation modes: point analysis and elemental mapping. Point analysis provided quantitative or semi-quantitative data on elemental composition at specific points or localized areas on the sample surface, representing local metal concentrations. Elemental mapping visualized the spatial distribution of elements over a broader surface area using color-coded maps, allowing evaluation of metal distribution patterns and sample homogeneity. The combination of these approaches enabled assessment of changes in elemental distribution following cryogenic grinding.

d. AAS analysis (wet digestion)

Washed hair samples were digested using a mixture of concentrated sulfuric acid (H_2SO_4 98%) and nitric acid (HNO_3 70%) according to standard protocols. The resulting solution was analyzed using the Thermo Scientific 3300 AAS to determine total Cd, Hg, and Pb concentrations. AAS served as a destructive quantitative reference method to validate the comparative results obtained from EDX.

Data Analysis

Cd, Hg, and Pb concentrations obtained from each analytical method were presented descriptively as mean values. Comparisons between methods (EDX intact hair, EDX powder, and AAS) were performed using the Friedman test, as the data were paired measurements from the same samples, the sample size was limited, and normal distribution could not be assumed. A significance level of $p < 0.05$ was applied. However, as this was an exploratory proof-of-concept study with a small sample size, statistical results were interpreted cautiously and supplemented by descriptive evaluation of differences and variations among analytical methods.

RESULTS AND DISCUSSION

The measured concentrations of Cd, Hg, and Pb shown in Table 1 reveal a consistent pattern: Atomic Absorption Spectrophotometry (AAS) tended to produce higher metal concentrations than Energy Dispersive X-ray (EDX) analysis, both in powdered and intact hair preparations. For Cd, the concentration determined by AAS

was 0.00656 mg/kg, whereas EDX yielded 0.00130 mg/kg in powdered hair and 0.00010 mg/kg in intact hair. For Hg, inter-method differences were relatively small, with AAS showing 0.00380 mg/kg, EDX intact hair at 0.00362 mg/kg, and EDX powdered hair at 0.00212 mg/kg. In contrast, Pb exhibited the largest disparity, with AAS measuring 0.12930 mg/kg, EDX intact hair 0.06978 mg/kg, and EDX powdered hair 0.00224 mg/kg.

These differences indicate that the analytical responses of each method are influenced by metal-specific characteristics, the form of elemental binding within the hair matrix, and sample preparation type. AAS, which relies on total digestion of the sample, permits dissolution and detection of metals present both on the surface and within internal structures of the hair, including the cortex and medulla (Richard, 1995; Hong et al., 2012). In contrast, EDX is an X-ray–based surface-sensitive technique that primarily reflects elemental composition at or near the hair surface, limiting its sensitivity to heterogeneous internal distributions (Mierzyńska et al., 2024; El-Bana et al., 2025).

Hair's structural and chemical properties as a biosorbent further influence metal distribution within its matrix. Human hair fibers consist of cuticle, cortex, and—when present—the medulla, with distinct physicochemical environments that affect uptake and retention of trace elements (Zhang et al., 2020). This heterogeneous structure contributes to spatial variability in elemental distribution, leading to differences in measurement depending on the preparation method and analytical technique employed.

Table 1. Heavy metal content in hair samples

Test Parameter	EDX (Powder)	EDX (Intact)	AAS	AAS/EDX Powder Ratio	AAS/EDX Intact Ratio	Relative Variation (%)
Cadmium (Cd)	0.0013	0.0001	0.00656	5.05	65.60	129.50
Mercury (Hg)	0.00212	0.00362	0.00380	1.79	1.05	29.01
Lead (Pb)	0.00224	0.06978	0.1293	57.72	1.85	94.73

Table 1 illustrates that disparities among methods varied by element. For Cd, AAS values were approximately 5.05 times higher than EDX powdered hair and 65.60 times higher than EDX intact hair. Hg showed smaller inter-method ratios—1.79 relative to powdered hair and 1.05 relative to intact hair—indicating closer agreement among methods. In contrast, Pb exhibited the greatest disparity with AAS values approximately 57.72 times higher than EDX powdered hair and 1.85 times higher than EDX intact hair, suggesting that Pb distribution is highly dependent on sample preparation and metal localization within the hair (Richard, 1995; Zhang et al., 2020).

Relative variability expressed by the coefficient of variation (%) was 129.50% for Cd, 29.01% for Hg, and 94.73% for Pb. The high variation for Cd and Pb demonstrates greater measurement dispersion among methods, whereas Hg was comparatively stable across techniques. The pronounced variation in Pb associated with EDX intact hair may be attributed to Pb's strong affinity for functional groups within keratin, such as sulfhydryl and carboxyl groups, which are heterogeneously distributed along the hair shaft, creating localized accumulation "hotspots" (Zhang et al., 2020; El-Bana et al., 2025).

Because EDX analyzes localized micro-areas on the hair surface, results are strongly influenced by the specific spot chosen for analysis. Regions with elevated Pb deposition may yield disproportionately high signals compared to other regions on the same hair strand, explaining the high variation in Pb for intact hair compared to

powdered samples (Mierzyńska et al., 2024). External contamination, including dust, soil particles, environmental aerosols, cosmetic residues, or handling during sampling, can also produce exogenous signals detectable by surface-sensitive EDX analysis (El-Bana et al., 2025).

In contrast, powdered sample preparation via cryogenic grinding produces a more homogeneous particle distribution, making EDX measurements more representative of the overall hair matrix. Fragmentation of hair layers allows signals derived from both internal and external structures to be measured more evenly, reducing bias from localized surface hotspots. Cryogenic grinding at low temperatures also minimizes losses of volatile elements such as Hg during preparation (Hong et al., 2012). Therefore, cryogenically ground powders tend to yield more homogeneous and consistent descriptive results compared with intact hair.

The Friedman test indicated no statistically significant differences in metal concentrations among methods ($p > 0.05$). However, caution is warranted given the limited sample size ($n = 5$), which reduces statistical power and increases the risk of Type II error. High Pb dispersion in EDX intact hair further increases variability, lowering sensitivity to detect inter-method differences (Richard, 1995; Zhang et al., 2020). Thus, these findings should be considered preliminary evidence for cryogenic grinding's effect on measurement representativeness, requiring validation with larger sample sets.

Overall, AAS consistently produced the highest concentrations across all metals analyzed, while EDX powdered hair yielded values numerically closer to AAS than EDX intact hair. This suggests that homogenization via cryogenic grinding improves the representativeness of metal distribution in the hair matrix and reduces heterogeneity effects on surface-sensitive measurements (Hong et al., 2012; Mierzyńska et al., 2024).

Limitations include small sample size, high Pb variability, and potential external contamination affecting EDX intact hair results. As an initial laboratory evaluation, these findings cannot yet be generalized to population-level exposures. Future studies with larger sample sets, standardized sampling protocols, and validation using certified reference materials (CRM) are necessary to confirm method accuracy and consistency (Richard, 1995; Hong et al., 2012; Zhang et al., 2020).

Despite these limitations, the study highlights the potential of cryogenic grinding to improve hair-based biomonitoring of heavy metals, particularly in high-exposure regions such as small-scale gold mining areas in Sekotong. Sample homogenization enhances representativeness and consistency for Hg, Pb, and Cd analyses and offers operational advantages including reduced strong acid usage, lower hazardous waste, and greater preparation efficiency compared with wet digestion methods (Hong et al., 2012; El-Bana et al., 2025).

CONCLUSION

This study demonstrates that the Atomic Absorption Spectroscopy (AAS) method yields higher concentrations of Cd, Hg, and Pb compared to Energy-Dispersive X-ray Spectroscopy (EDX), owing to its ability to detect metals through complete matrix destruction of hair samples. Sample preparation using cryogenic grinding improved sample homogeneity and representativeness, resulting in EDX measurements of powdered hair that were closer to AAS results than those obtained from intact hair. Although the differences between methods were not statistically significant ($p > 0.05$), cryogenic grinding shows potential for enhancing sample preparation quality in hair-based heavy metal analysis and warrants further development for environmental and

health biomonitoring applications. Descriptive results from this study indicate that cryogenic grinding may improve the representativeness and reliability of hair-based heavy metal analyses, making it a promising approach for more robust and sustainable biomonitoring methodologies.

RECOMMENDATION

Cryogenic grinding has potential as an alternative sample preparation technique for hair-based heavy metal analysis due to its ability to enhance sample homogeneity and produce more representative readings, particularly for volatile elements such as Hg. This approach should be considered for biomonitoring populations in regions at risk of heavy metal exposure to support more comprehensive exposure assessments. However, further validation with larger sample sizes, the use of certified reference materials (CRM), and reproducibility testing are necessary to strengthen the method's reliability. Additionally, standardization of preparation procedures and the enhancement of laboratory cryogenic facilities are required to ensure that this method can be applied consistently, safely, and efficiently in hair-based heavy metal analysis.

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