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# Hair Lead Levels as an Alternative Indicator to Measure Lead Level in Children Body

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**Abstract** Informal recycling of lead-acid batteries produces uncontrollable pollution with a negative impact on health, particularly for children living near recycling areas. This study aimed to determine the correlation between blood lead levels (BLL), the gold standard to measure lead levels in human body, and hair head levels (PbH) as an alternative indicator. The study was conducted in 2014 as part of environmental pollution research. The research was focused on informal recycling of lead-acid batteries with cross-sectional design involving children (aged 7 to 13 years) who lived close to lead-acid battery recycling activities in Depok City, Indonesia. Blood lead level was measured using anodic stripping voltammetry method and hair lead level using atomic absorption spectrometry. The measurements resulted in an average BLL of 8.22 µg/dL and PbH of 8.23 µg/g. The correlation between blood level and hair level is strong with coefficient correlation of 0.543 ($p<0.05$). The linear equation displays a positive pattern (y=1,573x-4,705), suggesting that when BLL increases, PbH increases as well. Hair lead levels can be used as an alternative indicator to measure lead levels in children.

**Keywords** Hair Lead Levels, Blood Lead Levels, Recycling Lead Acid Batteries, Children

## 1. Introduction

Lead is a dangerous heavy metal that accumulates quickly in living things and cannot be broken down [1]. Lead has extensive applications in the automotive, manufacturing, and mining industries [2]. Global use of lead is still dominated by the manufacture of lead-acid batteries (86%), with lead products generated through mining and recycling lead [3]. Up to date, the process of recycling lead from lead-acid batteries has not been well organized, while the demand is high. Therefore, opportunities for recycling on a small scale are prevalent. In 2014, the Indonesian Commission for the Elimination of Leaded Gasoline mapped the locations of informal used battery recycling activities in the Greater Jakarta area (including Depok), and 71 locations were identified as sites for informal recycling of used batteries and collection points for used batteries [4].

Ecologically friendly processing techniques are not used in the informal recycling of used batteries [5], resulting in environmental pollution that causes long-term effects on living things, especially people who live close to recycling sites [6], [7]. Lead in the form of fine particles (particulate matter) enters human body through the respiratory tract, and some falls on the surface of food and beverages in a form that is easily soluble and absorbed by human body [8], [9]. Upon entering human body, lead can be distributed

through blood circulation and accumulate in various organs, including hair [10,11].

Children are extremely susceptible to lead poisoning since they are more likely to put objects in their mouths and therefore absorb more lead [12]. On the other hand, an immature immune system is a factor that affects lead accumulation in children [13]. The risk of disease occurrence in children is closely related to high acute lead exposure because children's brains and nervous systems can absorb four to five times more lead than adults [11], [14]. The effects of lead that can damage the nervous system in children are more potent than in adults since children's nervous systems are more sensitive [15]. Lead exposure can cause various nervous system disorders, such as headaches, nausea, tremors, and numbness [16]. Children with high BLL are prone to anemia because high BLL can interfere with the heme biosynthetic system [17], [21] which serves to form red blood cells and can shorten the life of erythrocytes, hence the risk of anemia [18].

Environmental pollution exerts a detrimental role on human public health, with different human side effects. In this perspective, heavy metals' exposure can be considered a powerful threat, due to their persistence and versatile profile. For these reasons, they are described as epigenetic hazards, similar to other mutagenic, teratogenic, and genotoxic compounds [19]. Among heavy metal compounds, lead is harmful at any exposure level, by inducing significative effects on neurodevelopment and cognitive skills in children [20].

Early detection of lead poisoning in children is necessary so that preventive measures can be taken to reduce negative health effects of lead exposure. However, some children have an excessive fear of having lead levels checked because of the taking of blood. Lead has cumulative toxic properties and biomagnification for human health. Blood levels of lead in specific amounts can be distributed to soft tissues and accumulate in hair, nails, and teeth [21,22]. Human hair is a precious physical feature of the human body, essential for skin protection and thermic regulation. Human hair is sensitive to aging and environmental dynamics, specifically when induced by genetic and epigenetic factors. In this perspective, a primary role is reserved to heavy metal compounds [23]. Therefore, an analysis using hair samples was conducted to determine lead levels which can be an alternative way to measure biological indicators to observe a history of lead exposure in children due to its accessibility and practicality. This study aimed to determine the correlation between BLL and PbH in children aged 7-13 in informal used battery recycling areas.

# 2. Material and Method

### 2.1. Research Location and Design

The research was conducted in Depok City, West Java Province in 2014. Beji and Pancoran Mas Districts were selected due to the presence of informal recycling used battery activities in the areas. The study population included all children aged 7-13, who lived within the radius of 1,000 meters from a used battery recycling location and had resided more than 5 years in the area. The list of children was obtained from the health officer who worked for government-owned public health center in the area. Upon receiving the list, all children in the list were invited to take part in the research, and 70 children agreed to participate. All research subjects had expressed their consent in this research, and since the subjects were children, consent from parents or guardians were requested as well. Parents or guardians were invited to come to the health office with their children. The purpose of the research was explained and respondents' parents allowed their children to participate directly and signed the consent form. Research data has been published several times, including: "The relationship between BLL and blood Hb levels" [24], "the relationship between air lead levels and the incidence of anemia" [17], and "the relationship between air lead levels and BLL" [25].

### 2.2. Blood Sample Collection

Blood samples were collected from research subjects between 8 a.m. and10 a.m. The skin surface where venous blood was drawn was cleaned with soap and water, dried with a tissue, and wiped with 70% alcohol. Blood was taken from antecubital vein as much as 10 ml using a size 25 needle and inserted into the EDTA royal blue tube [26]. The EDTA tube was shaken 8-10 times to prevent blood clots. Each specimen tube was labeled for identification and stored at 4°C [27]. Lead Care Analyzer II machine was subsequently used with anodic stripping voltammetry (ASV) method to measure Pb in the blood [28,29]. The accuracy of the Lead Care Analyzer II in measuring BLL is between 0 and 65 µg/dl.

### 2.3. Hair Sample Collection

The hair lead level refers to the amount of lead in the hair, excluding surface contamination in the hair. Therefore, prior to processing, the children's hair was washed to ensure that the hair lead level was purely from internal part of hair. Hair samples were taken as much as 1 gram from the occipital area of the scalp using sterilized scissors. Hair was cut near the scalp approximately 2-5 cm from the last hair growth. Samples were quickly transferred to coded plastic bags, tightly sealed, and stored for pre-treatment. All specimens were stored in a dry, ventilated room in a desiccator and eventually delivered to the laboratory for analysis. The hair samples were cut until each piece measured 0.5 cm. Hair samples were cleaned by washing with detergent, rinsed with demineralised water, and rinsed with acetone solution. At the drying stage, the hair samples were placed in the oven for 2-4 hours with a stable temperature of 70±5°C. The dried hair samples were stored

in a clean and dry place in a polyethylene bag.

Before analyzing hair samples using atomic absorption spectrometry (AAS), the samples were first destroyed. The destruction step consisted of measuring 0.5 grams of hair samples into a beaker, adding a solution of 15 ml of HCl and 5 ml of $NHO_3$ covered with a tight watch glass, and homogenizing. The solution was heated using a hot plate for 30 minutes until the solution boiled. The watch glass lid was opened, and, on a water bath, the solution was evaporated, and 12.5 ml of HCL solution was added. Subsequently, the solution was re-heated until homogenization occurred and put back into the water bath to cool. A 50 ml volumetric flask was prepared to transfer the solution from the beaker while rinsing using distilled water until the sample was completely dissolved and stopped at the mark of the volumetric flask. Sample preparation had been completed and the sample was ready for analysis using the AAS (Atomic Absorption Spectrophotometry) machine. Prior to the use of the AAS machine, the machine was started for 5-10 minutes until the machine condition was stable, after which the standard solution was entered to standardize the AAS tool. After the standardization process was complete, the hair sample solution was entered into the viret for analysis in the AAS machine. Each sample was repeated three times to obtain satisfactory precision and accuracy. The measurement results were recorded on a computer connected to the AAS machine, and downloaded when the analysis was complete. The analysis of Pb levels in hair was carried out by an accredited laboratory with trained and skilled analysts [30], [31].

### 2.4. Data Analysis

The normality of the BLL and PbH data was tested with the skewness and standard error values (SE skewness). If the skewness test results are divided by the standard error ≤ 2, then the variable is normally distributed [32,33]. A simple Pearson correlation test was performed to determine the relationship between BLL and PbH. A strong association between the two variables was shown by the value of r = 0.51 to 0.75, while a very strong relationship was indicated by the value of r = 0.76 to 1.00 [34].

## 3. Results

### 3.1. Characteristics of Respondents

A total of 70 samples met the normality test criteria in this study, with 67 respondents (95.7%) were women and 48 respondents were children aged 7-10 (68.6%).

**Table 1.** Characteristics of Respondents (n=70)

| Variable | n | (%) |
|---|---|---|
| Gender | | |
| Female | 67 | 95.7 |
| Male | 3 | 4.3 |
| Age | | |
| 7-10 years | 48 | 68.6 |
| 11-13 years | 22 | 31.4 |

### 3.2. Lead Exposure Levels in Children's Blood and Hair

The measurements resulted in an average blood lead level of 8.22 µg/dL, with the highest blood lead level of 14.6 µg/dL and the lowest value of 3.80 µg/dL. The average lead content in hair was 8.23 µg/gr, with the highest value of 26.62 µg/gr and the lowest value of 0.01 µg/gr. The normality test results of the Pb in blood and Pb in hair variables were 1.94 and 1.58, indicating the value of ≤ 2, and hence normal distribution for both variables.

**Table 2.** Lead Exposure Levels in Children's Blood and Hair and Their Distribution (n= 70)

| Variable | Mean | Min.- Max. | 95% CI | Skewness | SE skewness | Normality Score |
|---|---|---|---|---|---|---|
| Pb in blood (µg/dL) | 8.22 | 3.80-14.60 | 8.05-9.12 | 0.555013 | 0.286750 | 1.94 |
| Pb in hair (µg/gr) | 8.23 | 0.01-26.62 | 5.75-10.74 | 0.452735 | 0.286750 | 1.58 |

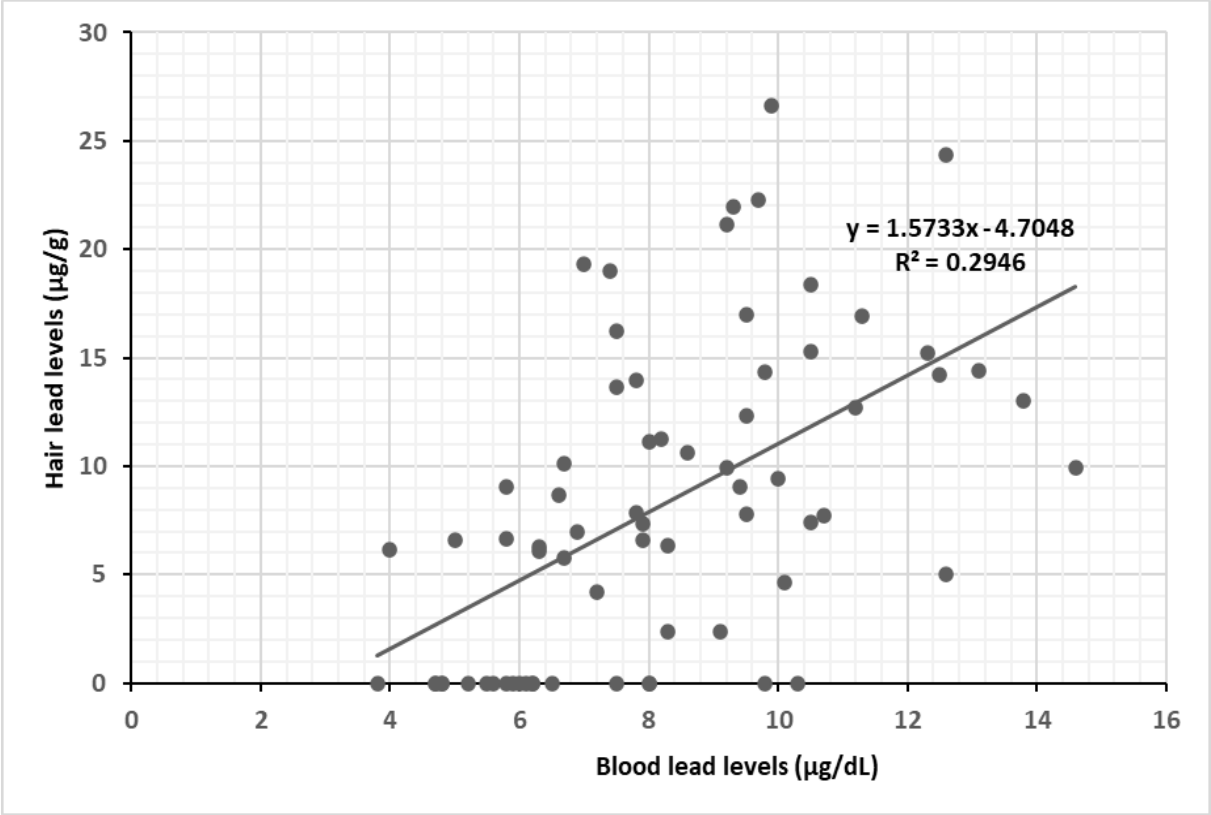


**Figure 1.** Correlation Between BLL and PbH

**Table 3.** Correlation Equation Between BLL and PbH

| Variable | r | $R^2$ | Line Equation | *P* |
|---|---|---|---|---|
| PbB vs PbH | 0.543 | 0.295 | Pb Hair = 1,573*Pb Blood – 4.7048 | <0.001 |

### 3.3. Correlation Between BLL and PbH

Figure 1 shows the results of the linear regression equation test analysis. The results of the analysis indicate that there is a strong relationship between BLL and PbH (r = 0,543). In Table 3, the positive pattern of the linear equation shows that PbH increases with the increase in BLL. The obtained line equation can explain 29.5% of the variation in the increase in PbH caused by an increase in BLL. Statistical analysis shows a significant association between BLL and PbH (p<0.001).

## 4. Discussion

### 4.1. Findings

The informal recycling of lead-acid batteries in residential areas can impact the health of nearby residents who live close to the recycling areas due to exposure to lead, which is toxic to the body [35]. The measurement findings revealed that children residing close to the lead-acid battery recycling sites had an average BLL of 8.22 µg/dL, which was higher than the CDC's 5 µg/dL recommendation but less than the WHO's recommended level of 10 g/dL [36,37]. This finding was less than the Blacksmith Institute's measurement in the Curug District, in which the average BLL value was 24.18 µg/dl [38].

The measurement results of PbH were also directly proportional to BLL, which was 8.23 µg/g, with the lowest value of 0.01 µg/g and the highest value of 26.62 µg/gr. These results showed the high lead content in children's hair in the lead-acid battery recycling areas in the Depok City area with an average value of 4.0 µg/g [39]. Similar results were found in a study conducted in Spain on 478 children aged 6-14, in which the lead concentration in girls' hair was 10.54 µg/dl, and 6.55 µg/dl in boys' hair (P < 0.001) [40]. Lead in hair is a critical source indicating direct deposition from the environment [41]. It is consistent with a study conducted to examine the effectiveness of using hair as an alternative matrix to detect chronic toxic exposures among subjects exposed to lead at work and non-work [42].

The main finding of this study is that there is a strong relationship between blood lead concentrations and PbH (r=0.543; P < 0.001). This finding is in line with the results

of studies in Sardinia, Italy ($r = 0.4351$; $p < 0.001$) and the city of Saratov, Russia ($r = 0.45$, $p<0.05$), each of which stated that there was a significant correlation between BLL and PbH [43,44]. The studies involved a sample of 330 children in Italy and 189 children in Russia.

Studies in north-central Italy (Sassuolo) and Poland (Miasteczko Laskia) reported a relatively low but statistically significant correlation between BLL and PbH. In Italy, 210 children aged 7 were included in the study sample, and the analysis results obtained a significant correlation between BLL and PbH ($r=0.125$, $p=0.005$) [45]. Meanwhile, the study in Poland found a significant correlation between BLL and PbH with a relatively low r-value ($r=0.270$, $p=0.00145$) [46].

Lead analysis using blood and hair samples is a mutually supportive initial screening. Lead analysis in hair can corroborate blood analysis as evidence of long-term exposure [39]. Due to slow nature of hair growth, the elements measured in hair represent a prolonged (chronic) accumulation of various elements of exposure, while sample analysis of blood levels describes acute exposure [47]. Supported by statements in the same study regarding the relationship between BLL and PbH, the researchers stated that lead levels caused the most detected associations between BLL and PbH [44]. Thus, based on the results of the study, it was found that there was a strong correlation between BLL and PbH among children in the lead-acid battery recycling areas in the City of Depok, Indonesia.

Previous publications have reported a strong and statistically significant association of increased BLL with airborne lead exposure in children living in used battery recycling areas [25]. Although the level of lead exposure in the environment was still below the WHO recommendation, the lead in the ambient air was 0.4 g/m3, and the lead content in the soil was 13.95 g/g. Children living close to informal battery recycling sites should have their blood lead levels checked frequently to avoid lead-related risks [11] because the clearance of lead from the body is metabolically much slower to occur as a result of continued lead exposure over many years. From the analysis of respondents' characteristics, almost all children who live near informal used battery recycling sites have lived in the area for more than 5 years (100%). This finding explains that high lead levels in the environment can cause high lead levels in blood and hair in children due to long-term and continuous exposure.

### 4.2. Limitation and Suggestion

The limitation of this study is the small sample size, so it does not represent the level of lead pollution at the district/city level. Research can only describe the occurrence of lead pollution in environments where there is active informal recycling of used batteries. Using hair as a biomarker sample for measuring lead in hair is more accessible to obtain than taking blood from children; this can be a consideration for making hair an alternative biomarker in determining indicators of lead pollution in children from polluted living environments.

## 5. Conclusions

This study identified a strong association between BLL and PbH ($r=0.543$; $P<0.0001$). The lead exposure value found in the hair biomarker sample was 8.23 µg/gr, and 8.22 µg/dL in the blood. Lead analysis using blood and hair samples is a mutually supportive initial screening. It is assumed that the high BLL and PbH values are due to the high lead levels in children due to prolonged and sustained exposure. Lead in blood is measured to identify exposed individuals, and monitor the progress of medical treatment, while measurement of lead in hair can serve as an initial assessment to identify areas contaminated with heavy metals.

## Authors' Contributions

Conceptualization: BR, SS, AS, UH; Data curation: BR, SS, AS, UH; Formal analysis: BR, SS, AS, UH; Methodology: BR, SS, AS, UH; Visualization: BR, SS, AS, UH; Writing-original draft: BR, SS, AS, UH; Writing-review and editing: BR, SS, AS, UH.

## Conflict of Interest

There is no conflict of interest in the publication of this manuscript.

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