ORIGINAL ARTICLE

Blood Lead Levels in Women and Sources of Exposure in Drinking Water and Fish in Pemali District Indonesia

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ABSTRACT

Introduction: Lead, a heavy metal originating from the tin industry in Pemali District, has polluted the waters and biota. Lead contamination has been exposed to humans, which will have an impact on women's reproductive health. This research aims to analyse the relationship between the drinking water source and aquatic animal food intake and women's blood lead levels. **Materials and methods:** This research uses analytical methods with a cross-sectional approach from July to October 2022 and involves a sample total of 91 women aged 30—49 years in Pemali District. Respondents were selected through purposive sampling, with the inclusion criteria being living in the research location area for ≥20 years and being willing to be a respondent. Exclusion criteria were pregnant or breastfeeding respondents and active or passive smokers. The data were analyzed in SPSS version 25 using chi-square and logistic regression tests. **Results:** The drinking water source and aquatic animals that are most consumed are refilled drinking water depot (60.4%) and mackerel (47.3%). The bivariate test showed a significant association between protected dug wells (p=0.015), gourami (p=0.015), and cork fish (p=0.038) with the respondent's blood lead levels. Based on the multivariate test, variables influencing blood lead levels were protected dug well (OR=0.24) and gourami (OR=8.699). **Conclusion:** The blood Pb level measurement results showed that all respondents had blood Pb levels >10 μg/dl. Two variables significantly affect blood Pb levels: protected dug well with a negative direction (B=-1.426) and gourami with a positive direction (B=2.163).
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Keywords: Blood lead level, Drinking water, Fish, Lead exposure, Woman

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INTRODUCTION

Lead or plumbum (Pb) is a naturally occurring metal that can be released into the air. One of them comes from the mining of lead [1]. Lead is removed from the atmosphere by rain and transferred to soil or comes in contact with surface water and becomes the highest type of heavy metal contained in water in mining [2]. Lead originates from the environment through bioaccumulation in water and food, ultimately consumed by humans [1,3,4], about 10–15% through adult ingestion [5,6].

The Centers for Disease Control (CDC) has determined that no exposure to lead can be said to be in the safe level category for humans [7,8]. According to the World Health Organization (WHO), the threshold value of lead content in the blood is 10-25μg/dl, but the concentration of blood lead that does not have negative effects on health has not been identified. Blood lead concentrations as low as 10 μg/dL are associated with a variety of effects, including cardiovascular disease in adults [9].

More than 95% of blood lead is bound to erythrocytes and is mainly excreted from the human body through urine, and 90% is deposited in bones [10]. Bone lead stores are mobilized during periods of increased bone turnover, such as during pregnancy and lactation, and lead is released into maternal blood and breast milk [11]. So, blood lead testing is a beneficial type of diagnosis to look at external lead exposure and is a direct clue to the amount of lead entering the body [12]. Moreover, lead from smoke exposure is also risky behavior for blood lead levels [11,13].

As an obesogen, Pb interferes with the work of hormones, so it is called Endocrine Disrupting Chemicals (EDCs).

EDCs cause weight gain, leading to adipogenesis and lipid accumulation [14]. Pb also alters molecular mechanisms related to glucose regulation and affects blood pressure [15]. Lead that accumulates in the body causes acute and chronic toxicity, genetic disorders, neurotoxicity, cardiovascular disorder (CVD), and cancer, and it affects almost all organ systems [16]. Pb collected in the body causes impaired kidneys, nerves, digestion, brain, and skin function. As much as 90% of Pb in the blood will bind to erythrocytes and block hemoglobin synthesis, which can result in anemia and a shorter red blood cell lifespan [17]. The health impact of increasing blood lead levels will increase the cost of treatment in healthcare facilities.

Lead accumulates in the body, causing acute and chronic toxicity, including reproductive and genetic disorders [18–20]. Blood lead levels in women can cause health problems, including preeclampsia and hypertension in pregnant women [21]. Women with blood lead levels of 10 μg/dL face complications such as premature birth, low birth weight, infertility, miscarriage, neurological defects in early childhood, and maternal hypertension. The level of lead bioaccumulation in blood in the range of 10–15 μg/dL in women of childbearing age directly affects the developing fetus [1]. Studies in Indonesia related to lead showed a significant relationship between lead levels in the bone in mothers and the incidence of Low Birth Weight [22]. In Indonesia, data or examination of lead levels in humans has not become a service priority, including in areas with high exposure, such as in Pemali District in Bangka Belitung Islands Province.

The Pemali District area in Bangka Regency is a tin mining area that has been going on for hundreds of years and contaminates waters and biota [23]. Based on the measuring results, lead levels in community drinking water sources, aquatic animals, and processed foods based on aquatic animals consumed by the community are still above the established quality standards [24],[25]. Women in the study area became the primary consumers of water and biota because their status was primarily that of housewives who lived indoors and were protected from other exposures, such as cigarettes. Therefore, this study aims to analyse the relationship between the type of drinking water source and aquatic animal food consumed with blood lead levels in women.

MATERIALS AND METHODS

Study design and population

This study used a cross-sectional design involving 91 respondents in Pemali District who were selected through purposive sampling based on inclusion and exclusion criteria. The inclusion criteria for respondents was women aged 30 to 49 to ensure that respondents lived in the study area or adjacent locations in Bangka Belitung Province with ≥20 years of Pb exposure. Women who were active or passive smokers was excluded from the study. Respondents were chosen based on women's participation in Posyandu activities organized by Pemali Puskesmas in 2022. In 2022, Pemali Puskesmas' Posyandu activities involved 795 women. Of 230 women who met the requirements, 91 agreed to participate and signed the informed consent. Samples were chosen based on the Slovin formula:

$$n = \frac{N}{(1 + Ne^2)}$$

$$n = \frac{795}{(1 + 0.1^2)}$$

$$n = 88.827$$

Notes:
n = sample size
N = population size
e = acceptable margin of error

Data collection

All respondents completed a questionnaire containing informed consent, respondent characteristics (age, occupation, education, income), and choice of drinking water source. Intake of aquatic animal food data was collected through interviews by enumerators. Computed laboratory staff took blood samples using a vacutainer and put them in three ml Ethylene Diamine Tetra acetic Acid (EDTA) tubes, two tubes for each respondent. All variable data were collected between July and September 2022.

Technique for measuring aquatic animal food intake

Aquatic animal food intake was collected from a 3x24-hour food recall questionnaire, converted in grams, and calculated into an average amount of daily consumption [26]. For statistical analysis purposes, the aquatic animal food intake was grouped into two categories based on the median value because the data was abnormally distributed (p=0.000).

Lead analysis in blood samples

The analysis method for blood lead levels uses an atomic absorption spectrometer (AAS) [27]. Blood sampling was carried out in a porcelain dish with a size of five ml of EDTA blood, heated over the stove for four hours, and then roasted in the muffle furnace for 18 hours by cooling it to room temperature. The test was carried out by adding one ml of 1:1 HNO_3 and five ml of deionized water, which was left overnight and filtered with the Whatman-1 filter paper. The absorption in lead was measured at a wavelength of 283.3 nm. The categorization of blood lead levels was based on median values (47.5 µg/dl) because the data was abnormally distributed (p=0.003).

Statistical analysis
The data were analyzed in SPSS version 25 using chi-square and logistic regression tests. The chi-square test looked at the relationship between the drinking water source and intake of aquatic animal food with blood lead levels using a 95% confidence level and is significant at p≤0.05. Variables with p≤0.25 will be analyzed multivariate using logistic regression to obtain the variables most associated with blood lead levels.

Ethical clearance
This study was approved by the Health Research Ethics Committee, Faculty of Public Health Jember University No. 245/KEPK/FKM-UNEJ/VIII/2022.

RESULTS

Respondent's characteristics
Table I shows that most respondents were aged 30-39 years (52.7%), had occupation status as housewives (86.8%), had last education at the senior high school level (56%), and had income in the range of <Rp. 3.624.884 (69.2%). The measurement results show that blood lead levels range from 11–94 µg/dl. These results show that all respondents had blood Pb levels >10 µg/dl. Therefore, the categorization of blood lead levels was based on median values (47.5 µg/dl) because the data was not normally distributed (p=0.003). As many as 50.5% of respondents had low blood lead levels.

Table I. Respondent's Characteristics

Respondent's Characteristics	n (%)
Age (years)	
30-39	48 (52.7)
40-49	43 (47.3)
Occupation	
Housewife	79 (86.8)
Apart from housewives	12 (13.2)
Education	
Elementary school	12 (13.2)
Junior high school	15 (16.5)
Senior high school	51 (56)
Diploma	6 (6.6)
Bachelor	7 (7.7)
Income (rupiah)	
< Rp. 3.624.884	63 (69.2)
≥ Rp. 3.624.884	28 (30.8)
Blood lead level (µg/dl)	
Low	46 (50.5)
High	45 (49.5)

Types of drinking water source and blood lead level
Table II shows respondents most consumed water from refilleded drinking water depots (60.4%). Refilled drinking water is also the type of drinking water with the most consumers with high blood Pb levels (27 respondents). Based on the percentage of consumers with the highest blood Pb levels, refilled drinking water is the type of drinking water in fourth place (49.1%) after pump-dug wells (68.2%), local mineral water (53.8%), and local water companies (50%). There is an association between protected dug wells and blood lead levels in women (p=0.015). Most respondents who consumed water from protected dug wells had low blood Pb levels (71.4%).

Table II: Types of Drinking Water Source and Blood Lead Levels

Types of Drinking Water Source	Blood Lead Level			P-Value*
	Total (%)	Low (%)	High (%)	
Protected dug wells				
No	63 (100)	26 (41.3)	37 (58.7)	0.015
Yes	28 (100)	20 (71.4)	8 (28.6)	
Pump dug wells				
No	69 (100)	39 (56.5)	30 (43.5)	0.076
Yes	22 (100)	7 (31.8)	15 (68.2)	
Boreholes				
No	82 (100)	41 (50)	41 (50)	1.000
Yes	9 (100)	5 (55.5)	4 (44.5)	
Local Water Companies				
No	85 (100)	43 (50.6)	42 (49.4)	1.000
Yes	6 (100)	3 (50)	3 (50)	
Drinking Water Supply System Management Agencies				
No	88 (100)	44 (50)	44 (50)	1.000
Yes	3 (100)	2 (66.7)	1 (33.3)	
Refill Drinking Water Depots				
No	36 (100)	18 (50)	18 (50)	1.000
Yes	55 (100)	28 (50.9)	27 (49.1)	
Local mineral water				
No	78 (100)	40 (51.3)	38 (48.7)	0.966
Yes	13 (100)	6 (46.2)	7 (53.8)	

*Chi-square

Intake of aquatic animal food and blood lead level
Table III shows that the aquatic animal food that is most consumed in the high category is mackerel (47.3%). Mackerel and *Clarias gapienus* are aquatic animal foods that have the most consumers with high blood Pb levels (20 respondents). Suppose aquatic animal food is ranked based on the percentage of high-category consumers with the highest blood Pb levels. In that case, Clarias gariepinus (55.6%) is in fourth place, and mackerel is in sixth place (46.5%). There is an association between gourami (p=0.015) and cork fish (p=0.038) with blood lead levels in women. Most respondents who consumed gourami (84.6%) and cork fish (78.6%) had high blood Pb levels.

Table III: Intake of Aquatic Animal Food and Blood Lead Level

Food Intake	Blood Lead Level				p-value*
	Total (%)	Low (%)	High (%)	Range (Median)	
Mackerel					
Low	48 (100)	23 (47.9)	25 (52.1)	1.4 – 102.8 (17.1)	0.748
High	43 (100)	23 (53.5)	20 (46.5)		

CONTINUE

Table III: Intake of Aquatic Animal Food and Blood Lead Level. (CONT.)

Food Intake	Blood Lead Level				p-value*
	Total (%)	Low (%)	High (%)	Range (Median)	
Shrimp					
Low	52 (100)	23 (44.2)	29 (55.8)	0 – 25.7 (2.0)	0.238
High	39 (100)	23 (56)	16 (44)		
Squid					
Low	47 (100)	20 (43.5)	27 (60)	0 – 22.8 (3.2)	0.172
High	44 (100)	26 (59.1)	18 (40.5)		
Shell					
Low	46 (100)	19 (41.3)	27 (58.7)	0 – 3.3 (0.0)	0.116
High	45 (100)	27 (60)	18 (40)		
Clarias gariepinus					
Low	55 (100)	30 (54.5)	25 (45.5)	0 – 34.2 (0.0)	0.467
High	36 (100)	16 (44.4)	20 (55.6)		
Nile tilapia					
Low	60 (100)	32 (53.3)	28 (46.7)	0 – 43.1 (0.0)	0.605
High	31 (100)	14 (45.2)	17 (54.8)		
Gourami					
Low	78 (100)	44 (56.4)	34 (43.6)	0 – 38 (0.0)	0.015
High	13 (100)	2 (15.4)	11 (84.6)		
Pangasius hypopthalmus					
Low	80 (100)	43 (53.8)	37 (46.2)	0 – 28.6 (0.0)	0.185
High	11 (100)	3 (27.3)	8 (72.7)		
Cork fish					
Low	77 (100)	43 (55.8)	34 (44.2)	0 – 31.4 (0.0)	0.038
High	14 (100)	3 (21.4)	11 (78.6)		

*Chi-square

Multivariate analysis

Variables qualifying for admission to multivariate tests are p≤0.25 (protected dug well, pump dug well, shrimp, squid, shell, gourami, *Pangasius hypopthalmus*, and cork fish). Table IV shows two variables most related to blood lead levels: protected dug well (p=0.008; B=-1.426; OR=0.24) and gourami (p=0.011; B=2.163; OR=8.699). The value of B for protected dug wells is negative, which means that the impact between protected dug wells and blood lead level is the opposite. Respondents who consumed water from protected dug wells were only 0.304 times at risk of having high blood Pb levels. The value of B for gourami is positive, meaning the impact between protected dug wells and blood lead level is unidirectional. Respondents who consume gourami in the high category will have blood lead levels 8.699 times higher than those who consume gourami in the low category.

Table IV: Multivariate logistic regression analysis of factors associated with Metabolic Syndrome among women from high lead exposure

Variable	B	SE	Exp (B)	Sig.***
Protected dug wells	-1.426	0.534	0.240	0.008
Gourami	2.163	0.846	8.699	0.011

**Logistic regression

DISCUSSION

Types of drinking water source and blood lead level

The results of this study showed that based on the type of drinking water source consumed, the variable related to respondents' blood lead levels was the source of protected dug wells (p=0.015). Respondents who consumed protected dug well drinking water sources had lower blood lead levels than respondents who did not. Based on the measurement results, water from protected dug wells has the minimum lead content compared to other water sources, which is as much as 0.012 mg/L. These results differ from studies of well water in Seoul, reporting that lead often exceeds quality standards, with concentrations ranging from 2,000–9,000 mg/L. This causes lead levels in the blood of respondents in this study to be higher in people who drink well compared to those who drink other water sources (tap water, pure water, or mineral water) [28].

Data from the *Puskesmas* Pemali shows that all protected dug wells used by respondents were groundwater that met clean water's physical, chemical, and biological requirements. Based on the results of observations, the respondent's protected dug well has a depth and wall construction that meets standards for preventing pollutants. The average depth of respondents' wells is 8–12 meters, while a depth of 6 meters from the water surface can reduce water pollution [29]. Most wells have also been covered with cement so that they are watertight and prevent the entry of chemicals into the water [30]. Good well construction can prevent heavy metals from entering well water; on the other hand, a dug well that has no floor, is cracked, and does not have a cover will increase the risk of contaminants entering the well [31].

These results reinforce the studies that state a significant relationship between blood lead levels and the quality of clean water sources in women. [32]. Different results were shown in studies in rural agricultural areas that showed that household well water sources were not significantly associated with blood Pb levels. This is because no water samples contain lead levels above the detection limit of 0.001 mg/L [33].

Intake of aquatic animal food and blood lead level

Food intake associated with blood lead levels is gourami and cork fish, which are types of freshwater fish. As for marine fish, none had any association with blood Pb levels. If we look at the results of measuring Pb content in marine fish, only mackerel contains Pb above the standard, while shrimp, squid, and shells are still below the permissible standard. The research results in Brazil showed that Pb in shrimp, squid, and shells was not detected above national established limits [34].

Research on the Andean River in Peru shows that lead is one of the Potential Toxic Elements (PTE) in river water. These levels of PTE may be of concern when considering long-term chronic exposure through bioaccumulation and bioamplification in the food chain [35]; long-term exposures to PTEs via the consumption of foods can lead to carcinogenic, teratogenic, and mutagenic damage to the nervous system and heart [36]. The results of this study strengthen other research that states that the mean concentration of lead (mg/kg) observed in Rupsha River freshwater fish specimens exceeded Europian Union (0.1 mg/kg) and Bangladesh Ministry of Fisheries and Livestock (0.3 mg/kg) guidelines [37]. Another research related to freshwater fish consumption in Bangladesh also states that there is a human health risk in consuming fish collected from the Buriganga River [38]. The results of a study in Bosnia and Herzegovina revealed that freshwater trout fish consumption (17.7%) contributed the most to lead exposure to food, besides tuna (25%) and sardines (24.6%) [39].

Most respondents who consumed gourami (84.6%) and cork fish (78.6%) also had high blood Pb levels. Another study states that lead concentrations in cork fish (6.32 mg/kg) contributed the heaviest metal exposure to freshwater fish besides tilapia (8.64 mg/kg) and *Carassius carassius* (6.852 mg/kg) [40]. This is related to gourami and cork fish as omnivorous animals when cork fish feed on zooplankton, zoobenthos, and plants from the surface water body, and gourami often feed on insects, larvae, crustaceans, algae, and detritus in sediment [41]. Omnivorous habits can accumulate hazardous substances if the organisms from the lower trophic levels in the food chain are contaminated with such dangerous substances as heavy metals and biotoxins [42].

Multivariate Analysis

The results of multivariate tests showed that two variables that most influenced respondents' blood lead levels were protected dug well and gourami. The multivariate test results showed a negative efficiency value (p=0.008 ; B=-1.426; OR=0.24), meaning respondents who consumed protected dug well water had lower blood lead levels than those who did not. Multivariate tests are consistent with bivariate test results; therefore, it can be concluded that water from protected dug wells is safer for consumption. The majority of polluting sources in the form of former tin mining are located far from residential areas. This is in line with other studies that state that women who live near hazardous waste sites or smelters have a higher risk of blood lead levels [11]. Protected dug wells do not use pipes to channel water to the surface. The use of iron-based pumps has the potential to channel lead residues into the human body due to corrosion. Corrosion is the process of dissolving part of the pump-forming material into drinking water. Corrosion of pumps and equipment causes leaks and deterioration of the chemical and microbial quality of water and has a direct impact on the concentration of lead and copper elements in water [31].

The multivariate test results showed a positive efficiency value (p=0.011 ; B=2.163 ; OR=8.699), meaning respondents who consumed gourami had higher blood lead levels than those who did not. Another study states that banded gourami has the highest Metal Pollution Index (12.675) and highest concentrations of Pb (3.81 mg/kg) compared to other freshwater fish that live in Loktak Lake, India. [43]. These results are supported by research in Bangladesh, which states that the Biota-Sediment Accumulation Factor (BSAF) values for lead in gourami are more significant than in other fish because they ingest sediment and omnivore-feeding behavior [41]. Bottom-dwelling fishes exhibit a greater concentration of trace metals than pelagic fishes. Species inhabiting the lower zone of the water column show the highest levels of heavy metals [39].

This cross-sectional study only describes the women's blood lead level at a certain time. However, living in the study area with ≥20 years of Pb exposure was set as inclusion criteria to avoid bias in these research results. Future researchers can examine the content of other heavy metals in the Pemali Regency mining area.

CONCLUSION

The types of drinking water and aquatic animal food that respondents most consume are refilled drinking water depots and mackerel. The bivariate test showed a significant association between protected dug wells, gourami, and cork fish and the respondent's blood lead levels ($p<0.05$). Based on the multivariate test, two variables influenced blood lead level: protected dug well with a negative direction (OR=0.24) and gourami with a positive direction (OR=8.699). Respondents must consume drinking water from protected dug wells and reduce gourami intake to reduce the risk of increasing blood lead levels.

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