## Lead content of human milk in lowland and highland agricultural areas

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### Lead Content of Human Milk in Lowland and Highland Agricultural Areas

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**Abstract.** Human milk is a golden source of nutrition for infants, especially at the age of 0-6 months. The risk of food contamination at this age will have an impact on the health of the infant, both in the short and long term. Currently, the presence of lead in agricultural areas is a contaminant from agricultural activities. Human milk containing lead can cause tissue damage, inhibit the formation of red blood cells and also inhibit growth. This study aims to compare the lead content of human milk from breastfeeding mothers in agricultural areas in the highlands (43 mothers) and lowlands (28 mothers). Anthropometric measurements were performed on the mother. Blood lead level (BLL) was analysed using atomic absorption spectrophotometry (AAS), while human milk lead (HML) used ICP OES. Mann-Whitney test was used for data analysis. The average age of the mother was  $26\pm5$  years, weight  $60.5\pm15.7$  kg, height  $158.2\pm70.7$  cm, Hb level  $12.5\pm1.9$  µg/dL, HML  $19.27\pm15.9$  µg/dL, and BLL (highland)  $32.34\pm13.3$  µg/dL. The mean age of babies was 99 days and 40% of babies were boys. It was found that there are differences in infant age, Hb level and HML of breastfeeding mothers. In conclusion, there is a significant difference in the lead content of human milk from agricultural areas in the lowlands compared to the highlands.

#### INTRODUCTION

Many researchers have discovered the importance of human milk in infant growth and prevention from infections, allergies, asthma, diabetes, obesity and also perform better development in cognitive and behavior during childhood [1]. Human milk contains immune-protective components and nutrients that infants require while their antibodies are maturing, in addition to providing support for growth [2].

Human milk has also been investigated in terms of its various components. Human milk contain approximately 87 to 88 percent water, as is generally known. It contains calories 65-70 kcal/dL, lactose 7.8 g/dL, 1.2 g/dL protein, and 3.2-3.5 g10 fat, depending on the stage of breastfeeding, mother nutritional intake, and environmental factors. Furthermore, the protein content of human milk from mothers who delivered birth prematurely is higher [3].

During lactation, transport proteins assist in the delivery of vitamins, minerals, and immunoglobulins from the mother's blood through the cell membrane to the mammary glands. Unlike, protein, fat, and lactose must be synthesized first from glucose, amino acids, triglycerides, or fatty acids derived from maternal nutritional intake or reserves from adipose tissue or muscle [4].

Human milk, on the other hand, can act as a carrier or conductor of harmful chemicals from the environment or from the mother during breastfeeding or pregnancy storage. It is worth noting that human milk is the primary food source for newborns, and its consumption during infancy has an impact on health during the subsequent growth period. As a result, the presence of chemicals that accumulate in maternal tissues and can be passed on to infants during breastfeeding is a public health concern that must be addressed. The nature and features of these nutrients, as well as the nutritional content and technique of transferring these nutrients into human milk, have a role in the

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transfer of hazardous chemicals. Human milk's fat content has been examined 2) r its ability to bind to fat-soluble harmful substances including persistent organic pollutants. Methylmercury, dioxins, polychlorinated biphenyls, chlorinated pesticides, polybrominated flame retardants, and p18 uorinated chemicals used as repellants are examples of pollutants. The most commonly studied hazardous heavy metals are lead (Pb), mercury (Hg), and cadmium (Cd) [5].

Heavy metal residues, such as lead, have been identified in agricultural areas, according to studies. Lead is frequently utilized in industry, fuel, plumbing, herbicides, and fertilizers, among other things. Lead can also be absorbed by plants, such as vegetables, and stored in food [6]. Lead can be breathed as well as damaging agricultural land since it is spread in the air during agricultural activities. As a result of the significant exposure to lead in the agricultural environment, employing materials suspected of containing lead is also a factor to consider.

The amount of lead in human milk is strongly linked to the mother's diet and nutritional status throughout pregnancy and lactation [7]. Lead is a heavy metal that interacts readily with minerals like Fe, Zn, and Ca, as well as proteins, particularly those containing sulfhydryl groups [8], [9], [10]. The mechanism of lead absorption into the body was discovered to be linked to the mineral's state in the mother's body. Lead is mobilized into the mother's blood and then human milk during the breastfeeding process due to the mechanism of deconstructing calcium stores from bones [11].

Previous research has not explored at the specific nature of lead and maternal internal variables, as well as other probable causes that cause lead to be present in human milk. Geographical differences may be related to agricultural practices in terms of the use of check calculated compounds which will further have different impacts on the lead content in human milk and related factors. The goal of this study is to compare the amount of lead in human milk from breastfeeding mothers in agricultural areas in the highlands and lowlands, as well as other factors that influenced it.

#### MATERIAL AND METHODS

This research is a cross-sectional study design carried out in the agricultural area of Central Java Province, namely onion farming in the city of Brebes Regency as a representative of lowland agriculture and Sumowono and Bandungan districts in Semarang Regency as vegetable farming areas in the highlands. The subjects of this study were breastfeeding mothers with infants aged 0-9 months with inclusion criteria willing to join the study, still breastfeeding, gave birth to normal weight infants (>2500 g), not twins, and were local residents living in the study area.

At the time of data collection, anthropometric measures of mothers and infants were taken to acquire data on weight, height, while data at birth water from maternal and child health books. Maternal nutritional status was determined using a BMI cutoff of 18.5 kg/m2 based on the BMI categorization for Asia [12].

Venipuncture was used to obtain blood samples from research participants in order to measure blood lead, calcium, and hemoglobin levels. For blood lead samples, roughly 5 cc of blood was extracted with heparin, collected in metal-free tubes, centrifuged to avoid clotting, and then sent to the GAKY laboratory of the Faculty of Medicine, Diponegoro University for AAS blood lead analysis. Blood calcium levels and hemoglobin levels were measured in the CITO laboratory, Semarang. 20

A minimum of 5 mL of human milk was collected using a manual breast pump that had been sterilized and placed in a sterile glass bottle before sample preparation. The prepared sample was then analyzed for lead content using ICP-OES at Centre for Industrial Pollution Prevention Technology laboratory, Semarang.

In this study, the profiles of mothers and infants were analyzed in guivariate analysis. Bivariate analysis was used to compare the two groups (highland and lowland) on data of the mother's (age, weight, height and Body Mass Index/BMI) and significance by sex, weight, and length at birth), human milk lead levels, hemoglobin, and calcium levels. The Spearman rank test was used to investigate the relationship between variables. Logistic regression was used to perform multifactor analysis. The relationship was evaluated using a significance level of 0.05 [13], [14].

This study was approved for conduct after receiving ethical approval from the Health Research Ethics Commission, Faculty of Public Health, Diponegoro University, as evidenced by certificate number 545/EA/ KEPKFKM/2019. Mothers who participate in this study are those who have agreed to the information about how the study will be conducted. Their identity is kept private.

#### **RESULT AND DISCUSSION**

Central Java Province has agricultural areas in both the highlands and the lowlands. This geographical disparity is also linked to the types of plants produced. According to BPS-Statistics Indonesia on data of agricultural statistic for Horticulture, Brebes district is located in lowland and Central Java's largest shallot producer, while Semarang district is located in the highlands and provides vegetables to the Central Java region, including mustard greens, chilies, tomatoes, and other vegetables.

Over the last ten years, research has revealed that pesticides used in agricultural areas can be a source of lead pollution in the environment. Lead is, in fact, one of the compounds found in pesticides and fertilizers. It can be found in pesticides alongside other heavy metals such as As and Cd. Lead is thought to aid in the growth and increase of biomass in fertilizers [15], [16]. Previous research has shown that economic activities have an impact on commodities, resulting in a variety of health issues. Excessive pesticide use harms the environment and jeopardizes the health of the people who live near agricultural land [17], [18].

Previous research has discovered that lead content is detectable not only in the blood, but also in human milk [19]. A comparison of lead content in breast milk was performed in this study as an illustration of food safety aspects in the infant's main diet. Infants are a vulnerable group in terms of health because they are in the process of developing antibodies and growing rapidly, which determines their health and quality of life as adults. It was reported that the median blood lead content of infants above 4.5 g/dL at birth was significantly shorter at 24 months of age compared with other infants with lower blood lead levels [20].

Variables	Value
Infant's profile	
Median age (months old) (minmax.)	3 (0-9)
Median birth weight (gram) (minmax.)	3200 (2000-4000)
Median birth length (cm) (minmax.)	49 (45-62)
Boy (n, %)	24 (40)
Maternal profile	
Mean age (years old) $(\pm SD)$	$26\pm 6$
Mean weight (kg) (±SD)	$59 \pm 10.1$
Mean height (cm) $(\pm SD)$	$151.2 \pm 4.5$
Median Body Mass Index (kg/m <sup>2</sup> ) (minmax.)	24.9 (18.34-45)
Median Haemoglobin (µg/dL) (minmax.)	12.7 (7.2-16.5)
Mean Sistole (mmHg) (±SD)	$115 \pm 13$
Mean Diastole (mmHg) (±SD)	$73 \pm 9$
Mean Calcium (mg/dL) (±SD)	$9.08 \pm 0.55$
Median lead content in human milk (µg/dL) (minmax.)	17 (1.9-75)

In general, breastfeeding mothers are at a reproduction general general between a normal BMI and give birth to a baby girl (Table 1). Based on the research, it was found that there were significant differences in the levels of lead in human milk from Brebes Regency compared to Semarang Regency (Table 2). Other factors also appeared to be significantly different, except for maternal anthropometry, maternal age, maternal body mass index and infant length and weight.

Blood pressure levels in breastfeeding mothers in the Brebes area were significantly higher. Lead affects the excitability and contractility of the heart, modifies the compliance of vascular smooth muscle tissue, and works directly on the component of the central nervous system that regulates blood pressure. This is thought to be associated with increased oxidative stress, dysfunction 13 the nitric oxide (NO) system, inflammation, dysregulation of vasoactive hormones caused by lead exposure [23]. The relationship between lead concentrations in bone and the risk of high blood pressure has been investigated in hypertensive individuals in Boston, Massachusetts. There is a significant relationship with resize thypertension with a relative risk of 1.19 (p=0.04) [24]. Age and gender also played a role in this relationship. Blood lead levels were positively associated with increases in systolic and diastolic blood pressure in those aged 20 to 44, and young women are more likely than young men to be in relationships [21].

TABLE 2. Differences in lead content in human milk and other factors in the agricultural areas of Brebes Regency and Semarang	ş

Variables	Brebes Regency (n=28)	Semarang Regency (n=43)	p-value
Infant's profile			
Median age (months old) (minmax.)	1 (0-3)	4 (1-9)	<0.001ª
Median birth weight (gram) (minmax.)	3200 (2410-4000)	3200 (2000-3900)	0.741
Median birth length (cm) (minmax.)	48 (46-62)	49 (45-54)	0.380
Boy (n, %)*	8 (28.6)	16 (37.2)	-
Maternal profile			
Mean age (years old) (±SD)	26±5	27±6	0.161
Mean weight (kg) (±SD)	56.5±7.4	60.6±11.33	0.078
16 an height (cm) (±SD)	151.0±4.9	151.3±4.3	0.815
Median Body Mass Index (kg/m <sup>2</sup> ) (minmax.)	24.37 (20.6-31.39)	26.46 (18.34-45.44)	0.073
Median Haemoglobin (µg/dL) (minmax.)	10.9 (7.2-12.8)	13.7 (11-16.5)	<0.001 <sup>b</sup>
Mean Sistole (mmHg) (±SD)	109±11	$118 \pm 14$	0.003 <sup>a</sup>
Mean Diastole (mmHg) (±SD)	69±7	76±10	0.002 <sup>a</sup>
Mean Calcium (mg/dL) (±SD)	8.5±0.29	9.46±0.28	<0.001 <sup>a</sup>
Median lead content in human milk $(\mu g/dL)$ (minmax.)	32.06 (17-75)	11.55 (1.9-75)	<0.001 <sup>a</sup>

\*missing data 11 infants

a significant difference, T-test

<sup>b</sup> significant difference, Mann-Whitney test

There are 36 % of breastfeeding mothers experiencing anemia and most of them (88.5%) come from the Brebes area. Research has shown that lead exposure can affect the formation of red blood cells through the mechanism of **26** bibtion of erythrocyte  $\delta$ -aminolevulinic acid dehydratase [22]. Research on pregnant women in Brebes found that the average blood lead level in pregnant women was four times higher than the standard (10 µg/dL) [23]. As a result, the findings of this study add to the picture that anemia caused by lead exposure in the Brebes area may have started during pregnancy and continued until breastfeeding, or even before pregnancy.

Because of the anemia that frequently occurs in pregnant women and breastfeeding mothers, lead is more easily absorbed by the body. This absorption mechanism was discovered to be related to the mother's mineral state. When a person is iron deficient, the expression of Divalent Metal Transporter 1 (DMT-1) in the duodenum increases because the body's efforts to obtain iron at the molecular level are sufficient. Unfortunately, because they have the same valence, this condition also increases lead absorption. In one experiment, increased levels of DMT1 in human cells resulted in a more than 7-fold increase in lead transport compared to controls [10]. Thus, the condition of anemia can further aggravate the absorption of lead in the body and can damage other tissues and organs.

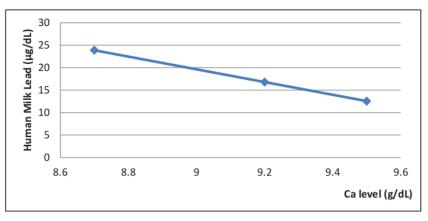


FIGURE 1. Association between calcium level and lead in human milk (p<0.001)

Under normal circumstances, the body has a mechanism in place to keep calcium levels in the blood stable, which is facilitated by hormones. When calcium levels in the blood are low, the body will increase absorption from the intestines, improve absorption in the kidneys, and perform bone disassembly (bone resorption). Calcium requirements in breastfeeding mothers involve not only the mother's own calcium requirements, but also calcium requirements in human milk [7]. Unfortunately, due to the ability of lead to mimics calcium, lead can also be mobilized into the blood via calcium metabolism in women who exclusively breastfeed [24],[9]. As a result, lead will be present in the blood and will be transferred into breast milk. As a result, lead will be in the blood and will be further delivered into human milk. The level of lead trans 27 ed into breast milk may also be related to the mother's breastfeeding practice. A study in breastfeeding moth 12 found that blood lead levels were higher in women with partial breastfeeding compared to those who did not. Women who breastfeed exclusively have higher blood lead levels than mothers who breastfeed partially [25]. However, our study did not detail the types of breastfeeding practices and experiences of mothers.

In total, the lead content in human milk from the two regions has an average far exceeding that recommended by the US CDC-WHO which is  $3.5 \ \mu g/dL$  [26]. Based on the transferred into human milk, it can be seen in Figure 1 that there is a significant relationship between blood calcium levels in breastfeeding mothers and lead levels in their milk with a negative correlation (r= -0.603). This shows that mothers with low calcium levels have a higher risk of delivering lead to their milk that may be stored in the bones during pregnancy or even earlier.

#### CONCLUSION

It can be concluded that there is a significant difference in the lead content of human milk from agricultural areas in the lowlands compared to 23 highlands. Mothers in Brebes have lower Hb and Ca, and higher blood pressure and lead content in their milk. The results of this study become an alarm for our agricultural behaviour and require health restoration for vulnerable groups including breastfeeding mothers.

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