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# Heavy Metals Levels in Breast Milk of Lactating Mothers Working in Heavy Metals Contaminated Factories

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#### Abstract

The objectives of this research were (1) to investigate the levels of Cd, Cr, Cu, Pb, Mn, and Zn in the breast milk of lactating mothers who working in industrial area (2) to investigate associated factors related with heavy metals in breast milk. 44 breast milk samples were analyzed by ICP-MS, the average Cd was  $0.31\pm1.42~\mu g/L$ , Cr was  $14.06\pm46.81~\mu g/L$ , Cu was  $9.99\pm4.59~\mu g/L$ , Pb was  $8.64\pm18.67~\mu g/L$ , Mn was  $7.18\pm25.72~\mu g/L$ , Zn was  $16.06\pm10.33~\mu g/L$ . Chi square presented that Cr, Pb, Mn was associated with period of work experience, Cr, Mn, Zn was associated with green leafy vegetable consumption (p<0.05). Binary logistic regression presented positively significant association between Pb in breast milk of factory workers and working in factory experience (95%CI was 1.085-38.699), and also showed positive significant relationship between Cu in breast milk and working hours per week of factory workers (95%CI was 1.019-14.758). Thus this study can serve as a representative of heavy metals exposure of lactating mothers and also provide the database of heavy metals in breast milk of industrial factory workers.

Keywords: Heavy metal; Breast milk; Factory worker; Associated factor

#### 1. Introduction

Breast milk is major food for infants and globally recommended to mothers to feed breast milk to infant since the first six months at least or until two years old (Bansa et al., 2017; Mieczan, 2014). Breast milk contains useful nutrients such as proteins and antibodies to protect infant from infections (Francesco et al. 2008). Some are necessary for good health, but high amounts or high concentration or long period of heavy metals may cause adverse health effects to human health, both acute or chronic effect. (Ferner, 2001). Heavy metals can get in the human body through all three exposure route; inhalation, dermal, ingestion, such as water, air, food or skin absorption. Humans can expose to heavy metals in manufacturing, pharmaceutical, electronic industrial, or residential surrounding (Roberts, 1999). The chronic effects can cause by continuous exposure during daily life or routine occupational exposure, even in low concentration and or low amount, but exposure repeat again and again will lead chronic. (Dupler, 2001; Eck and Wilson, 1989).

Exposure to heavy metals of lactating mothers who working in heavy metals contaminated factories can cause adverse health effects not only to mothers but also babies who breast milk feeding. Several research reported the exposure to heavy metals that may affect to fetuses, babies, and children, also may cause adverse health effects to child development. They investigated that early exposure to lead, mercury, and cadmium

exposure was correlated to infant adverse health effects, such as endocrine disorders, neurological, developmental, and thyroid hormone status. Similarly, lead can cause low birth weight, neonatal length decreasing, also lead can affect to learning disabilities, attention deficit disorders of newborn babies (Iijima *et al.*, 2007; Caserta *et al.*, 2013).

# 2. Materials and Methods

This was a cross sectional study during year 2018 to 2019, this study interviewed the participants and measured the heavy metals concentration of breast milk among lactating mothers who working in the industrial areas in central region, Thailand.

### 2.1 Participants

The participants were recruited during March, 2018. The 44 participants were from industrial factories located in Central of Thailand; which were jewelry, computer, and electronic materials factories. This research also was approved and received certificate documents from the Ethic Review Committee for Research-Involving Human Research subjects - Health Science Group, Chulalongkorn University (COA. 173/2562).

#### 2.2 Sample Collection and Analysis

Face-to-face interview was used to get the personal information of participants which are general characteristics, exposure factors, and occupational factors. All participants were lactating mothers who working in the industrial areas, they were collected the breast milk samples and contain in glass bottles, cover with para-film to avoid evaporation and contamination, keep in -20°C. All 44 breast milk samples were digested with nitric acid (HNO<sub>3</sub>) and hydrochloric acid (HCL) by microwave digestion (Wongsasuluk et al., 2017; Wongsasuluk et al., 2018)., then analyze the concentrations of Cd, Cr, Cu, Pb, Mn, and Zn by inductively coupled plasma mass spectrometry (ICP-MS) (LOD was 0.001 µg/L) The %relative accuracy was ranged from 98% to 103%.

### 2.3 Data Analysis

Descriptive statistics including the average, median, range, and SD were figured out. Chi Square and binary logistic regression analysis were applied to investigated the associated factors.

## 3. Results and Discussion

# 3.1 Characteristics of Participants

The 44 participants were from industrial factories located in Central of Thailand; which were 20 (45.4%) from jewelry factory, 16 (36.4%) from computer factory, 8 (18.2%) from electronics materials factory. The results of interview found the average age of participants was 32.6+4.8 years, average BMI was 22.5+3.0 kg/m<sup>2</sup>, average personal income was 23,454+22,886 baht or 780+762 USD. For the education, most of participants were graduated high school (47.8%). The marital status was found married 86.4%, only 11.4% had underlying disease which were allergy, 13.6% of participants had anemia during pregnancy. According to the results of personal behavior, none of participants was smoking, while 45.5% of participants had family smoking. Only 13.6% was alcohol drinking, 11.4% was eating seafood every day, and 79.5% was eating green leafy vegetable every. The characteristics of job presented that the average current job experience was 6.9+3.3 years, the current factory working experience was 8.2+4.1 years. For their current job, 27.3% of them had directly expose to heavy metals or heavy metals vapor/fume during working. The average working hour per day was 9.2+1.4 hours/day, average working hours per week was 50.2+11.2 hours/week. 90.9% of participants did not change their duty during breastfeeding. For personal protective equipment (PPE) using, the most PPE that participants usually were rubber gloves and cotton mask, both of them found 43.2% of participants used.

# 3.2 Concentrations of Heavy Metals in Breast Milk factory women worker (n=44)

All of the breastmilk of lactating mother samples were used to analyze the concentrations of six heavy metals which were; lead, chromium, copper, cadmium, manganese, and zinc. The concentrations of heavy metals in 44 breastmilk of factory workers were measured, the average concentration of Cd was 0.31+1.42 µg/L, median of Cd was 0.001 μg/L or less than limit of detection (<LOD), and ranged from <LOD to 7.46 µg/L. For Cr, the average concentration of Cr was 14.06+46.81 µg/L, median of Cd was  $0.001~\mu g/L$ , and ranged from <LOD to 287.80 µg/L. For Cu, the average concentration of Cu was 9.99+4.59 µg/L, median of Cd was 11.78 μg/L, and ranged from <LOD to 22.45 µg/L. The average concentration of Pb was 8.64+18.67 µg/L, median of Pb was 0.001 µg/L, and ranged from <LOD to 86.04 µg/L. The average concentration of Mn was 7.18+25.72 µg/L, median of Mn was 0.001 µg/L, and ranged from <LOD to 147.40 μg/L. For Zn, the average concentration was 16.06+10.33 µg/L, median of Zn was 13.16 μg/L, and ranged from <LOD to  $60.14 \, \mu g/L$ .

As a results of all concentrations of six heavy metals, this study compared the different of each heavy metals concentration by the product of factories (or factory type). The results showed that the highest Cd concentration in breastmilk found in computer factory workers (max 7.46 µg/L, ave mean. 0.84+2.30 μg/L), the highest Cu concentration found in jewelry factory workers (max 22.45  $\mu$ g/L, ave. mean 10.08+5.30  $\mu$ g/L), the highest Cr concentration found in jewelry factory workers (max 287.80 µg/L, ave. mean  $24.24+66.66 \mu g/L$ ), the highest Pb concentration found in computer factory workers (max 86.04 µg/L, ave. mean  $14.88+26.89 \mu g/L$ ), the highest Mn concentration found in jewelry factory workers (max 147.40 µg/L, ave. mean 9.34+32.98  $\mu$ g/L), similarly, the highest Zn concentration also found in jewelry factory workers (max 60.14 µg/L, ave. mean  $17.99+13.21 \mu g/L$ ). The comparison of breast milk among three types of factories workers by percentage of detected and

non-detected heavy metals in breast milk, found that breast milk from jewelry factory workers showed 5% Cd detected, 100% Cu detected, 30% Cr detected, 25% Pb detected, 20% Mn detected, and 100% Zn detected. Samples from computer factory workers presented 12.5% Cd detected, 100% Cu detected, 18.8% Cr detected, 37.5% Pb detected, 12.5% Mn detected, and 100% Zn detected. For electronic materials factory workers, the results showed 0% Cd detected, 100% Cu detected, 0% Cr detected, 37.5% Pb detected, 0% Mn detected, and 100% Zn detected. The different types of factories affected the level of heavy metals concentrations in breast milk, the jewelry factories presented the majority of heavy metals in breast milk that might from a process of welding solder such as ring welding.

#### 3.3 Associated Factors

The results found that the associated factors of Cr were personal income (p=0.021), anemia during pregnancy (p=0.011), seafood consumption (p=0.029), green leafy vegetable consumption (p=0.005), current job experiences (p=0.021), and current factory working experiences (p=0.002). Similarly, the associated factors of Pb were current job experiences (p=0.022) and current factory working experiences (p=0.003). For Mn, the associated factors found anemia during pregnancy (p=0.025), green leafy vegetable consumption (p=0.001), and current factory working experiences (p=0.022). In addition, the associated factor of Zn found green leafy vegetable consumption (p=0.001) (Table 1). The similarity of these heavy metals, found that the majority associated factors of heavy metals in breastmilk of factory workers was working experience; long period of working in factory and also green leafy vegetable consumption related to the heavy metals in breastmilk. In term of binary logistic regression computation, the results presented a positively significant association between Pb in breast milk of factory workers and working in factory experience (95%CI was 1.085-38.699), and also showed positive significant relationship between Cu in breast milk and working hours per week of factory workers (95%CI was 1.019-14.758).

Table 1. Associated factors with heavy metals in breastmilk

Factors		Cr≤Med. (n=35)		Cr>Med. (n=9)		p-value
		n	%	n	%	
Personal	≤ 15,000	14	63.6	8	36.4	0.021F
Income	> 15,000	21	95.5	1	4.5	
(Baht)						
Anemia in	Yes	2	33.3	4	66.7	$0.011^{F}$
pregnancy	No	33	86.8	5	13.2	
Seafood	Every day	4	80.0	1	20.0	0.029 <sup>F</sup>
consumption	At least every week	19	95.0	1	5.0	
	Not regular eating	12	63.2	7	36.8	_
Green leafy	Every day	31	88.6	4	11.4	$0.005^{F}$
vegetable	At least every week	3	37.5	5	62.5	
consumption	Not regular eating	1	100	0	0	
Current Job	≤ 6.5 years	14	63.6	8	36.4	0.021 <sup>F</sup>
experiences	> 6.5 years	21	95.5	1	4.5	
(Years) Current	< 9 voors	14	60.9	9	39.1	0.002F
Current factory work	≤ 8 years > 8 years	21	100.0	0	39.1 0	0.002r
experiences	o years	21	100.0	U	U	
(Years)						
Factors		Pb≤Med.		Pb>Med.		p-valu
		(n=		(n=		F
		n	%	n	%	
Current Job	≤ 6.5 years	11	50.0	11	50.0	0.022
experiences	> 6.5 years	19	86.4	3	13.6	
(Years)						
Current	≤ 8 years	11	47.8	12	52.2	0.003
factory work	> 8 years	19	90.5	2	9.5	
experiences						
(Years)						
Factors		Mn≤Med.		Mn>Med.		p-valu
		(n=38)		(n=6)		
		$\mathbf{n}$	%	n	%	
Anemia in	Yes	3	50.0	3	50.0	0.025F
pregnancy	No	35	92.1	3	7.9	
(participants)						
Current	≤ 8 years	17	73.9	6	26.1	0.022 F
factory work	> 8 years	21	100	0	0	
experiences	-					
/TT \						
(Years)		Zn≤Med.		Zn>Med.		
Factors						p-valu
		(	n=22)	(n=	=22)	p-varu
						p-varu
	Every day	(	n=22)	(n=	=22)	
Factors	Every day At least every week	n (	n=22) %	(n= n	=22) %	0.001 <sup>F</sup>

F = Fisher's exact test

<sup>\*=</sup> significant, p-value < 0.05

This study measured the concentrations of Cd, Cr, Cu, Pb, Mn, and Zn, not only this study that found the heavy metals contaminated in human breastmilk but also other research in world wide. The study of Pb and Cd in breastmilk of lactating mother in Lebanon, presented that they detected the average of cadmium was 0.87+1.18 µg/L and the average of lead was 18.18+13.31 μg/L. The comparison between Lebanon and this study, the concentrations of Cd and Pb found in this research was slightly less than Lebanon. In addition, their results of regression analysis showed that green leafy vegetable was not associated while potato consumption was associated with lead contamination (Basil et al, 2018). The study about levels of mercury, lead, and cadmium in the breast milk of lactating healthy mothers who were not exposed to heavy metals by occupation and living in different 8 environmentally polluted areas of the Slovak Republic (N=158). Their results presented that average cadmium, lead, and mercury concentration in breast milk samples were 0.43, 4.7 and 0.94 mg/kg, respectively (Monika U. and Vlasta M., 2005). The research in Taiwan also investigated the concentrations of Cd and Pb in early stage of lactating mother, presented that heavy metals concentrations were separated and measured in the four stages: stage 1 colostrums (day 1 to day 4 postpartum), stage 2 transitional milk (day 5 to day 10 postpartum), stage 3 early mature milk (after day 10 to day 60 postpartum), and stage 4 mature milk (after day 60 postpartum). The concentrations of Pb of four stage of lactating mothers ranged from 22.36 ng/mL to 0.45 ng/mL. The average of lead concentration were 13.22+3.58 ng/mL, 8.92+2.60 ng/mL, 11.72+2.58 ng/mL, and 2.93+1.70 ng/mL, for four stage respectively (Chao et al., 2014).

The study in Saudi Arabia about the concentrations of breast milk of lactating women living in Al-Ehssa and Riyadh regions who were not exposed to heavy metals by occupation, the result of their study presented that the average of concentrations of Cd and Pb in all tested breast milk samples were 1.732+1.691 µg/L and 31.671+45.663 µg/L respectively (Iman *et al.*, 2003). The study

about heavy metals levels found in breast milk of lactating mothers who living in Zarrinshahr, where is an industrial locations of Iran. The results presented that the average Cd and average Pb in breast milk samples were  $2.44 \pm 1.47 \,\mu\text{g/L}$  (ranged from 0.62 to  $6.32 \mu g/L$ ) and  $10.39 \pm 4.72 \mu g/L$  (ranged from 3.18 to 24.67 µg/L), respectively. Moreover, they found a positive correlation between the Pb concentration in breast milk and age of lactating women (P < 0.05). In addition, the concentration of Cd in breast milk samples significantly increased (P < 0.05) in lactating women who were exposed to smoking either active or passive (Rahimi et al., 2009). Cadmium can be mostly found in heavy metals contaminated factories. After human expose to cadmium, it can cause both acute and chronic adverse health effects. The target organs of Cd are kidneys, lungs, liver, placenta, bones, and brain. Acute symptoms are abdominal pain, breathing difficulty, nausea, and vomiting. Chronic diseases are renal disease, obstructive lung disease, and fragile bones, also include cardiovascular disease, learning disorders, migraines, alopecia, emphysema, osteoporosis, arthritis, poor appetite, growth impairment, loss of taste and smell, and anemia (Roberts, 1999). Similar with cadmium, copper also affect to many target organs in human body after exposure. The target organs of copper are nervous system, male and female reproductive system, connective tissue, also liver and kidney damage (Eck and Wilson, 1989). Even zinc is essential mineral for human but expose to high dose of zinc might cause health effects to human body. Acute symptoms after expose to zinc are nausea, abdominal pain, and vomiting. Other effects also include dizziness, lethargy, and anemia (Porea et al., 2000; Plum et al., 2010).

Some study support that expose to heavy metals can cause the heavy metals in breastmilk. Kadriye *et al.*, 2015 mentioned that majority of Pb contaminated in breast milk might not cause by exposure to heavy metals of lactating mother during lactation. But it might come from Pb that was stored in bones of mothers. Pb concentrations in breast milk, cord blood, and maternal blood

were highly influenced by bone metabolism of mothers rather than the accumulation from dietary Pb ingestion. During the 2<sup>nd</sup>-half of pregnancy, fetal skeletal development while mobilization of calcium occurring in bones of mothers, Pb go through to maternal blood circulation. Approximately 94% of the Pb burden is found in the osseous tissues, majority in the Pb-phosphate and Pb-carbonate forms, in adults. The half-life of Pb in the blood accumulation system is 30 days while 27 years in the bones. Hence, adolescent or girls exposed to Pb since youth or immaturity get to the reproductive age with significant Pb burden. Although discontinues exposure to Pb, but Pb might be stored then may cause high blood Pb levels persistently (Rebocho et al., 2006). The safety limits for heavy metals levels in breast milk have not been established. There are dissimilarity of breast milk levels, depend on many different factors such as the time of breast milk collecting; morning or evening, the period or stage of lactation, also environmental factors; location of residence, transportation, occupation (Emel O. et al., 2011). Similar with this research results, there were many studies presented that the statistics results of significant associated factors related with heavy metals in breastmilk were smoking, fish and seafood intake, alcohol drinking, and some of study found heavy metals in green leafy vegetables. (Llobet et al., 2003; Leotsindis et al., 2005; Gundacker et al., 2000; Gundacker et al., 2002; Gundacker et al., 2007; Esther et al., 2011; Chao et al., 2014; Basil et al., 2018). For the conclusion of this study, there were discovered that the majority associated factors of heavy metals in breastmilk of factory workers were working experience, long period of working in industrial factory related to the heavy metals in breastmilk. The level of heavy metals in breast milk of lactating mothers can be used as database of heavy metals exposure of workers in heavy metals contaminated factories. This study suggested that the health risk assessment of babies who breast milk feeding should be studied for further research.

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