The Comparison of Urinary Cadmium (UCd) and Urinary Lead (UPb) between 2007 and 2015 in a Population Living in a Zinc Contaminated Area

Sanhawat Chaiwong1*, Wikrom Chanthanao2, Pramote Loeskhampom3, Phongtape Wiwatanadate4 and Suthipong Sthiannopkao5

1School of Public Health, Walailak University, Na Khon Si Thammarat 80160, Thailand
2Department of Disease Control, Ministry of Public Health, Nonthaburi 11004, Thailand
3Tak Provincial Health Office, Tak 63000, Thailand
4Faculty of Medicine, Chiang Mai University, Chiang Mai 52000, Thailand
5LNU-MSU College of International Business, Liaoning Normal University, Dalian, Liaoning, China

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* Corresponding author:
E-mail: c_sanhawat@hotmail.com

ABSTRACT
This paper compares urinary cadmium (UCd) and lead (UPb) between 2007 and 2015 in a population living in an area of zinc contamination and classified in terms of year, subdistrict, gender and gender broken down by age. A total of 441 participants from zinc contaminated areas gave urine samples in 2007 and again in 2015 for analysis of cadmium and lead concentrations. Urine was divided into 2 parts for: 1) cadmium and lead analysis by ICP-MS and 2) urinary creatinine (Cr) measurement by the modified Jaffe’s reaction method. The statistical analysis includes mean, frequency and percentage, paired t-test and ANOVA. The results show a statistically significant decrease in the urinary concentrations of cadmium and lead in 2015 compared to 2007 for: 1) all subdistricts, 2) year, 3) age group, 4) gender and 5) gender by age. The reduction was greater in gender by age of females than in that of males, but this was not statistically significant. The conclusion illustrates that UCd and UPb in terms of years, sub districts (Prathadpadeang, Mae Tao and Mae Ku), gender, and gender by age (a cross tabulation of gender and age) show a statistically significant decrease from 2007 to 2015.

1. INTRODUCTION
In nature, heavy metals occur as natural constituents of the earth crust and are persistent environmental contaminants since they cannot be degraded or destroyed. (Lenntech, 2004; UNEP and GPA, 2004). In rocks, they exist in different chemical forms including sulphides and oxides, and some exist as both sulphide and oxide ores. Therefore, sulphides of lead, cadmium, arsenic and mercury naturally occur together with sulphides of iron (Habashi, 1992). Mining activities and other geochemical processes often result in the generation of acid mine drainage (AMD) (Peplow, 1999; Lenntech, 2004; UNEP and GPA, 2004; USDOL, 2004; Wenk and Bulakh, 2004; Higgins, 1971). It is well known that waste from mining industries contains large amounts of toxic elements in the form of heavy metals that can pollute the environment (Tansengco et al., 2018). Lead and cadmium are by-products of refining sphalerite and are released into the environment through zinc mining. Mae Tao, downstream of a zinc mine, is located at the tailing mine of Mae Tao creek and has been affected by severe environmental changes. Waste water from mining and refining drained directly into the Mae Tao creek and severely contaminated the soil along the river. Residents of the Mae Tao area are likely to have experienced higher exposure to contaminants than people who were living in Prathadpadeang and Mae Ku. This is because eating local plants, animals, and meat produced in the contaminated areas are major sources of cadmium and lead exposure in humans. In 1984, zinc mining was established in the Prathadpadeang subdistrict, upstream of Mae Tao creek. This creek is used for agriculture, provides water for 21.4 km² of paddy fields and affects 12 villages with a total population of 12,075 (Swaddiwudhipong et al., 2007). In 1999 the International Water Management Institute (IWMI) found cadmium was potentially polluting rice and soil in these areas (Padungtod et al., 2006; Phaenark et al., 2009). Further studies found that 7.2% of the...
population had cadmium contamination levels of more than 5 µg/g Cr (Swaddiwudhipong et al., 2007). Sources of food were also affected; rice contaminated with cadmium registered as high 1,800 times the regulatory standard (Simmons et al., 2005).

Blood lead and blood cadmium are a good indicators of recent exposure, but they are not as effective for long-term exposure because 20-40% of lead and 15% of cadmium enter into soft tissue and bone. Cadmium and lead remain within the human body for a decade or more, particularly in the kidneys. The level of cadmium in urine is therefore considered a good indicator of the body burden after long-term exposure and has become the way it is most often measured. Absorbed lead is excreted primarily in urine and feces; sweat, saliva, hair and nails, and breast milk are minor routes of excretion (Kehoe, 1987; Rabinowitz et al., 1980; O’Neill, 1993; Noonan et al., 2002). The highest concentrations of urinary cadmium and lead were found in subjects living in Mae Tao. This is consistent with findings from earlier studies as elevated levels of cadmium and lead in urine generally occur in areas with sphalerite ore (ZnS) (Alloway and Ayres, 1993). In the Mae Sot area, those 16-60 years of age have a renal dysfunction rate of 2.3% (Padungtod et al., 2006). This study therefore focused on the concentrations of urinary cadmium in children 9-15 years old, who were born between 1993 and 1999 and lived downstream of the zinc mine. The length of time the children were exposed to cadmium was nearly one half of cadmium’s half-life (10-30 years), the time that elimination of cadmium from the body via urination requires (Satarug and Moore, 2004). The kidney, then, is the critical organ for this elimination (O’Neill, 1993). The studied areas were in three sub districts, namely Prathapadeang - located downstream of the zinc mining community - and Mae Ku and Mae Tao - the further downstream communities - and which cover the twelve villages in Figure 1. The purpose of this study was to determine and compare urinary cadmium and lead concentrations in residents between 2007 and 2015.

2. METHODOLOGY

2.1 Population

The sample population was comprised of 441 subjects who lived in the Mae Sot district of Tak province which is situated in northern Thailand. The cadmium contaminated areas included rice fields irrigated with water from the Mae Tao and Mae Ku creeks; these creeks run through a zinc mining area and flow to twelve villages in three subdistricts: Mae Tao (6 villages), Mae Ku (3 villages) and Prathapadeang (3 villages) (Chaiwong et al., 2013).

2.2 Criteria for inclusion in the sample population:

1) Born during 1992 to 1999.
2) Urine cadmium and lead measured in 2007.
3) Living in a zinc contaminated area in three subdistricts: Prathapadeang (village 1, 3 and 4), Mae Tao (village 1 - 6) and Mae Ku (village 6, 7 and 8).

![Figure 1. Overview of studied areas with cadmium contamination (Chaiwong et al., 2013)](image_url)
2.3 Materials and methods

The study protocol was approved by the Tak provincial health office ethical committee.

2.3.1 The sample collection

Urine was collected by village health volunteers (VHVs). The target population was then briefed and given guidance in urine collection procedures by VHVs. Urine was collected in a polypropylene container and immediately preserved by a 65% concentration of double distilled nitric acid in a ratio of 10:1 (urine sample : nitric acid) for cadmium and lead extraction. Cadmium and lead were changed from their organic form to their inorganic form.

2.3.2 Reagent preparation; 5 μg/L of Rh and Ir: Pipetted 0.50 μL of 1000 ppm stock solutions, added 30.00 mL of 2.00% of double distilled HNO₃, adjusted with 18 MΩ DI water to 1000 mL, and stored at 4-8°C (CDC, 2003).

2.3.3 Sample preparation; 9000 µL of diluent, 900 µL of 18 MΩ deionization water and 100 µL of urine were drawn into a 20 mL polypropylene tube. Then, the solution was centrifuged at 3,500 rpm for 10 minutes. Finally, 20 mL of the supernatant was aspirated to a sample cup. The determination of cadmium and lead in urine was performed with inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500 series) (CDC, 2003).

2.3.4 Biochemical analysis: The urine collected for creatinine measurement was analyzed by Jaffe’s reaction. Urinary creatinine was measured with the Roche/Hitachi cobas c system, with reagents potassium hydroxide R1 (80 mmol/L), phosphate R1 (12 mmol/L), and picric acid SR 50 (4.4 mmol/L), at pH>13. The urine was diluted with distilled water in a ratio of 1.25 (urine sample: distilled water). A standard material (c.f.a.s., Cat. No.759350) was used to calibrate the automated systems before running the samples. The quality control was done with Precinorm U Cat. No. 171743 and Precipath U Cat. No. 171778 (Roche Diagnostics, Thailand).

2.3.5 Statistical analysis

Data are computed by automatic programs. Data are analyzed in two ways;

- Descriptive statistics: frequency, percentage and mean.
- Analytical statistics were divided into 2 parts:
  1) Paired t-test is used to compare between the two groups in terms of year, age and gender (p<0.05).
  2) Analysis of variance (ANOVA) is used to compare the level of urinary cadmium and urinary lead among 3 sub districts and gender by age (p<0.05).

3. RESULTS AND DISCUSSION

Table 1 shows the total means of UCd among all 3 subdistricts had urinary cadmium and urinary lead greater in 2007 than in 2015. The differences for both urinary cadmium and urinary lead are statistically significant (p<0.05). Urinary cadmium and urinary lead concentrations of subjects in 2007 were greater than in 2015. The differences for both urinary cadmium and urinary lead are statistically significant (p<0.05).

Table 1. Urine biochemical profile comparing mean contamination between 2007 and 2015 categorized by subdistrict.

| Sub district     | N   | Urinary cadmium (µg/g Cr) | Urinary lead (µg/g Cr) |
|------------------|-----|--------------------------|------------------------|
|                  |     | 2007         | 2015         | 2007         | 2015         | X       | SD    | X       | SD    | X       | SD    |
| Prathadpadeang   | 139 | 0.105a       | 0.101        | 0.005b       | 0.007        | 0.731a   | 1.033   | 0.011b   | 0.040   |
| Mae Tao          | 209 | 0.130a       | 0.115        | 0.009b       | 0.018        | 0.943a   | 1.528   | 0.038b   | 0.127   |
| Mae Ku           | 93  | 0.059a       | 0.119        | 0.012b       | 0.022        | 1.406a   | 0.898   | 0.053b   | 0.270   |
| Total            | 441 | 0.126a       | 0.113        | 0.009b       | 0.014        | 0.974a   | 1.154   | 0.032b   | 0.154   |

Data presented as mean adjusted creatinine (Cr). Superscripts a and b indicate statistical significance obtained from paired t-test between 2007 and 2015 of urinary cadmium and urinary lead (p<0.05).
There is reason to think that the transmissions of heavy metal contaminants depends on the plant-to-food chain transfer coefficient (TC) value. Hellstrom et al. (2007) shows that the consumption of locally grown vegetables and root crop are an important exposure pathway and that a statistically significant relationship exists between concentrations of heavy metals in urine and eating homegrown products. The difference in concentrations of cadmium and lead in the environment depends on the transfer coefficient value (the concentration of metal in the aerial portion of the plant relative to the total concentration in soil). Lead has the lowest coefficient (0.01-0.1) reflecting its relatively poor absorption in soil colloid. The result is contamination in the soil and transfer to plants grown in the area. Cadmium in the soil ranged from 1.13 to 94.00 mg Cd/kg soil. For comparison, the permissible standard for Thailand is 0.15 mg Cd/kg soil (Padungtod et al., 2006). A previous study conducted by The Association of Toxicology of Thailand (2007) examined the cadmium contaminations in the paddy fields in three subdistricts. Elevated levels of lead, then, may be absorbed via the food chain (Alloway and Ayres, 1993), while the highest coefficient (1.0-10.0) of cadmium is directly absorbed by root plants and accumulates elsewhere in the environment. In this replication study, urinary cadmium concentration in contaminated areas was found in the population in the expected pattern: those living nearer the areas of zinc contamination having higher concentrations than those living farther away from them. Urinary lead concentration, however, did not correlate with geographic proximity. These findings can be explained by the population’s consumption of plants and animals grown in the contaminated areas.

The key factor in explaining the differences between 2007 and 2015 is the decrease in the half-life of cadmium and lead, estimated to be 10-30 years (Songprasert et al., 2015; Maret, 2017). It might be eliminated from the body via urine during the intervening period. Furthermore, the measured levels can be explained, in part, by the community public policy process for solving environmental problems in contaminated areas. A health risk management plan was previously instituted by the government. The plan set out to first stop the movement of heavy metals, including cadmium and lead, along the plant-to-human food chain. As a consequence, all rice grown in the contaminated areas was bought to burn. Further, the government supported the production of non-food crops such as sugar cane, decorative palm and rubber plant to replace rice cultivation (Padungtod et al., 2006). In addition, the medical intervention program done by Chaiwong et al. (2010) reported the effect of education programs based on health belief models for prevention of cadmium exposure among high-risk female students. The results showed that the experimental group had a prevention cadmium exposure mean score significantly higher than the control group (p<0.01), and also the experimental group had a significantly higher mean score of prevention cadmium exposure after receiving the education program compared to before (p<0.01). The health risk management plan implemented in the contaminated area appears to be a contributing factor to the reduction in cadmium and lead levels.

Table 2 summarizes urine biochemical profiles as compared between two age groups at two time periods. Urinary excretion of cadmium and lead was notably higher in both groups in 2007 compared to 2015 (p<0.05). Mean urinary cadmium and lead of 13-15 year olds in 2007 were higher than 9-12 year olds in 2007. There were no statistically significant differences. In 2015, 13-15 year olds again had higher concentrations of urinary cadmium compared with 9-12 year olds; in contrast the 9-12 year olds had higher concentrations of urinary lead than 13-15 year olds in 2015. As with the cadmium result, there were no statistically significant differences (p>0.05).

Table 2. The means of UCd and UPb in groups aged 9-12 years old and 13-15 years old in 2007 and again in 2015

| Population                  | N   | UCd (µg/g Cr) | SD  | UPb (µg/g Cr) | SD  |
|----------------------------|-----|--------------|-----|---------------|-----|
| 9-12 years old in 2007     | 255 | 0.118<sup>a</sup> | 0.104 | 0.955<sup>a</sup> | 1.108 |
| 9-12 years old repeated in 2015 |     | 0.007<sup>b</sup> | 0.013 | 0.023<sup>b</sup> | 0.111 |
| 13-15 years old in 2007    | 186 | 0.135<sup>a</sup> | 0.122 | 0.997<sup>a</sup> | 1.219 |
| 13-15 years old repeated in 2015 |     | 0.008<sup>b</sup> | 0.011 | 0.014<sup>b</sup> | 0.017 |

Data presented as mean adjusted creatinine (Cr). Superscript a and b indicate statistical significance obtained from analysis of variance (ANOVA) between two age groups in 2007 and in 2015 of urinary cadmium and urinary lead (p<0.05).
Diet and supplements have been reported as preventing lead and cadmium absorption into body. Garlic, a local plant used for flavor, is recommended for consumption. Garlic can provide three types of medicinal compounds: 1) organo-sulphur compounds such as diallyl tetrasulfide, providing antioxidants, 2) sulphur-containing amino acids and compounds with free carboxyl and amino groups, helping to excrete Pb or Cd from the body, and 3) sulphur-containing amino acids such as S-allyl cysteine and S-allyl mercaptocysteine, helping to prevent Cd and Pb intestinal absorption. (Reddy et al., 2011; Ola-Mudathir et al., 2008; Farag et al., 2010). Tomatoes can produce metal chelating proteins and phytochelatins when exposed to heavy metal ions (Tito et al., 2011; Steffens et al., 1986). Nwokocha et al. (2012) show oral administration of tomatoes significantly reduced the accumulation of heavy metals (Cd, Pb and Hg) in the liver of rats. Vitamin B1 has been shown to potentially prevent absorption of Pb, and its pyrimidine ring mediates its interaction with Pb which may cause an increase in Pb excretion (Reddy et al., 2010; Sasser et al., 1984). Vitamin B6 has also been found to be effective in reducing accumulation of Pb in tissues and in reduction of inhibition of ALAD activity. This function is likely to be attributed to the ring nitrogen atom in its structure which can chelate Pb before it is absorbed (Tandon et al., 1987). Links with the study of Ryu et al. (2004) showed that the intestinal absorption of cadmium (Cd) is influenced by body iron (Fe) status in laboratory animals and humans.

Table 3 illustrates urine biochemical profiles as compared between two sex groups of subjects across two time periods. Urinary excretion of cadmium and lead in 2007 was recognizably higher than in 2015 in both groups (p<0.05). However, urinary excretion of cadmium and lead in males in 2007 was higher than that of females in 2007. The difference was not statistically significant (p>0.05). Urinary excretion of cadmium and lead of males in 2015 was higher than that of females in 2015. Again, there were no statistically significant differences (p>0.05).

Table 3. The means of UCd and UPb of male and female in 2007 and repeated measurement in 2015

| Population                  | N  | UCd (µg/g Cr) | UPb (µg/g Cr) |
|-----------------------------|----|--------------|---------------|
|                             | X  | SD.          | X             | SD           |
| Male in 2007                | 205| 0.127ª        | 1.016ª        | 1.242        |
| Repeated male in 2015       |    | 0.011ª        | 0.023ª        | 0.078        |
| Female in 2007              | 236| 0.124ª        | 0.930ª        | 1.072        |
| Repeated female in 2015     |    | 0.006ª        | 0.017ª        | 0.023        |

Data presented as mean adjusted creatinine (Cr). Superscripts a and b indicate statistical significance obtained from analysis of variance (ANOVA) between two sex groups in 2007 and in 2015 of urinary cadmium and urinary lead (p<0.05).

In 2007, urinary cadmium level measured among girls, especially in the age group 13-15 years, indicated that girls had more cadmium absorption than boys in their bodies. It is possible that the iron is lost in blood via menstruation and increases both cadmium and lead absorption in their body. In humans with depleted iron stores like females, up to 8.9% absorption of CdCl2 has been observed; the absorption was up to four times higher than in humans with normal iron stores (Flanagan et al., 1978), but in 2015 both UCd and UPb decreased. This can be explained by males having more muscle than females, resulting in higher serum creatinine. Creatinine is a chemical the body makes to supply energy, mainly to muscles. A high amount of creatinine in male urine might be caused by muscle building as creatinine is a chemical waste produced by muscle metabolism. Also, the comparison of gender showed the elimination of creatinine to be 14% - 26 mg per kg of body mass per day for men and 11 to 20 mg per kg of body mass per day for women (McPherson et al., 2006). A similar study by Yabe et al. (2018) shows that lead and cadmium excretion in urine of children was lower in younger children (0-3 years old) than older children (4-7 years old).

Table 4 shows the total mean UCd and UPb of gender by age in 2007 were higher than in 2015 at a statistically significant level (p<0.05). In terms of cadmium excretion in urine, females by age in 2007 had higher levels than males by age in 2007, but the differences were not statistically significant (p>0.05). In terms of lead excretion in urine, females...
by age in 2007 was higher than males by age in 2007, but in 2015, males by age had higher concentrations than females by age in 2015, but there were no significant differences (p>0.05).

### Table 4. The means of UCd and UPb of gender by age in 2007 and 2015

| Population                  | N  | UCd (µg/g Cr) | UPb (µg/g Cr) |
|-----------------------------|----|---------------|---------------|
| Males by age in 2007        | 241| 0.124<sup>a</sup> | 0.930<sup>a</sup> |
| Males by age in 2015        | 0.011<sup>b</sup> | 0.023<sup>b</sup> | 0.111 |
| Females by age in 2007      | 200| 0.127<sup>a</sup> | 1.017<sup>a</sup> |
| Females by age in 2015      | 0.006<sup>b</sup> | 0.017<sup>b</sup> | 0.112 |

Data presented as mean adjusted creatinine (Cr). Superscripts a and b indicate statistical significance obtained from analysis of variance (ANOVA) between gender by age groups in 2007 and in 2015 of urinary cadmium and urinary lead (p<0.05).

The decrease of Pb in females might be a consequence of transference to the placenta and other tissues in the body. The bones and teeth of adults contain about 94% of their total lead body burden; in children, the figure is approximately 73% (ATSDR, 2007). Lead in mineralizing tissues is not uniformly distributed. It tends to accumulate in bone regions undergoing the most active calcification at the time of exposure. Concerning comparison of trace elements concentrated in bone and intervertebral disc tissue, Kubaszewski et al. (2014) showed that the element concentrations identified in bone are comparable. In the case of Pb, Ni, Mo, Mg, and Zn, the concentration in the bone was 2 to 25.8 times higher than that observed in the disc. In disc tissue, fewer samples had trace element concentrations below the detection threshold. Brito et al. (2014) illustrated the magnitude of Pb uptake in cortical and trabecular bones in healthy animals and animals with altered balance in bone turnover and the impact of exposure to Pb on serum markers of bone formation and resorption.

Furthermore, we can use urine to replace blood for assessment of occupational exposure to lead (Moreira and Neves, 2008). Punshon et al. (2016) showed the correlation between elements and potentially toxic metals (Cd, Pb, Hg and Mn) which can cross to the placenta depend on nutrient concentrations (Zn and Se), potentially affecting the fetus. Norkaew (2011) reported statistically significant differences of cadmium concentration in blood, urine and placenta tissue of pregnant women inside polluted areas compared to those outside polluted areas. Levin (1981) studied maternal cadmium exposure in pregnant rats injected with CdCl₂. The result was fetal death and placenta necrosis.

### 4. CONCLUSIONS

The comparisons of UCd and UPb of subjects in terms of years (2007-2015), sub districts (Prathadpadeang, Mae Tao and Mae Ku), gender (in 2007 and 2015), and gender by age (a cross tabulation of gender and age) showed statistically significant decreases from 2007 to 2015. Factors decreasing Cd and Pb in urine from 2007 to 2015 were: 1) dietary, medicinal plants (garlic and tomato) and vitamins (B1 and B6) can decrease cadmium and lead absorption in the human body, 2) heavy metal transferred as lead to placenta, 3) creatinine excretion from body mass growth of males, 4) transfer coefficient (TC) to varieties of plants in zinc contaminated areas to the food chain, and 5) education intervention programs such as community public policy processes for solving cadmium and lead contamination problems in the environment.

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