Human Hairs, Nails, and Urine as Biomarker of Human Exposure Related with Heavy Metals Contaminated in Drinking Water in Agricultural Area

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Environmental Management
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การใช้ผม เล็บ และปัสสาวะ เป็นตัวชี้วัดทางชีวภาพต่อการรับสัมผัสโลหะหนักที่ปนเปื้อนในน้ำดื่มในพื้นที่เกษตรกรรม

นางสาวปกเกศ วงศาสุลักษณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Field of Study	Environmental Management
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ปกเกศ วงศาสุลักษณ์ : การใช้ผม เล็บ และปัสสาวะ เป็นตัวชี้วัดทางชีวภาพต่อการรับสัมผัสโลหะหนักที่ปนเปื้อนในน้ำดื่มในพื้นที่เกษตรกรรม (Human Hairs, Nails, and Urine as Biomarker of Human Exposure Related with Heavy Metals Contaminated in Drinking Water in Agricultural Area) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ศรีเลิศ โชติพันธรัตน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. วัฒน์สิทธิ์ ศิริวงศ์, 178 หน้า.

การศึกษานี้ได้ทำการประเมินระดับความเสี่ยงต่อสุขภาพของมนุษย์จากโลหะหนักปนเปื้อนในน้ำบาดาลบ่อตื้น โดยใช้ผม เล็บ และปัสสาวะ เป็นตัวชี้วัดทางชีวภาพ เพื่อเปรียบเทียบความแตกต่างระหว่างกลุ่มชาวบ้านที่ดื่มน้ำบาดาล และกลุ่มชาวบ้านที่ดื่มน้ำประปา ผลการศึกษาพบว่า น้ำใต้ดินมีสภาพเป็นกรดทั้งในฤดูแล้งและฤดูฝน โดยในฤดูแล้งน้ำใต้ดินมีค่า pH 5.28+1.15 และ 5.16+4.19 นอกจากนี้ ผลการศึกษาพบว่าชาวบ้านในพื้นที่ศึกษานี้มีอัตราการดื่มน้ำต่อวันสูงถึง 4.21±2.73 ลิตร/วัน ซึ่งสูงกว่าค่ามาตรฐานกว่าสองเท่า ซึ่งเป็นสาเหตุหนึ่งที่ทำให้พบความเสี่ยงต่อสุขภาพจากการดื่มน้ำใต้ดินปนเปื้อนโลหะหนักสูงขึ้น ผลการประเมินความเสี่ยงต่อสุขภาพในฤดูแล้งพบว่า 24.14 % ของกลุ่มชาวบ้านที่ดื่มน้ำใต้ดิน มีความเสี่ยงต่อการเกิดมะเร็งจากสารหนู และ 27.59 % มีความเสี่ยงต่อโรคที่ไม่ใช่มะเร็ง อีกทั้งพบว่า 13.79% ของชาวบ้านที่ดื่มน้ำใต้ดิน พบความเสี่ยงต่อโรคที่ไม่ใช่มะเร็งจากสารตะกั่ว ในฤดูฝน พบความเสี่ยงต่อโรคมะเร็งจากสารหนู 17.24% ของชาวบ้านที่ดื่มน้ำใต้ดิน และ18.97% ต่อโรคที่ไม่ใช่มะเร็ง นอกจากนี้ 36.21% ของชาวบ้านที่ดื่มน้ำใต้ดิน พบความเสี่ยงต่อโรคที่ไม่ใช่มะเร็งจากสารตะกั่วอีกด้วย จากการศึกษาตัวชี้วัดทางชีวภาพ ได้แก่ ผม เล็บ และปัสสาวะพบว่า ตัวอย่างจากกลุ่มชาวบ้านที่ดื่มน้ำบาดาล มีปริมาณโลหะหนักทั้ง 4 ชนิด ได้แก่ สารหนู แคดเมียม ตะกั่ว และปรอท สูงกว่ากลุ่มชาวบ้านที่ดื่มน้ำประปาอย่างมีนัยสำคัญทางสถิติ ค่าเฉลี่ยของสารหนูในปัสสาวะของกลุ่มผู้ดื่มน้ำบาดาล ได้แก่ 36.97 µg/L ซึ่งสูงกว่าค่ามาตรฐาน (35 µg/L) แตกต่างจากกลุ่มผู้ดื่มน้ำประปา ที่พบค่าเฉลี่ยสารหนูในปัสสาวะเพียง 19.30 µg/L ค่าเฉลี่ยของสารหนู แคดเมียม ตะกั่ว และปรอท ในผมของกลุ่มผู้ดื่มน้ำบาดาล ได้แก่ 0.091, 0.613, 18.26, and 87.27 ug /gH ตามลำดับ ในขณะที่ผู้ดื่มน้ำประปามีค่า 0.077, 0.076, 14.851 and 15.43 ug/gH. สำหรับผลการศึกษาเล็บพบค่าเฉลี่ยของสารหนู แคดเมียม ตะกั่ว และปรอท ในกลุ่มผู้ดื่มน้ำบาดาลมีค่า 0.378, 0.192, 61.640, 2.281 ug/gN ซึ่งทุกโลหะหนักสูงกว่าค่าเฉลี่ยของกลุ่มผู้ดื่มน้ำประปาที่มีค่าเฉลี่ย 0.257, 0.150, 23.500, 1.030 ug/gN ตามลำดับ ผลการศึกษาปัจจัยเกี่ยวข้องที่มีสัมพันธ์กับปริมาณโลหะหนักในตัวชี้วัดทางชีวภาพ โดยหา odd ratio และ binary logistic regression พบว่า การดื่มน้ำใต้ดินเป็นปัจจัยเสี่ยงที่ก่อให้เกิดสารหนูในปัสสาวะเกินมาตรฐาน (OR=43.50, 95%CI: 5.60-337.91, และ OR_{adj}=70.77, 95%CI: 7.86-634.83) รวมไปถึงการสะสมของสารหนูในเล็บอีกด้วย (OR=2.99, 95%CI: 1.31-6.80, และ OR_{adj} =3.58, 95%CI: 1.28-10.01) ดังที่กล่าวมา การศึกษานี้แนะนำให้หลีกเลี่ยงการดื่มน้ำบาดาลโดยตรง น้ำบาดาลควรผ่านการกรองก่อนดื่ม สำหรับการศึกษาต่อไปเกี่ยวกับการใช้ตัวชี้วัดทางชีวภาพ การศึกษานี้แนะนำให้ใช้ปัสสาวะสำหรับตรวจวัดการรับสัมผัสรายวันของสารหนู ตะกั่ว แคดเมียม ปรอท และใช้เล็บ เป็นตัวชี้วัดทางชีวภาพสำหรับการรับสัมผัสระยะยาวต่อสารหนู ตะกั่ว แคดเมียม และผมเหมาะสมเป็นตัวชี้วัดการรับสัมผัสสารปรอท

สาขาวิชา การจัดการสิ่งแวดล้อม
ปีการศึกษา 2559

ลายมือชื่อนิสิต ....
ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

5487778820 : MAJOR ENVIRONMENTAL MANAGEMENT
KEYWORDS: GROUNDWATER / RISK ASSESSMENT / BIOMARKER / HEAVY METALS

POKKATE WONGSASULUK: Human Hairs, Nails, and Urine as Biomarker of Human Exposure Related with Heavy Metals Contaminated in Drinking Water in Agricultural Area. ADVISOR: ASSOC. PROF. SRILERT CHOTPANTARAT, Ph.D., CO-ADVISOR: ASSOC. PROF. WATTASIT SIRIWONG, Ph.D., 178 pp.

Urine, hairs and nails were used as biomarkers to compare between the groundwater drinking group and the non-groundwater drinking group in intensively agricultural areas in Ubon Ratchathani province, Thailand. The shallow groundwater is acidic with average pH values of 5.28+1.15 and 5.16+4.19 in dry and wet seasons, respectively. The results showed an average drinking rate of approximately 4.21±2.73 L/day, which is twice as high as the standard. Due to the high drinking rate and groundwater contaminated with heavy metals (As, Cd, Pb, Hg), during the dry season, the results showed that 24.14 % of the groundwater drinking participants found As carcinogenic risk and 27.59 % found As non-carcinogenic risks as well as 13.79 % of the participants had a Pb non-carcinogenic risk. Similarly, during wet season, the results revealed that 17.24% of groundwater drinking persons found As carcinogenic risk and 18.97% found As non-carcinogenic risk as well as 36.21% of the participants found Pb non-carcinogenic risk. Interestingly, the concentrations of As, Cd, Pb, and Hg in urine, hairs and nails of the groundwater drinking group were significant higher than the other group. The average As concentration in the urine of the groundwater drinking participants was 36.97 μg/L exceeding the urine standard (35 μg/L), while the other group was 19.30 μg/L. The average concentrations of As, Cd, Pb and Hg in hairs of the groundwater drinking group were 0.091, 0.613, 18.26, and 87.27 ug/gH, respectively, while non-groundwater drinking group were 0.077, 0.076, 14.851 and 15.43 ug/gH. For nails, the average concentrations of the groundwater drinking group of As, Cd, Pb and Hg were 0.378, 0.192, 61.640, 2.281 ug/gN, while non-groundwater drinking group were 0.257, 0.150, 23.500, 1.030 ug/gN, respectively. Finally, the associated factor of odd ratio and binary logistic regression of As in urine found potential risk factor was the groundwater drinking (OR=43.50, 95%CI: 5.60-337.91, and $OR_{adj=}$70.77, 95%CI: 7.86-634.83). For As in nails, potential risk factor also found the groundwater drinking (OR=2.99, 95%CI: 1.31-6.80, and $OR_{adj=}$3.58, 95%CI: 1.28-10.01). As a result, this study suggested that groundwater should be avoided directly consume and should be treated especially before use as drinking water. For the future study, urine is suggested to be the biomarker related with daily exposure to As, Cd, Pb, and Hg. Furthermore, for long term exposure, nail is suggested for As, Cd, and Pb bio-monitoring, while hair is suggested to be the biomarker for Hg exposure.

Field of Study: Environmental Management
Academic Year: 2016

Student's Signature ....
Advisor's Signature ....
Co-Advisor's Signature ....

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CHAPTER I
INTRODUCTION

1.1 Overview

Recently, one of the most important environmental issues is heavy metal contamination in the environment and their exposure to humans. Heavy metals are of particular concern due to their strong toxicity even at low concentrations (Marcovecchio et al., 2007). Heavy metals occur in water bodies of natural origin (e.g., due to eroded minerals within sediment, the leaching of ore deposits and extruded volcanic products) and of anthropogenic origin (e.g., solid waste disposal, industrial or domestic effluents, harbor channel dredging). The release of heavy metals into the environment such as in groundwater occurs from various human activities: metallurgy and refining industries, coal combustion, diesel and fuel oil, sewage are a few example sources (Marcovecchio et al., 2007, Michalak et al., 2012, Jamal et al., 2013). Some of the metals are essential to sustain life; calcium, magnesium, potassium and sodium must be present for normal body functions. Also, Co, Cu, Fe, Mn, Mo and Zn are needed at low levels as catalysts for enzyme activities (Adepoju-Bello et al., 2009). Although some trace elements are essential to our health, many others are potentially harmful, such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), and lead (Pb). They can induce toxic, carcinogenic effects or oxidative stress when exposure is excessive, even at long-term low dose levels of intake. Ingestion of local food such as vegetables, meat, fish or water and also dust inhalation are the main ways of exposure to metals and metalloids. It is therefore plausible that food, drinking water, and air from areas characterized by distinct types of geological substrates can contribute differently to the dietary intake of trace elements. However, excess exposure to heavy metals can result in toxicity. The release of heavy metals into the environment occurs from various contaminated sources such as metallurgy and refining industries, landfill and landfill leaching, coal combustion, mining, diesel and fuel oil, industrial estates. The heavy metal pollutants can also contaminate the surrounding environment and further be transported to other surrounding areas, such as rice fields and corn fields. Moreover, some studies have shown that heavy metals can be transported to aquatic habitats, water, and sediment, affecting the aquatic biota to show heavy metal bioaccumulation; the results of research has found that the heavy metal contents in local fish and shellfish, for example, closely correlate with their concentrations in the water and sediment (Siriwong, 2006, Inmaculada et al., 2010).

Heavy metals have the potential to reach in the soil and then contaminate the surface and groundwater, which causes adverse effects on human health (Chotpantarat et al., 2011, Wongsasuluk et al., 2014). The potential toxicity of contaminants is generally determined by the composition of the elements involved. These heavy metals and metalloids can be dispersed and accumulated in plants and animals and so taken into and accumulated within the higher trophic levels, including within the human food chain. The important point of heavy metal contamination is the metals' toxicity even at low concentrations. The known adverse health effects of heavy metals include, for example, allergies, hyperpigmentation and the induction of cancer caused by As and Cd, due to their absorption in the gastrointestinal system. Arsenic is a toxic element for humans and is commonly associated with serious health disruptions. The principal manifestations of arsenicism affecting health are melanosis, keratosis and different forms of cancer (skin, bladder, lung, liver and prostate among others). The most common form of massive and chronic exposure caused is from the consumption of contaminated drinking water. Bangladesh, India, Mongolia, China, Taiwan, Mexico, Argentina, Chile and Thailand are countries where arsenic poisoning appears as a public health problem, resulting mainly from the consumption of As-contaminated water (Yanez et al., 2005).

Humans are exposed to a variety of chemicals released into the environment as a consequence of anthropogenic activities. In the last few decades, the human bio-monitoring approach has been increasingly used to reflect the relationship between environmental exposure, body burden, and possible adverse health effects.

Human bio-monitoring is defined as a direct measurement of human exposure to environmental contaminants by measuring the substances or their metabolites in body fluids and tissues. The basic human bio-monitoring principles have been derived from occupational medicine where the approach has been used since the early 1930s for exposure assessments and the health protection of exposed workers (Milena et al., 2012).

Heavy metals are bio-accumulated in the human body by three main routes, which are inhalation, dermal absorption and ingestion. Their compounds are accumulated mostly in bones, parenchymal organs, myocardium, skin, and hair (Michalak et al., 2012). Heavy metals can cause serious health effects with varied symptoms depending on the nature and quantity of the metal intake (Adepoju-Bello and Alabi, 2005). Concern about the metals and metalloids in environment exposure and affect to human health has motivated the scientific community to find trustworthy tools and methods for assessing the impact of toxic metals emission from anthropogenic sources or naturally anomalous levels of metals in water, soil and air. There are various procedures for measuring exposure to toxic substances. Biological monitoring has been widely operated, with blood, urine, feces, hairs and nails being the most regularly analyzed biological materials to determine the levels of many metals (Barbosa et al., 2005). With respect to blood and urine, the metal concentrations of which decrease rapidly after exposure; meanwhile, hairs and nails appear to be of greater value in evaluating past and ongoing exposure to high levels of metals (Gellein et al., 2008).

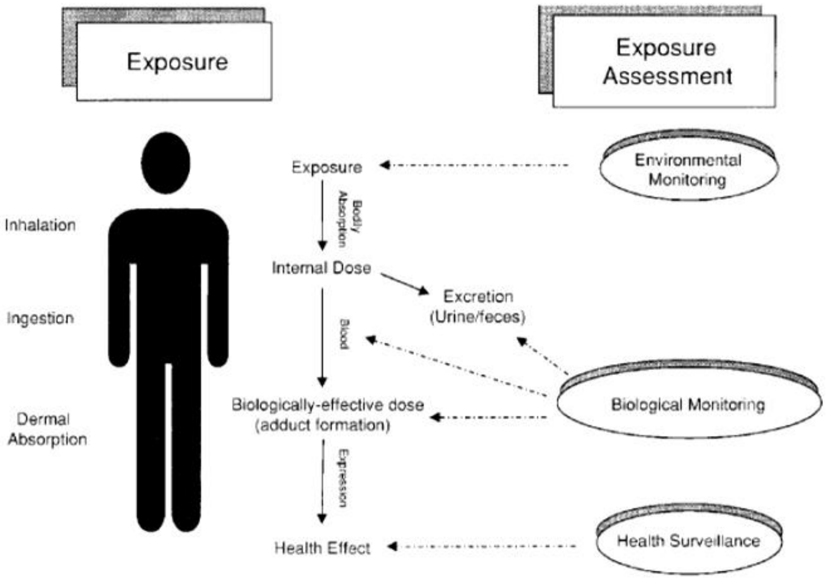

Figure 1. 1 Schematic representation of the pathways of a toxicant, from exposure to the induction of health effects (Barr, 1999)

Human bio-monitoring refers to a scientific procedure of evaluated human exposure to environmental xenobiotics, based on the sampling and analysis of human tissues and body fluids. The results investigated by bio-monitoring are stated to provide more precise data than estimations based on measurements of chemical concentrations in the environment (Sylwia et al., 2013).

There are various routes by means of which metals may be excreted from the body and there are therefore many ways of assessing human exposure to metals and metalloids (Varrica et al., 2014). Human bio-monitoring is a well-established method for measuring human exposure to chemicals and therefore provides a useful tool for the protection of human health. Whereas human bio-monitoring has been used for a long time in occupational health for the surveillance of workers, it is increasingly being implemented in the field of the environment and public health policy. Human bio-monitoring data provide helpful information on overall exposure because they integrate all routes of exposure, inter-individual differences in terms of absorption and metabolism, as well as lifestyle, which are the main factors influencing the body burden (Catherine et al., 2014). There are many applications in which hair analysis was used to document historic chemical and element use or exposure, such as suspicious death, discrimination between single and chronic exposure, and crimes committed under the influence of chemicals. Moreover, hair testing for drugs has been successfully performed several months after death, even following exhumation. Hairs have also been used to identify drugs in Egyptian mummies. Nicotine was recently reported to have been detected in hair samples of pre-Columbian mummies. Similar to hair, nails provide a stable material for detecting chemical and element exposure and can be a complement to hair in the detection of chemicals (Chen et al., 2014).

Arsenic (As) is a well-known human carcinogen. Once ingested, soluble forms of arsenic are readily absorbed from the gastrointestinal tract and distributed in all body systems through the blood. Although the blood arsenic level can come down to a minimum level within a few hours of ingestion and mostly excretes via the urine within three to four days, a certain part of it accumulates in many parts of the body, especially in keratin-rich biological derivatives of the ectoderm such as hair, scales, and nails of the chronically As-exposed population.

There are two types of keratins: alpha-keratins and beta-keratins. The alpha-keratins are relatively rich in cysteine residues and thus contain many disulfide cross-bridges. In addition, they contain most of the common amino acids. Nails contain hard and brittle keratins of up to 22% cysteine, while hair contains softer and more flexible keratins consisting of 10–14% cysteine (Badal et al., 2003). Hair and nails are keratinous biological materials that have a similar likelihood of accumulating drugs. The major advantage of keratinous biological materials in chemicals analysis compared to body fluids is that they have a larger surveillance window. This makes keratinous biological materials an important detection tool for toxicologists. Keratinous biological materials, such as hair and nails, offer a substantially longer retrospective window of detection compared to other body fluids. Furthermore, human scalp hair has been used as an alternative biological material for blood and urine in bio-monitoring environmental and occupational exposures of various pollutants, since its sampling is considered less invasive, more convenient to store and transport, and less hazardous to handle (Wang et al., 2009).

There are many pathways for releasing heavy metals from human activities into the environment such as through metallurgy, the refining industries, landfills and landfill leaching, coal combustion, mining, industrial estates and also agricultural activities. After being released, heavy metals can be transported to the surrounding environment and reach humans by way of three exposure routes: inhalation, dermal exposure, and ingestion, which can cause adverse health effects to humans. After heavy metal exposure and the phagocytosis process of cells, heavy metals are accumulated via the blood circulation system and excreted through sweat, urine, feces, hair, and nails. The half-life of arsenic in the blood and bones are 1-2 days and 1 month respectively, the half-life of cadmium in the blood is 2.5 months, and it is about 30 years in bone. The half-life of Hg in blood is 2 months and it is 20 years in bone. Meanwhile, the half-life of Pb is 1 month in blood and about 20-30 years in bone. But some studies have found As in hair after more than a hundred years. Pascal et al. (2007), for instance, studied the arsenic in two specimens of Napoleon's hair. Napoleon died and his hair samples were found to still contain 40 times the accepted threshold (of 1 ng/mg) of arsenic (Pascal et al., 2007). About 90%-95% of all intaken heavy metals accumulated in the blood, and 75%-80% is excreted via urine, while 15% is excreted via feces, sweat, breast milk, the hair, and nails. The other 5%-10% of intaken heavy metals is collected in the human body in three components. About 1% attaches with red blood cell (RBCs), about 4% collects in soft tissue such as the kidney and liver, and about 90%-95% collects in the bones and teeth (Tosukhowong, 2014). Hairs are composed of compact protein, hard keratin. Hair has a high affinity and relation for metals, according to the cystine which is approximately 14% of its total composition. Sulphur atoms in cystine or to sulphydryl (SH) groups are found bound with heavy metals in hair in other amino acids (Katz, 1988). Like hair, nails are composed of compact protein. Nail plates consist of hard translucent keratin, which containe metals. The bulk of the keratin is derived from lunula and the nail matrix. Urine is the main route of excretion and produced by the kidneys; it contains the byproducts of metabolic processes: salts, toxins, and water that end up in the blood (Nathalie et al., 2012).

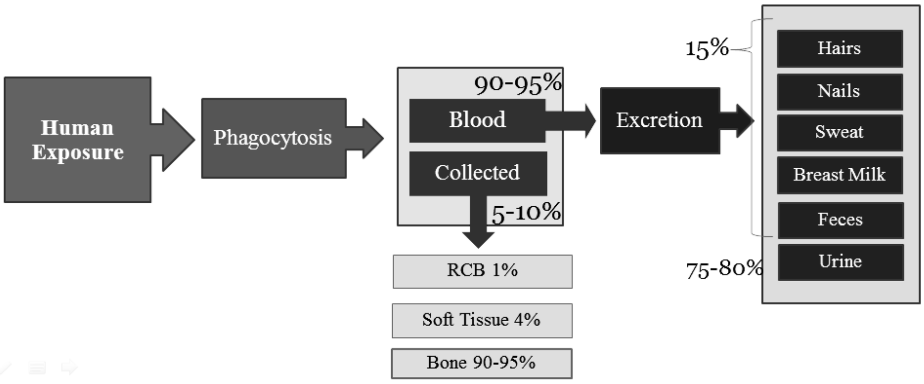

Figure 1. 2 Heavy metal excretion

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There are many adverse health effects from heavy metal exposure. In the example of arsenic (As), the health effects are generally delayed and found from low arsenic concentrations in the drinking water supply. Arsenicosis is the effect of arsenic poisoning that usually occurs over a long period of time such as 5 to 20 years. Drinking arsenic contaminated water over a long period results in various health effects, including skin problems, skin cancer, cancers of the bladder, kidney, and lung; diseases of the blood vessels in the legs and feet; and also type 2 diabetes mellitus, high blood pressure, and reproductive disorders. Not only arsenic, but also mercury (Hg), lead (Pb), and cadmium (Cd) can cause hypertension and diabetes. Mercury, cadmium, lead and other heavy metals have a high affinity for sulfhydryl (-SH) groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants, with subsequent decreased oxidant defense and increased oxidative stress (Houston, 2007; WHO, 2014; Wang et al., 2014).

1.2 Background and Justification

Intensive Agricultural Area in Ubon Ratchathani Province, Thailand

The study area is an intensive agricultural area, which the location of the biggest chilli pepper farm in Thailand. This site is located at Mueang district, Ubon Ratchathani province, in the Northeast of Thailand. This area has high agricultural activities and can produce a large amount of agricultural products, especially rice and chillies. The Hua Rua sub-district alone, produces about 4,000 tons of chilli and had an annual income of 50 million baht from chillies from 2004 to 2005 (Norkaew, 2009). For instance, most of the local farmers frequently overdose their crops with pesticides and fertilizers to achieve the highest levels of production. Therefore, multiple loads of pesticides and fertilizers are applied to crops and have led to a widespread accumulation of heavy metals in agricultural soils. When water runs downward through the soil, heavy metal might dissolve from such soil and release downward until reaching a shallow aquifer system. Consequently, many loads of pesticides and fertilizers are applied to add in this site. A case in point is this agricultural study area; it was found to be contaminated by heavy metals, as shown in Table 1.1.

Table 1. 1 Concentrations found in fertilizers samples

	Concentrations in Fertilizers (mg/kg)								
Name	**Al**	**Cr**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Yara Mila_S1	36.7	0.501	0.254	0.606	1.28	0.165	0.0013	0.000738	0.131
Yara Mila_S2	40.2	0.521	0.263	0.66	1.73	0.173	0.0013	0.0007	0.129
กระต่าย_S1	0.862	0.0408	0.0295	0.0209	0.24	0.0987	0.0013	0.0007	0.0356
กระต่าย_S2	1.06	0.034	0.0254	0.0284	0.224	0.0985	0.0013	0.0007	0.0283
Top One_S1	96.5	1.12	0.405	0.299	9.67	0.629	0.0013	0.00132	0.176
Top One_S2	71.6	0.751	0.246	0.222	6.82	0.42	0.0013	0.000761	0.103
อะโกรเฟต_S1	1920	2.75	0.799	0.478	2.16	0.483	0.0013	0.000925	0.501
อะโกรเฟต_S2	1990	2.22	0.647	0.0391	1.87	0.376	0.0013	0.000773	0.38
Ave.	519.61	0.99	0.33	0.29	0 .3	0.28	0.0013	0.0008	0.19

In addition, some studies of this agricultural area have found adverse health risks, both non-carcinogenic risks and cancer risks, to the local people living in the area, from the heavy metal contaminated groundwater (Wongsasuluk, 2010, Wongsasuluk et al., 2014). Furthermore, since the major drinking water source in this study area is groundwater, even low concentrations can cause adverse effects. Thus this study can serve as a representative of health risks and provide data to the database of biomarkers (hair, nail, urine) of local people who are exposed to low concentrations of heavy metal at a contaminating site.

1.3 Research Questions

- What are the heavy metal concentrations in the drinking water, hair, nails, and urine of local people in the agricultural area?
- Does the groundwater and non-groundwater drinking supplies place the local people at risk?
- Are socio-demographic, personal, exposure, and environmental factors related to the heavy metals concentrations in the biomarkers?

1.4 Research Objective

The main objective of this study is to investigate the risks of human exposure and associated factors related to the heavy metal contamination in drinking water in this agricultural area.

Sub-Objectives:

- To investigate and compare the heavy metal concentrations in the drinking water, hair, nails, and urine of the groundwater drinking group and non-groundwater drinking group.
- To measure the human health risks associated with the heavy metals contaminating the drinking water in the agricultural area.
- To find the relation between biomarkers of exposure and heavy metal concentrations in the drinking groundwater.
- To determine associations among socio-demographic, personal, exposure, and environmental factors and the biomarkers.

1.5 Research Hypotheses

- The heavy metal concentrations in the drinking water, hair, nails, and urine of the groundwater drinking group are higher than in the non-groundwater drinking group.
- Human health risks related to the heavy metal contaminations in the drinking water of the groundwater drinking group are higher than in the non-groundwater drinking group.
- There is a correlation between the heavy metal concentrations in the biomarkers and heavy metal concentrations in the groundwater drinking supply.
- Associations can be made between the socio-demographic, personal, exposure, and environmental factors with the heavy metal concentrations in the biomarkers.

1.6 Scope of the Study

1) Study Area

Heavy metals contaminated in a groundwater site: An agricultural area in Mueang district, Ubon Ratchathani province, that is at risk from the presence of heavy metals. The study duration was in March 2015 and October 2015.

2) Sampling Technique

2.1) A total of 100 local people (calculated based on preliminary results and the PS Program, Power and Sample Size Program: using preliminary data calculated the sample size and the result was n=26 per group, 100 people for covering sample size number.), who were the target participants in the study area, were randomly selected among those who permanently live in this agricultural area.

2.2) Face-to-face interviews were used to collect personal information.

2.3) Hair and finger nail samples of all participants were collected using stainless steel hair scissors and stainless steel nail clippers, respectively.

2.4) Urine was collected in glass bottles.

2.5) The drinking water of the participants, which was groundwater and tap water, was sampled from their residences.

3) Analytical Technique

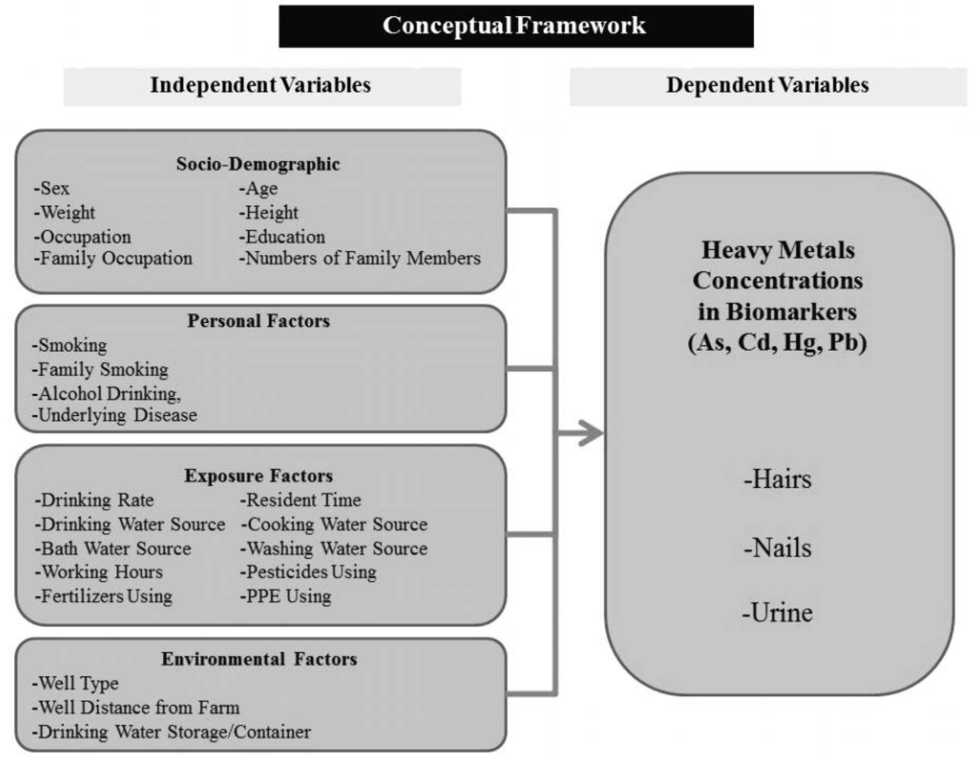
3.1) Sample preparation was performed following the International Atomic Energy Agency's recommendations (IAEA, 1985)

3.2) Microwave digestion was used to digest the hair and nails following the Milestone microwave digestion method.

3.3) Inductively coupled plasma mass spectrometry or ICP-MS (method following the Milestone Laboratory System, ETHOS) was used to find out the concentrations of arsenic, cadmium, lead, and mercury in the drinking water samples, hair and nails.

3.4) An atomic absorption spectrophotometer (AAS), based on the standard process of the American Conference of Governmental Industrial Hygienists (ACGIH), was used to find the concentrations of arsenic, cadmium, lead, and mercury in the urine.

1.7 Conceptual Framework



1.8 Operational Definition

Heavy Metals

The heavy metals in this study were defined as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). The study tried to identify the concentrations of these four heavy metals in biomarkers and drinking water by using a specific method of analysis in the laboratory.

Biomarkers

The biomarkers in this study are hair, finger nails, and urine; they were used to monitor the presence of heavy metals in the human body.

Participants

The participants in this study were randomly selected from the group of local volunteers who permanently live within study site, under inclusion and exclusion criteria.

Personal Protective Equipment (PPE)

The use of personal protective equipment included rubber gloves, a mask, boots, a hat and coveralls, to protect the human body and decrease exposure doses.

Drinking Water

Drinking water in this study was divided into two types: groundwater from a local shallow groundwater well and non-groundwater (i.e., local tap water).

Exposure Group

Native-born local people who generally drink groundwater and have lived in the study area for a long period of time.

Non-Exposure Group

Native-born local people who do not drink groundwater and have lived in study area for a long period of time.

Independent Variable

This study divided independent variables into four groups: socio-demographic factors (sex, age, weight, height, drinking rate, education, occupation, family occupation, number of family members), personal factors (smoking, family smoking, alcohol drinking, underlying diseases), exposure factors (resident time, drinking water source, cooking water source, bath water source, washing water source, working hours, pesticides use, fertilizer use, PPE use), environmental factors (well depth, well distance from a farm, drinking water storage or container).

Dependent Variables

In this study, dependent variables were defined as the heavy metal concentrations in the biomarkers, which were hair, finger nails, and urine.

1.9 Research Expected Outcomes

1. The heavy metal concentrations in the drinking water, hair, nails, and urine of the groundwater drinking supply group and non-groundwater drinking supply group.
2. A human health risk assessment associated with the heavy metals contaminating the drinking water in the agricultural area.
3. The correlation between heavy metal concentrations in the biomarkers and heavy metal concentrations in the groundwater drinking supply.
4. The associations between the socio-demographic, personal, exposure, and environmental factors and the heavy metal concentrations in the biomarkers.

CHAPTER II

LITERATURE REVIEW

2.1. Biomarkers

A **biomarker** is a measurable biological characteristic that indicates the concern severity or disease presence, and biomarker can be a substance that is presented into an human organism as a means to investigate organ function or other health appearances. Biomarkers are characteristic biological properties that can be find out and measured in parts of the body like the blood or tissue. Bio-monitoring is the assessment method of human chronic exposure to toxic and non-toxic metals. Due to the difficulties in sampling of invasive matrices, such as the blood or tissues of internal organs, non-invasive markers of exposure (urine, hair, nails) seem to be good alternative indicators of determining the potentially toxic doses of metals (Marcin et al., 2011).

2.1.1 Blood and Urine

Metals entering the human body are accumulated and diminish various physiological organ functions. Many diseases are associated with assessed metal presence in the body. Their bio-monitoring is necessary for assessing harm to the human body. Blood is the most regularly sampled to assess metal content. Analysis of heavy metals concentration in the blood is best suited for current high dose exposures. It could also be used to evidence chronic exposure. There has been found to be a temperate relationship between concentration of heavy metal in drinking water and in blood. Blood is a more difficult matrix to work with than urine, and blood samples are more difficult to gain for epidemiological study because of invasive sampling. Urine is the biomarker that has been the frequently used in epidemiological researches. The heavy metals level in urine has regularly been used as an indicator of current exposure because urine is the main route of excretion but total heavy metal analysis may also get interference from some foods such as seafood that has been recently ingested (Nathalie et al., 2012). In summary, urine, the main route of excretion and produced by the kidneys, contains the byproducts of metabolism--salts, toxins, and water--which end up in the blood. The urinary tract (Figure 2.1), which includes the kidneys, ureters, bladder, and urethra, filters and eliminates the waste substances from the blood.

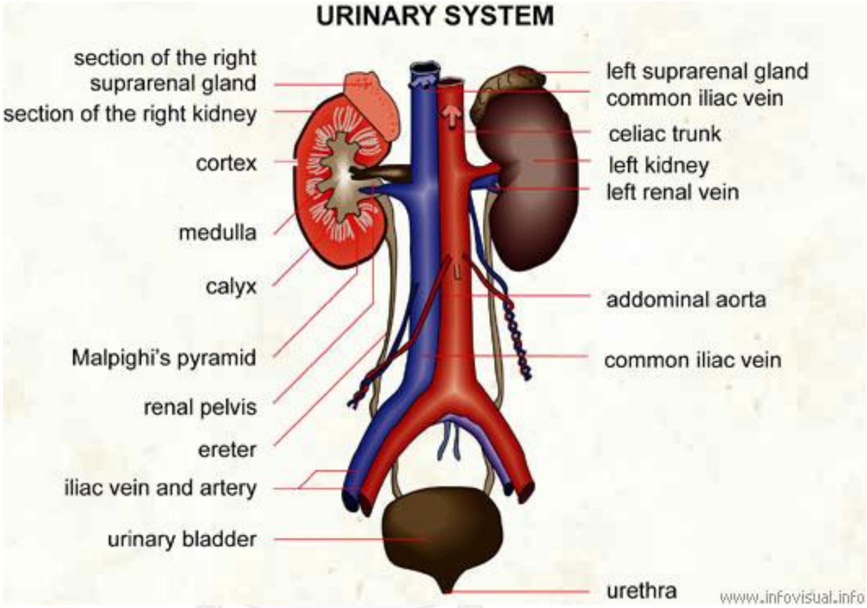


Figure 2. 1 Urinary System

Although blood and urine analyses are the most traditional approaches, they fluctuate in response to any change in physiological or environmental conditions. Hair and nails can provide a more permanent record of heavy metals associated with normal and abnormal metabolism and assimilation from the environment. In addition, hair and nails are easily collected, conveniently stored, and can be readily analyzed. Analysis of human hair and nails has thus become an important approach to understanding quantitative changes in certain elements inside the body (Krystyna, 2006).

2.1.2 Hair and Nails

The human biological materials accessible for sampling include blood and urine, but also hair and nails, which are derived from the ectoderm. Both hair and nails are composed of compact protein, hard keratin (Figures 2.2 and 2.3). Hair has a high affinity for metals, due mainly to the cystine that makes up approximately 14% of its total composition. Many metals found in hair are bound to sulphur atoms in cystine or to sulphydryl (SH) groups in other amino acids (Katz, 1988). Hair is substantially a cross-linked, part of crystalline, oriented, polymeric network that carries functional groups (e.g., acids, basic and peptide bonds) competent of compelling tiny molecules. Metals can bind to the hair structure through melanin. The quality and type of melanin incorporated into the hair shaft determine hair color. Melanins are polyanionic polymers containing negatively charged carboxyl groups and semiquinones at physiological pH. In consequence, they can bind cations by ionic interactions. Organic amines and metal ions have a high melanin affinity, because they are positively charged at physiological pH and interact with the melanin polymer by electrostatic forces between their cationic groups and the negative charges in the melanin polymer. The ionic binding may also be enhanced by other forces such as van der Waals attraction. Uncharged metals, such as elemental Hg, may also bind to the hydrophobic core of the melanin polymer in hair (Krystyna, 2006).

The measurement of concentrations of trace elements in hair is often used to assess heavy metal exposures in domestic environments. Because of the ease with which samples can be collected, transported, stored, and analyzed, hair analysis is valuable in screening individuals and populations for exposure to these metals. It can therefore be used to evaluate this exposure in an occupational environment and can serve as a screening test for heavy metal systemic poisoning (Krystyna, 2006). Krystyna and team reported that In healthy individuals, the concentration of Pb in hair may be from two to five times higher than in bone, approximately 10-50 times higher than in blood, and from 100 to 500 times greater than that in excreted urine. In the same way, the research of Marcin and team reported that hair is highly mineralized compared to blood and urine; for instance, the content of lead (Pb) is 10–50 and 100–500 times higher, respectively (Marcin et al., 2011).

Hair Anatomy

The cortex is the main component of hair shaft, which consists of melanin and keratin.

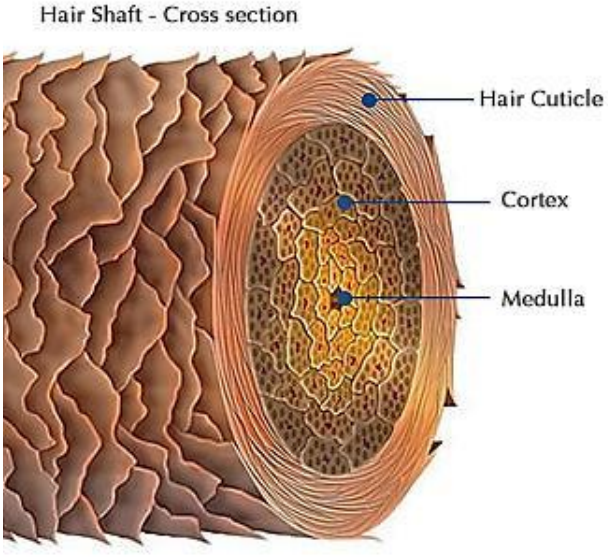


Figure 2. 2 Hair Anatomy (Bioxet, 2014)

Nail Anatomy

The nail plate consists of hard translucent keratin. The bulk of the keratin is derived from the lunula and nail matrix. Finger nails grow at average of 0.1 mm/day and a normal fingernail takes about 6 months to grow out completely. Toenails grow one-half to one-third the rate of finger nails; they are estimated to grow 0.03-0.05 mm/day and take 12-18 months to grow out (Melissa and Nriagu, 2006).

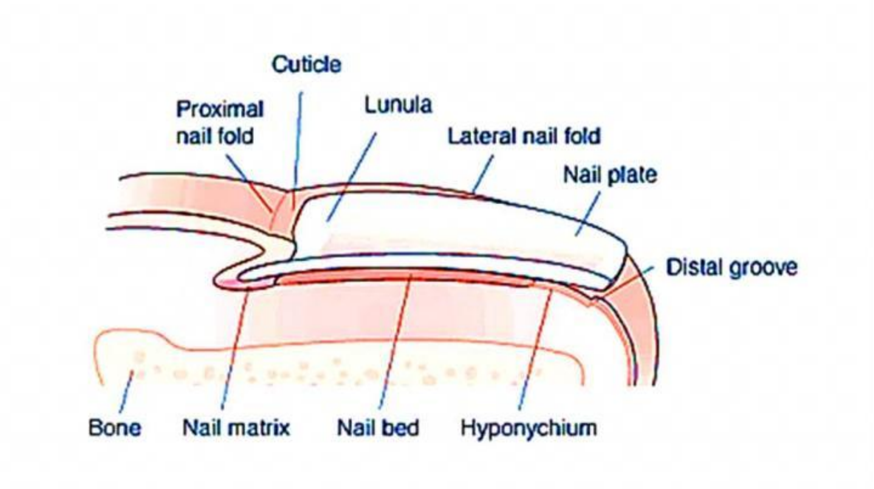


Figure 2. 3 Nail (Thailabonline, 2014)

Advantages of using hair and nails as biomarkers

1. Certain toxic metals accumulate or bio-concentrate in hair.
2. Elements especially arsenic (As) and mercury (Hg) are retained for many years and do not decrease as they do in blood and urine. Samples several hundred years old can be analyzed.
3. Samples are easily obtained with minimum legal problems–noninvasive method.
4. Hair and nails require only polyethylene bags or simple containers for storage, in a dry place, at room temperature. They do not require any refrigeration for storage or during transport. They need only to be protected from external contamination after collection.
5. Samples are easily transported because of their light weight and the fact they cannot leak like a fluid.
6. Standardized methods are available for the collection, transportation, storage, washing, preparation, and analysis of the samples.

7. Basic sampling equipment is required and the collection process is simple; no medical staff is required.

From the advantages and given and their ability to accumulate elements in their keratinous structures, human hair and nails can be considered reliable indicators of imbalances in the mineral content in the human body and reflect an individual's health status as integrated over a period of about half year (Pazirandeh et al., 1998, Sreenivasa Rao et al., 2002, Krystyna, 2004, Krystyna, 2006).

2.2 Heavy Metals

2.2.1 Health effects from heavy metal toxicity

The adverse health effects from exposure to toxic heavy metals are generally classified into two types: **acute effects** (which ranges from 14 days or less to intermediate effects at 15-354 days) and **chronic effects** (which lasts for more than 365 days) due to long term exposure and a long period of effect (Agency for Toxic Substances and Disease Registry, 2005). In addition, acute toxicity is normally from a sudden or unexpected exposure to high concentration heavy metal (e.g., from careless handling and contact, insufficient safety precautions, or an accidental spill or release of toxic material often in a laboratory, industrial, or transportation setting). Chronic toxicity results from repeated or continuous exposure, leading to an accumulation of the toxic substance in the human body. Chronic exposure may effect from contaminated food, air, water, or dust; living near a hazardous waste site; spending time in a contaminated area; maternal transfer in the womb; or from participating in hobbies that to contact with heavy metals or contaminated objects. Chronic exposure may happen in either the home or workplace. Symptoms of chronic toxicity are often similar to many common conditions and may not be suddenly recognized. Three main routes of human exposure comprise inhalation or breathing, dermal or skin or eye contact, and oral ingestion (WHO, 1998, International

Occupational Safety and Health Information Centre, 1999, Roberts, 1999, Dupler, 2001, Ferner, 2001, Wongsasuluk, 2010).

Table 2. 1 Human health effects from heavy metals

Heavy Metal	Human Health Effect	Oral RfD
As	Hyperpigmentation, keratosis and possible vascular complications (chronic effect)	2.3×10^{-6} mg/kg-day
Cd	Fragile bones, alopecia, anemia, migraines, growth impairment, and cardiovascular disease (chronic effect)	5.0×10^{-4} mg/kg-day
Cr	Gastroenteritis, yellow-green vomitus, hematemesis, hepatic necrosis, renal failure (acute effect)	3.0×10^{-3} mg/kg-day
Cu	Nausea, vomiting, diarrhea, liver damage, and kidney damage (chronic effect)	40 mg/kg-day
Hg	Digestive disturbances (acute effect) brain and kidney damage (chronic effect)	3.0×10^{-4} mg/kg-day
Pb	Mood swings, nausea, numbness, seizures, and weight loss (chronic effect)	3.5×10^{-3} mg/kg-day
Ni	Decreased body and organ weights (Chronic effect)	2.0×10^{-2} mg/kg-day
Zn	Stomach cramps, nausea, and vomiting (acute effect) anemia, pancreas damage (Chronic effect)	0.3 mg/kg-day

2.2.2 Bio-monitoring heavy metals

Assessment of the health hazards of environmental pollution is increasingly a major public health concern. Twenty metals are known to be toxic, and 10 of them are noxious to the human body because of their widespread production and use and their subsequent discharge and persistence in the environment. The toxic metals, upon accumulation in the human body, are detrimental to health in many respects. The 10 toxic metals are: Sb, As, Cr, Co, Cu, Pb, Hg, Ni, Sn, V, and Se. Of these, five metals, Pb, Cd, Hg, As, and Ni pose a threat to humans (Krystyna, 2006). This study will focus on four elements: arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). These heavy metals can cause many adverse health effects to human even when present in low concentrations.

Arsenic (As)

Arsenic is the most common cause of adverse health effects in humans because it can cause both cancer and non-cancerous effects. Arsenic may be also found in water supplies worldwide and thus widely affect humans. Target organs are the blood, kidneys, and central nervous, digestive, and skin systems. Symptoms of acute arsenic poisoning are sore throat from breathing, red skin at contact points, or severe abdominal pain, vomiting, and diarrhea, often within one hour after ingestion. Other symptoms are anorexia, fever, mucosal irritation, and arrhythmia. Cardiovascular changes are often subtle in the early stages but can progress to cardiovascular collapse. Chronic or lower levels of exposure can lead to progressive peripheral and central nervous changes, such as sensory changes, numbness and tingling, and muscle tenderness. A symptom typically described is a burning sensation ("pins and needles") in the hands and feet. Neuropathy (the inflammation and wasting of the nerves) is usually gradual and occurs over several years. There may also be excessive darkening of the skin (hyperpigmentation) in areas that are not exposed to sunlight, excessive formation of skin on the palms and soles (hyperkeratosis), or white bands of arsenic deposits across the bed of the fingernails (usually 4-6 weeks after exposure) (Roberts, 1999, Wongsasuluk, 2010).

The toxicity of arsenic decreases in the following order: arsine, inorganic As (III), organic As (III), inorganic As (V), organic As (V), arsonium compounds and elemental arsenic. Symptoms of As poisoning include diarrhea, vomiting, headaches, drowsiness, and convulsions. It should be noted that arsenic is distributed in all human tissues at levels ranging from 0.01 to 0.009 ppm wet weight down to a few ppt (parts per trillion, ng/g) in biological fluids. According to a research report (Rahman et al., 2000), normal hair contains small quantities of arsenic, from 50 to 400 ppm, but the level increases greatly during excess As intake. Its accumulation in hair during exposure is valuable in diagnosing arsenic poisoning.

Cadmium (Cd)

Cadmium can be mostly found in soils because insecticides, fungicides, sludge, and commercial fertilizers that use cadmium are used in agriculture. Inhalation accounts for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Target organs are the liver, placenta, kidneys, lungs, brain, and bones. Symptoms of acute cadmium exposure are nausea, vomiting, abdominal pain, and breathing difficulty. Chronic exposure to cadmium can result in chronic obstructive lung disease, renal disease, and fragile bones. Symptoms of chronic exposure could include alopecia, anemia, arthritis, learning disorders, migraines, growth impairment, emphysema, osteoporosis, loss of taste and smell, poor appetite, and cardiovascular disease (Roberts, 1999, Wongsasuluk, 2010). There is a study that confirms the effects of cadmium contamination in environment on humans. The report showed that cadmium is found in hair. A study in Poland found that the mean levels of cadmium (Cd) in hair were significantly higher in exposed children (0.44 ppm), those living in contaminated regions of the Copper Basin in Poland (around Legnica), than in the control group (0.23 ppm) (Krejpcio et al., 1999).

Lead (Pb)

Lead accounts for most of the cases of pediatric heavy metal poisoning. Target organs are the bones, brain, blood, kidneys, and thyroid gland. Acute exposure to lead is also more likely to occur in the workplace, particularly in manufacturing processes that include the use of lead (e.g., where batteries are manufactured or lead is recycled). Symptoms include abdominal pain, convulsions, hypertension, renal dysfunction, loss of appetite, fatigue, and sleeplessness. Other symptoms are hallucinations, headaches, numbness, arthritis, and vertigo. Chronic exposure to lead may result in birth defects, mental retardation, autism, psychosis, allergies, dyslexia, hyperactivity, weight loss, shaky hands, muscular weakness, and paralysis (beginning in the forearms). In addition to the symptoms found in acute lead exposure, symptoms of chronic lead exposure could include allergies, arthritis, autism, colic, hyperactivity, mood swings, nausea, numbness, lack of concentration, seizures, and weight loss (International Occupational Safety and Health Information Centre, 1999, Roberts, 1999, Wongsasuluk, 2010). There is a study that showed the influence of lead contamination in the environment on human. For example, according to Schuhmacher *et al.* family occupation (printers, mechanics, drivers, metal workers, machinists, and other related technical occupations) was one of the most significant factors influencing the lead content of children’s hair (Schuhmacher et al., 1991, Lekouch et al., 1999). Although the average Pb content was higher in an industrial area (9.38 ppm) than in an agricultural (non-exposed population) area (7.80 ppm), this difference was not statistically significant. Lekouch *et al.* also observed that Pb and Cd concentrations were significantly lower in the hair of children living in the control area than in a wastewater spreading field. This may be due to the fact that levels of trace elements in water, soil, plants, and animals of the municipal wastewater spreading field are very high. Children of lead workers may be exposed to lead dust transported home from the workplace on parents' shoes and clothing.

Mercury (Hg)

Mercury is a naturally occurring element that is found in air, water and soil. Exposure to mercury, at even small amounts, may cause serious health problems and is a threat to the development of a child. High exposures to mercury may result in damage to the gastrointestinal tract, the nervous system, and the kidneys. Both inorganic and organic mercury compounds are absorbed through the gastrointestinal tract and affect other systems via this route. However, organic mercury compounds are more readily absorbed via ingestion than inorganic mercury compounds. Mercury may have target toxic effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes. Symptoms of high exposures to mercury include skin rashes, dermatitis, memory loss, mental disturbances, tremors, emotional changes (e.g., mood swings, irritability, nervousness, and excessive shyness), insomnia, neuromuscular changes (such as weakness, muscle atrophy, and twitching), headaches, disturbances in sensations ("pins and needles" feelings, usually in the hands, feet, and around the mouth), changes in nerve responses, performance deficits on tests of cognitive function, lack of coordination of movements, and the impairment of speech, hearing, and walking. At very high exposures there may be kidney effects, respiratory failure and even death (USEPA, 2014). As mentioned, the target organ of mercury is the kidney; there has been a study in Sweden about the relation between trace elements in human hair and internal organs. This research reported that a positive correlation of high significance was observed between the mercury concentrations in hair and the kidney cortex (Muramatsu and Parr, 1988).

2.3. Related Studies

Medical research uses hair analysis to diagnose disease conditions and to define relationships between the concentrations of metals and various diseases. Thus scientists examine hairs to discover forms of poisoning due to the ingestion of abnormal doses of metals and try to identify the areas that require attention, due to the potential exposure of resident populations to metals and also to ascertain the occupational exposure to them. Despite the potential of hair analysis to quantify the relationship between human exposure and metal contamination, and although current analytical techniques have recently improved remarkably in determining the presence of metals in biological matrices, critical points still exist as regards to the interpretation of results and processing of reference values according to gender, age, ethnic origin, lifestyle and geographical area of residence (Dongarrà et al., 2012). Chemical elements accumulate and are eliminated by different routes, including the hair, where their levels can be determined. The level of trace elements is at detectable levels because hairs are highly mineralized tissues and can be sampled in relatively high mass. The literature presents hair as a suitable biomarker of exposure to many toxic elements: Ag, Al, As, Au, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Tl, and Zn. Since it is well documented that hair's mineral composition reflects chronic exposure, it has been applied in environmental bio-monitoring and occupational and forensic medicine (Michalak et al., 2012).

Assessing the effects of toxic heavy metals for past and long-term exposure requires the identification of a reliable biological indicator. Heavy metals in blood are widely used as indicator for current or recent exposure. Compared to other types of clinical specimens, scalp hair has different uses and even advantages over blood or urine, and each centimeter of scalp hair reflects approximately one month of past exposure. A considerable number of studies have revealed that head hair better reflects long-term environmental exposure than does blood and/or urine for toxic metals (Razi et al., 2012). Furthermore, hair sample can be a useful assessment tool as the measured contaminant levels can reflect exposure over a long-term period (weeks to years depending on hair length) and certain elements may be present in high concentrations in hair strands. This can be compared to blood and urine which often reflect the most recent exposures, and might only contain minute amounts of the chemicals of interest, making analysis a difficult challenge. Scalp hair has been used for the bio-monitoring of heavy metals on large cohorts, determining occupational exposure and exposure to local habitants in polluted areas (Wang et al., 2009).

Using blood as a biomarker for heavy metals exposure such as arsenic presented current accumulates in the hairs and nails. In addition, the hair and nail component and matrix becomes isolated from other metabolic processes in human body, sampling human tissues was an interesting alternative way with which to monitor long-term exposure in the past. Hair growth was more fast than nails and represented exposure that has happen for a months before collection. Finger nails growth was quicker than toenails, take an average of 6 months to grow out completely, and so can present exposure over a slightly earlier time period than hair samples. Many studies have shown that As in toenail and hair was good correlate with As concentration in drinking water (Andrew et al., 2008).

Moreover, blood plasma and hairs could be used to observed and compared the trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), Nickel (Ni), lead (Pb), and zinc (Zn) in gastrointestinal cancer patients and a control group. Although the correlation revealed considerably different patterns of trace elements in the patients and controls because of the uncontrolled growth of cells in patients accumulating more minerals to cope with requirements of the excessively dividing cells, the results showed that the average hair concentrations of Zn, Fe, Pb, Cu and Cd were notably higher in the patients than in the controls.

Pazirandeh and team measured arsenic concentrations in the hair of three groups in a village in western Iran. The value for the healthy population was 0.22 ppm. The distribution for the group with suspected arsenic poisoning showed that 75% had As levels in scalp hair (2.93 ppm) more than 20 times higher than the average of the healthy subjects. The distribution for the group with arsenic poisoning was also abnormal; it ranged between 0.5 and 14 ppm with an average value of 5.9 ppm (Pazirandeh et al., 1998). Similarly, a study in India measured hair As in victims from an As-affected area of West Bengal, India. Their study showed lower levels of Zn and Se in hair, a deficit that may aggravate As toxicity. The Mn, Ni, and Pb concentrations in tissue samples were high. In West Bengal, approximately 97% of the population drank 4-6 litres of water per day from wells and ate 750-1,000 g of rice with vegetables per day, both grown in water that was highly contaminated by As. They thus found that the drinking water and food were the primary sources of environmental exposure (Gautam et al., 2004). In the same way, Bozsai clearly shows that increased arsenic levels in the drinking water increased the As levels in hair. He found that subjects consuming water with 11-25 lg/L of As had elevated levels (2.1-3.0 mg/kg) of accumulated As in their hair. Hazardous levels (>3.0 mg/kg) in the hair could be found among those drinking water containing 51-75 lg/L of As. Finally, when As levels in drinking water exceeded 100 lg/L, there was a 10% rate of hazardous As accumulation in hair, and when they exceeded 200 lg/L, the rate reached 34% (Bozsai, 1992).

Although a review of the best biomarker to assess arsenic exposure via drinking water (Nathalie et al., 2012) suggests that arsenic in the hair and nails reflects the level of long term exposure, it was difficult to relate the level with the dose ingested, so they conclude that the urinary and nail biomarkers were useful to provide indications of internal doses for epidemiological studies. Not only heavy metal investigations were performed with hair but also acid: a researcher in China studied how hair, nails, and urine can be used for bio-monitoring of human exposure to perfluorooctane sulfonate and perfluorooctanoic acid and found a correlation among them. Nail was found to have a greater potential than hair and urine to be applied in human bio-monitoring of PFOS and PFOA in general populations (Jingguang et al., 2013).

A study in Japan was performed on the concentrations of mercury in hair, toenails, and urine related to fish intake rates. Their participants were women who were free from occupational exposure, and their estimated daily mercury intakes from fish and other seafood, using a food frequency questionnaire, found a positive correlation. They concluded that mercury resulting from fish consumption could explain total mercury levels in hair, toenails, and urine. This study's results supported the relationship between the heavy metal intake rate and heavy metal concentration in humans (Tomoko et al., 2007).

In Poland, human hairs were investigated to determine the possibility of using human hair as a biomarker with particular emphasis on persistent organic pollutants or POPs (Sylwia et al., 2013). Human hair and nails was reported to provide important analytical information on exposure to heavy metals such as mercury and lead; also nails were used to determine arsenic. In addition, this study showed the advantages of using human hairs as biomarker although invasive tissue and blood were considered a perfect sample for monitoring. The invasive sampling procedure may pose a risk to donors and adipose tissue is most likely to be obtained during medical surgery, while noninvasive biological samples such as hair cost less to sample because it does not require restricted measurements and the presence of qualified medical staff. Moreover hair has the possibility of long-term storage and a wide range of information on short-term and long-term exposure due to it being primarily being composed of keratin and its stability.

A study about biological and behavioral factors of biomarkers for arsenic exposure in a U.S. population reported that the concentrations of arsenic in the environment had effects on the concentrations of arsenic in urine and nails. The direct consumption of water, use of water in food preparation, and increasing tap water arsenic concentrations and consumption were associated with significant upward trends in the total arsenic concentrations in urine and nails (Rebecca et al., 2013). In addition, the research about chemical presence in the food web of freshwater ecosystems showed low concentration levels of DDT and derivatives in each food web compartment (i.e., water, sediment, aquatic plant, plankton, fish, and invertebrates). Magnification patterns (such as bioconcentration, bioaccumulation, and biomagnification) based on habitat and foraging behaviors of selected freshwater species indicated that DDT and derivatives can accumulate and be magnified through the food chain from the lowest up to the highest trophic level. The conclusion supported that chemicals contaminated in the environment can affect the biota via the food web (Siriwong et al., 2009).

Research related to the influences of groundwater arsenic and the consumption period in Cambodia focused on an analysis of the arsenic levels in human hair samples collected from villages in the Kandal province of Cambodia. It found a linear relationship between arsenic concentrations in human hair and in the local groundwater where arsenic (III) is the dominant species (Suthipong et al., 2010). Similarly to other studies in Cambodia, Jamal studied the use of hair arsenic as a biomarker not only for arsenicosis-related signs but also for associated symptoms. This study reported that contaminated groundwater from tube wells led the Kandal province to be a high arsenic-contaminated area; it moreover found that the most prevalent sign of arsenicosis was hypomelanosis, with a prevalence of 14.5% among all respondents and 32.4% among respondents with a hair arsenic levels of ≥1 μg/g. (Jamal et al., 2013).

In addition, research in Pakistan (Muhammad et al., 2009) found that hair could investigate not only arsenic’s effect from the direct consumption of contaminated groundwater but also from testing plants that grow in the area. A relationship between arsenic exposure through respiratory disorders in smokers from drinking water and smoking cigarettes made from tobacco grown in agricultural land irrigated by arsenic contaminated lake water was found: the arsenic levels in local cigarette tobacco were three- to four-folds higher than branded cigarettes. Furthermore, a review of the validity of human nails as a biomarker of arsenic found a case-control study that showed the relationship between toenail arsenic concentrations and bladder cancer risk among smokers, and also the increased risk of melanoma (Melissa and Nriagu, 2006). In the same way, a report from India that studied the arsenic contaminated in groundwater in West Bengal, India, with urine, hair, and nails found that 83% and 68% of the urine samples (n = 250) contain arsenic concentrations above 100 and 200 ug/L, respectively. Very good correlations linear regression between arsenic concentrations in water versus urine, hairs and nails samples were reported (Roychowdhury, 2010).

Hongmei (2011) studied urinary heavy metal levels among people exposed to an e-waste dismantling area and compared them to a green plantation area in China. This report discovered that both the occupational dismantling area people and non-occupational dismantling area people were higher than the control group. Furthermore, the correlations between urinary heavy metal levels and exposure factors in the exposure group revealed a positive relationship between the durations of dismantling and the levels of lead (Pb). Meanwhile, rice sources from a local village had a positive Pearson correlation with the levels of lead (Pb) and cadmium (Cd) (Hongmei et al., 2011). Likewise, research was performed on the urinary levels of arsenic and heavy metals in children and adolescents living in an industrial area of the Ria de Huelva, in Spain. Urine was used for bio-monitoring their exposure to arsenic and some heavy metals such as cadmium, chromium, copper, and nickel and compare them with the reference group formed by the remaining capital cities of the Andalusian provinces with small or non-existing industrial sources of heavy metal pollution nearby. This report recorded that in the Ria de Huelva, although the main determinants of the inter-individual variations in urinary metals were age, sex, area of residence, and frequency of intake of certain food items (mainly fish and shellfish), there were no significant differences in the concentrations of metal compounds between the case study and reference groups (Inmaculada et al., 2010).

A study in the United Kingdom aimed to understand arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of three different unexposed ethnic groups: Somali Black-Africans, Asians and Whites. The results presented that there was a significant difference in the arsenic levels in the fingernail and urine samples of one particular ethnic group compared to the other two groups even though they all resided in the same city (Eid et al., 2006). Likewise, research about trace elements in the scalp hair of children chronically exposed to volcanic activity at Mt. Etna, Italy, reported on the difference between living in towns located around the volcanic area and a reference area. The results revealed that the young people living in the Mt. Etna area were naturally exposed to enhanced intakes of arsenic (As) compare to individuals of the same age residing in other areas of Sicily characterized by different lithology not influenced by volcanic activity. The petrographic nature of the local rocks and the dispersion of the volcanic plume explain the differences. The most probable exposure pathways are ingestion through water and local food (Varrica et al., 2014).

Another study found that adults in the Nord-Pas de Calais region of France who located within 1 km of non-ferrous metal smelters had greater blood-lead levels than other who living fay away more than 1 km (Leroyer et al., 2001). To study the factors affecting cadmium burden and especially its association with industrial cadmium sources, this research measured this burden in children living in the same polluted area and in controls in areas without this type of soil pollution. They concluded that cadmium concentrations in both the blood and urine were higher among children living within 4 km of the zinc smelter than those living farther away.

As mentioned, hair and nails can be used as biomarkers for past exposure in humans and show long period exposure, which can cause chronic effects. In addition, urine studied to represent daily excretion. Moreover, correlations and comparisons between the heavy metal concentrations in hair, nails and urine could be the same or different due to the period of exposure. This research was focused on people who reside in intensive agricultural areas; it would study locals who live and work close to heavy metal contaminant exposure sites such as contaminated groundwater in agriculture areas, which produce rice and chillies, for example. This study compared exposure participants, who have been exposed to heavy metals over long periods of time through their groundwater drinking supply, with non-exposure or non-groundwater drinking participants. This study endeavors to find out the relationship between heavy metal accumulation in urine, hair, nail as a biomarker of exposure and heavy metals contaminating the groundwater drinking supply.

Table 2. 2 Heavy metals found in biomarkers from previous research

References	**Country**	**Site**	**Metal**	**Ave.Conc.±SD (Range)**		
				Hair (ug/g)	**Nails (ug/g)**	**Urine (ug/g creat.)**
Eid et al. (2006)	UK	Urban Area	As	0.12	0.18	19.3±24.5 (71.2-6.4)
Catherine et al. (2014)	Belgium	Urban Area	Cd	0.38		0.18 (0.77-0.006)
Katarzyna C. et al. (2010)	Poland	Industrial Area	As	0.33±0.83 3.96-0.65))		
			Cd	0.06±0.09 (0.49-0.05)		
			Hg	0.16±0.21 (0.80-0.03)		
			Pb	1.69±3.08 (10.89-0.00)		
Gautam et al. (2004)	India	As-rich Groundwater Area	As	0.73±3.43 (14.39-0.17)	1.28±7.24 (36.63-0.74)	
			Cd	0.17±0.40 (2.14-0.00)	0.09±0.32 (1.93-0.02)	
			Hg	0.08±0.88 (3.0-0.19)	0.04±0.45 (1.32-0.18)	
			Pb	1.56±8.03 (41.71-0.57)	2.04±10.99 (52.56-1.19)	

References	Country	Site	Metal	Hair (ug/g)	Nails (ug/g)	Urine (ug/g creat.)
Andrew et al. (2008)	Cambodia	As-rich Groundwater Area	As	0.52±2.43 (7.95-0.26)	0.33±1.96 (4.95-0.53)	
Li et al. (2011)	China	Mining Area	Hg	47.2±43.5 (123.0-6.28)		1140±698 (3190-22.5)
Wang et al. (2009)	China	Waste Recycling Area	Pb	96.4±85.3 (730-1.93)		
Varrica et al. (2014)	Italy	Volcanic Area	As	0.02±0.03 (0.16-0.00)		
			Cd	0.02±0.02 (0.11-0.00)		
			Pb	0.8±0.84 (5.07-0.00)		

CHAPTER III

METHODOLOGY

3.1 Study area

The study area is within a heavy agricultural area in Mueang district, Ubon Ratchathani province, in Northeast Thailand, at a longitude of 1695000-1704000 UTM and a latitude of 479000-469000 UTM (Figure 3.1). It was selected for its heavy metal contaminated groundwater. This site has been continuously and intensively farmed over a long period of time and remains one of the largest chilli farms in Thailand (Norkaew, 2009). This area has intensive agricultural activities and produces large amounts of agricultural products, especially rice and chillies. From the continued long-term agricultural activities, heavy metals have had the chance to reach deep into the soil and contaminate the surface water and also groundwater, which can put people at risk of adverse health effects (Chotpantarat et al., 2014). Local people consume shallow groundwater by pumping water up that has been exposed to heavy metal contamination.

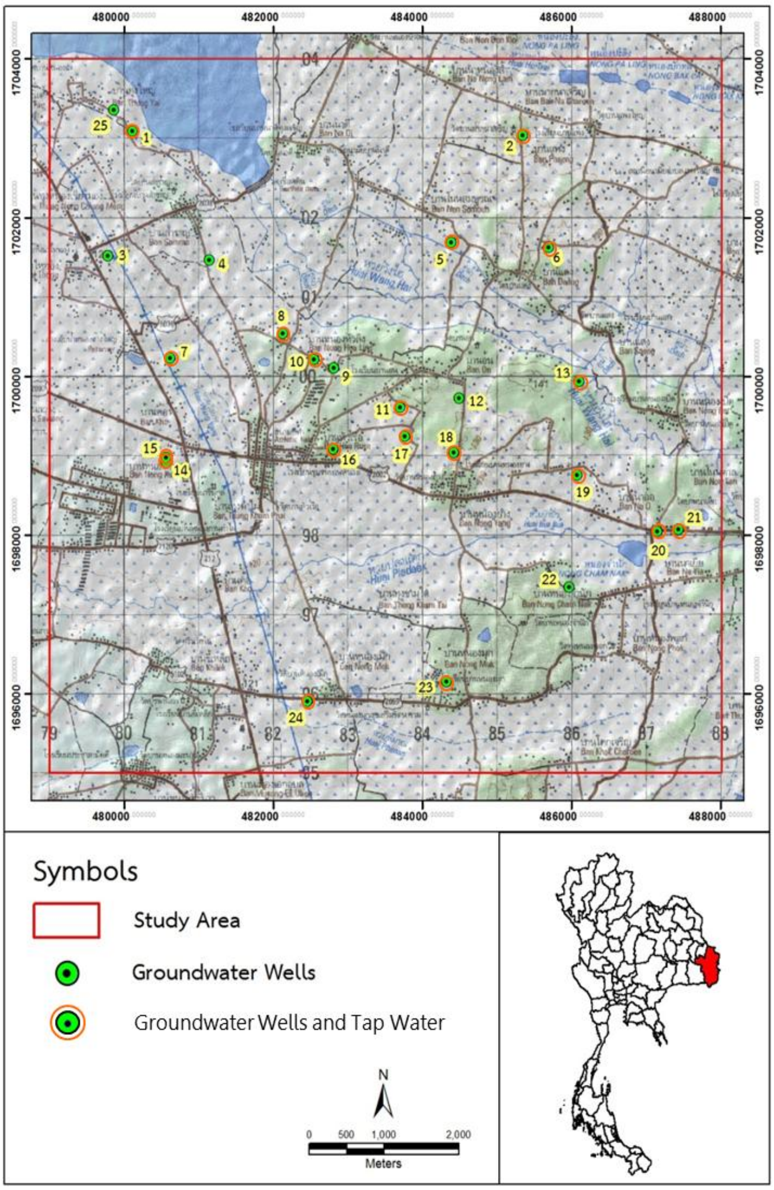


Figure 3. 1 The agricultural study area in Ubon Ratchathani province

Groundwater is the major water source of local people in this study area, and not only are there heavy metal contamination problems but also the pH of the groundwater is unsuitable for drinking. Srithongdee (2009) studied the distribution of pesticides in a shallow groundwater aquifer in an agricultural area of Hua Rua, Ubon Ratchatani, and found that the pH values of the shallow groundwater ranged from 3.68 to 4.88, and pesticides were not found in the shallow unconfined aquifer, probably indicating that the pH had been affected by the fertilizer applications. Similarly, Wongsasuluk (2010) studied the acidity of shallow groundwater and the average pH of shallow groundwater in this area was 4.72±1.09. In addition, another study from this study area identified adverse health risks, both non-carcinogenic risks and cancer risks (Figure 3.3) from heavy metals contaminating the groundwater of the local people living within this agricultural site (Wongsasuluk, 2010, Chotpantarat et al., 2014, Wongsasuluk et al., 2014). As mentioned, in terms of the hydrogeological characteristics, intensive farming appears to have the potential to incite the movement of agrochemicals from the ground surface to shallow groundwater systems (Figure 3.2).

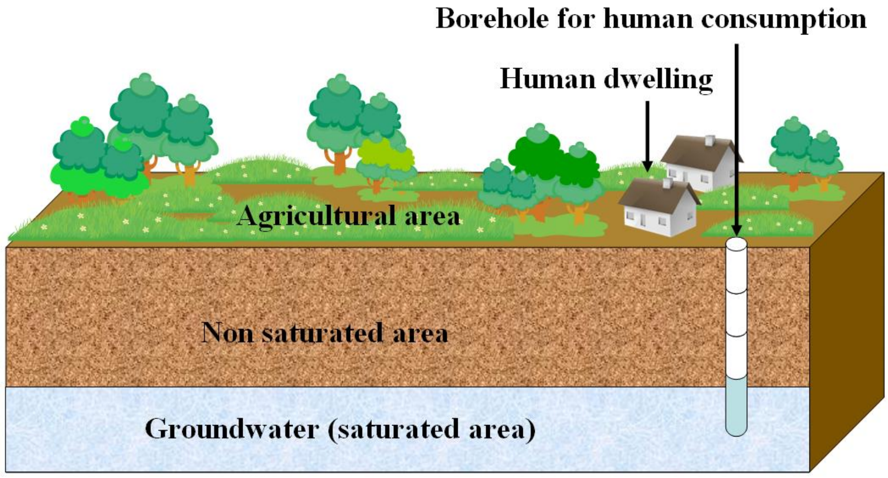


Figure 3. 2 Groundwater consumption by local people (Wongsasuluk, 2010)

The locals in this study area are mostly agriculturalist families. All their residents are located on their farms. The statistical data on the local people are provided as follows (Subdistrict Administrative Organization (SAO), 2013):

- Population: 9,011 people
    - Male: 4,468 people
    - Female: 4,543 people

- Total Household: 2,575 families
    - Agricultural Household: 1,491 families
- Agriculturalist: 7,092 people
    - Male Agriculturalist: 3,488 people
    - Female Agriculturalist: 3,604 people

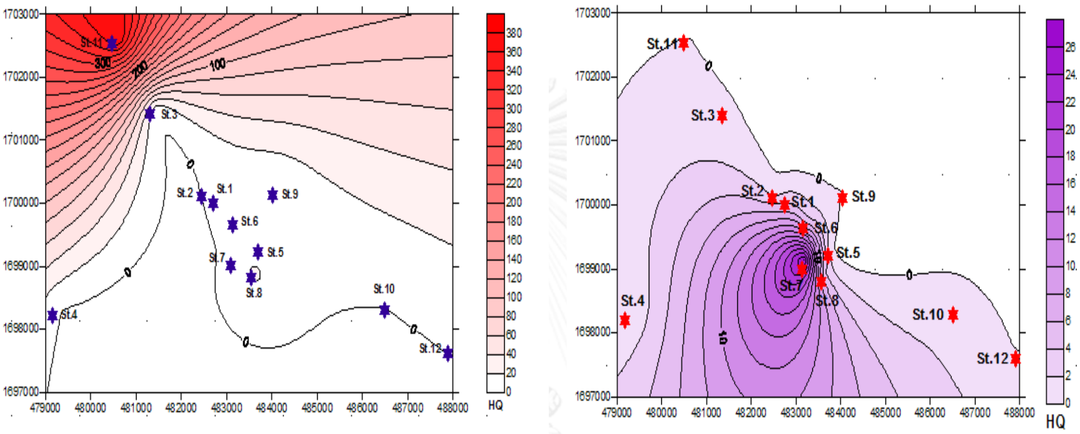


Figure 3. 3 As risk map and Pb risk map of the study area (Chotpantarat et al., 2014, Wongsasuluk et al., 2014)

3.2 Participants

Before collected samples from participants, this research was approved and got certificate documents from the Ethic Review Committee for Research-Involving Human Research subjects-Health Science Group, Chulalongkorn University, as attached documents in appendix A. In addition, the questionnaire of this research was evaluated with questionnaire of IOC (Index of Item-Objective Congruence) before used to interview with participants, the questionnaire was attached in appendix B.

The 100 participants of this study were divided into two types: the exposed group and non-exposed group. All 100 participants, who were the target participants in the study area, were randomly selected among those who originated from and permanently live in this agricultural study area and met the other criteria of this study.

1) Exposed Group (Groundwater Drinking): native-born local people who generally drink groundwater and have permanently lived in the study area since birth.

Inclusion Criteria of the Exposed Group

- Generally drink groundwater from shallow groundwater well in the study area.
- Older than 18 years of age (adults only).
- Native-born local people who permanently live in the study area.
- No migration or change of residence.
- No hair coloring, hair perming, hair re-bonding, or chemical activities within the past year (12 months).

Exclusion Criteria of the Exposed Group

- Stop drinking groundwater from the local well during this study.
- Migration to another place or change their residence location during this study.
- Do hair coloring, hair perm, hair re-bonding, or chemical activities during this study.
- Very sick and admit in hospital for a long period or die during this study.

2) Non-Exposed Group (Non-Groundwater Drinking): local people who do not drink the groundwater and have permanently lived in the study area since they were born or over a long period.

Inclusion Criteria of the Non-Exposed Group

- Do not drink the groundwater.
- Over 18 years of age (adults only).
- Native-born local people who permanently live in the study area.
- No migration or change of residence.
- No hair coloring, hair perming, hair re-bonding, or chemical activities within the past year.

Exclusion Criteria of the Non-Exposed Group

- Drinking groundwater from the local well during this study.
- Migration to another place or a change their residence (location) during this study.
- Any hair coloring, hair perming, hair re-bonding, or chemical activities during this study.
- Becoming very sick and being admitted to hospital for a long period or dying during the study period.

3.3 Sampling, Collection, and Preparation

There were 100 target participants. The sample size was based on a calculation of the preliminary results using the PS program, a power and sample size program. The result based on the preliminary data was n = 26 per group. Thus, 100 people were selected for this study. The target participants in the study area were randomly selected among those who permanently live in the area, and then face-to-face interviews were performed and samples of their biomarkers were collected.

For the manual sample size calculation,

$$n = 1 + 2C\left(\frac{SD}{X_1 - X_2}\right)^2$$

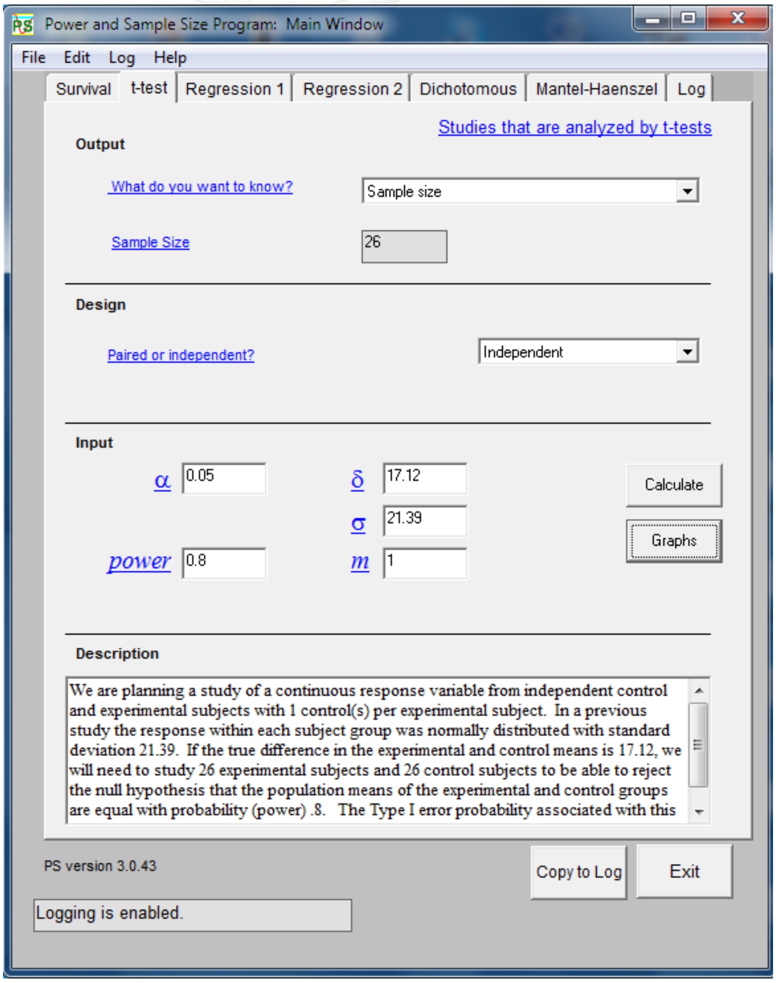
n = the sample size

C = the constant at α = 0.05 and 1-β =0.8, C = 7.85

X_1 and X_2 = the mean of the exposed and non-exposed group

(Carley and Harrison 2003; Snedecor and Cochran 1989)

For each participant, hair, nails, and urine were collected twice, at different six-month durations (based on the nails collected growth time). All biomarker samples were collected during two different seasons: during the months of March 2015, representing the dry season, and October 2015, representing the wet season.



What do you want to know? Sample size

Sample Size 26

Paired or independent? Independent

α 0.05

δ 17.12

σ 21.39

power 0.8

m 1

We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study the response within each subject group was normally distributed with standard deviation 21.39. If the true difference in the experimental and control means is 17.12, we will need to study 26 experimental subjects and 26 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) .8. The Type I error probability associated with this

PS version 3.0.43

Logging is enabled.

Figure 3. 4 Power and Sample Size Program (PS Program)

All participants were informed about the sample collections and face-to-face interviews ahead of time. Hair, nails, and urine from the target participants were collected, and a questionnaire was used in face-to-face interviews to collect personal information such as age, weight, underlying diseases, smoking behavior, and working hours per day.

For the purpose of collecting socio-demographic data on the participants, face-to-face interviews were used. The interview questionnaire consists of two main parts, and both the first and second parts consisted of open- and close-ended questions. The first part collected general information; the questions asks about the participant's background and personal information. The second part focused on health and exposure information to investigate the factors associated with the subject's heavy metal exposure (Table 3.1).

Table 3. 1 Questionnaire Information and Environmental Conditions

Socio-Demographic Factors	**Personal Factors**	**Exposure Factors**	**Environmental Factors**
-Sex -Age -Weight -Height -Occupation -Education -Family Occupation -Numbers of Family Members	-Smoking -Smoking Family Member(s) -Alcohol Drinking -Underlying Diseases	-Drinking Rate -Duration of Residence -Drinking Water Source -Cooking Water Source -Bath Water Source -Washing Water Source -Working Hours -Pesticides Use -Fertilizers Use -PPE Use	-Well Type -Well Distance from Farm -Drinking Water Storage/Container

The socio-demographic and other information of the local people may be associated to the heavy metal concentrations in hair, nails, and urine. Some studies have found differences in the heavy metal concentrations in biomarkers among different socio-demographic groups. With regard to gender, for example, Rakib et al. (2014) studied the arsenic mass fraction in human hair and nails through groundwater and found that the average arsenic (As) in males' hair was lower than in females' hair: male hair contained 0.93 μg/g and female hair contained 3.71 μg/g. Also the arsenic in males' nails was lower than in females' nails (1.40 μg/g for males compared to 2.03 μg/g for females). Similarly, Milena et al. (2012) conducted a study on human bio-monitoring (blood and urine) in the Czech Republic and suggest that one's sex can significantly influence the presence of mercury (Hg): their urine results showed that mercury levels in women were higher than in men. Male urine had 5.4 μg/g creatinine and female urine had 12 μg/g creatinine. The impacts of weight and height (Body Mass Index) also has been investigated. Mizanur (2011) studied association between arsenic (As) exposure and the BMI. It was found that the underweight group subjects had higher As concentrations in their urine than the normal weight and overweight group subjects. Likewise, on the whole the low BMI group participants had higher amounts of As in their urine than the high BMI group participants. The difference between smokers and non-smokers was investigated by Argelia et al. (2012). They measured the mercury (Hg), lead (Pb), and cadmium (Cd) levels in urine of Spanish adults and compare a group of smokers with non-smokers. This research found that the cadmium (Cd) excretion in urine of smokers was 31% higher than that of non-smokers. In the same way, Muhammad et al. (2009) investigated the arsenic (As) levels in the hair of smokers and non-smokers in Pakistan and found that arsenic levels in hair of smoker were significant higher than in non-smokers (non-smokers' hair contained 0.43+0.18 ug/g and smokers' hair contained 0.94+0.21 ug/g).

The participants were asked to fill in a questionnaire, which included questions on their hair lifestyle habits (hair coloring, hair bleaching, hair straighten, and hair re-bonding) and hair was sampled from the back of the head (upper neck) or behind the ear with the use of stainless steel scissors. Hairs from the first 1–2 cm from the scalp were sampled, which represented the past 1-2 months of exposure (Michalak et al., 2012). Finger nail samples were collected using stainless steel nail clippers. The hair and nail samples were sealed separately in labelled re-sealable polyethylene zipper storage bags and were not opened until they were in the laboratory for washing and cleaning and samples (Andrew et al., 2008). For urine sampling, the urine was collected in 100 ml glass bottles. The bottles were parafilm-wrapped to prevent leaking or evaporation and were then frozen at -4°C in sealed containers (Figure 3.5).

All drinking water samples were collected during two different seasons, which were during the months of March 2015 and October 2015. The groundwater drinking samples were pumped up from local shallow groundwater wells and collected after the water flowed for 2-3 minutes. Tap water was also collected after it was allowed to flow for the same amount of time. Then the pH was measured using a pH meter at the sampling locations. Samples were acidified with nitric acid (conc. HNO_3) to lower the pH, dissolve all heavy metals and prevent crystallization. After nitric acid was applied at the collection sites, the samples were transported to the laboratory in evaporation-prevention bottles.

Figure 3. 5 Sampling equipment: stainless steel hair scissors, stainless steel nail clipper, and glass bottle

Hair samples were washed with a proprietary baby shampoo under laboratory conditions and then with water. Shampoo washing is believed to remove the dust and particles (Sylwia et al., 2013). The shampoo selection was based on its composition among the metal cations, and only sodium was present. After the washing procedure, hair samples underwent purification from organic components (Marcin et al., 2011, Michalak et al., 2012). The hairs were cut into 5 mm pieces and the exposed hair samples were washed with acetone (Razi et al., 2012). The acetone washing method used was the one recommended by the International Atomic Energy Agency (IAEA, 1985), which is widely used in most studies as a pretreatment step to remove external contamination. In accordance with this method, hair samples were sequentially washed with acetone once, with deionized water three times, and once again with acetone at room temperature (Li et al., 2011). Before washing the nail samples, any visible dirt on the surface of the nails were removed manually. Then the nails were thoroughly washed using an ultrasonic bath with distilled water followed by MilliQ water and, finally, acetone. The washed samples were dried at 50ºC overnight in a drying oven (Gautam et al., 2004).

3.4 Analytical Technique

Following cleaning, 10 mg of each nail sample and 100 mg of each hair sample (Andrew et al., 2008) were purified of organic matter with concentrated nitric acid, 65% m/m (10mL), following the Milestone Digestion Method. They were spectrally purified in a microwave oven; the process assured the complete digestion of the hair and nail samples. Then, the concentration of heavy metals in the hair and nails were determined by inductively coupled plasma spectrometry-mass spectrometry (ICP-MS) (Gautam et al., 2004).

For the samples of urine, the participants were informed about the method of collection. Each sample was collected using a glass bottle with screw cap, parafilm cover and then immediately frozen for transport to the urine laboratory. The analysis of heavy metals was performed with an atomic absorption spectrophotometer (AAS). The urine concentrations of the heavy metals were adjusted by creatinine (e.g., CdU-cre, HgU-cre, PbU-cre) due to the fairly constant rates of creatinine excretion in the urine. However, since creatinine excretion is related to muscle mass and meat intake, the differences between the population groups, and in particular between men and women, were noted and evaluated. Adjustment with specific gravity is reported to be less affected by age, gender, body size and meat intake, and is considered to be an appropriate alternative when comparing populations groups with differences in terms of their creatinine excretion (Suwazono et al., 2005).

All drinking water samples were analyzed for four heavy metal concentrations, which were arsenic, cadmium, lead, and mercury, by inductively coupled plasma spectrometry-mass spectrometry (ICP-MS). Finally, the concentration of each heavy metal in the shallow groundwater and tap water was identified.

3.5 Quality Control for Sample Analysis

The nails and hair were prepared by following the preparation method prescribed by the International Atomic Energy Agency (IAEA). In terms of the instruments used for analysis, inductively coupled plasma mass spectrometry (ICP-MS) would be controls by using the standard laboratory (Center Laboratory of Thailand in Chachoengsao district) analyzing the concentrations of heavy metals in the hair and nail samples. For urine, all samples were analyzed by an atomic absorption spectrophotometer (AAS) following the standard method of the American Conference of Governmental Industrial Hygienists (ACGIH) for the purpose of quality control. Moreover, the creatinine was measured to adjust for other contaminated factors in the urine. The laboratory assessed the analytical chemical technique to document method validation that the AOAC Peer Verified Methods Program (1993) recommended.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) is the lowest concentration level that can be determined to be statistically different from a blank (with 99% confidence). The limit of quantitation (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence. The detection limit of ICP-MS is 0.001 µg/L for all heavy metals (As. Cd, Pb, Hg), while the detection limits of an AAS are 0.01, 0.01, 0.02 and 0.05 µg/L for As, Cd, Pb, and Hg, respectively. The LODs were calculated by SD*3.

3.6 Data Analysis and Statistical Method

The personal information of participants obtained from face-to-face interviews was summarized and were used to evaluate the human health risk assessment and their associated risk factors. The results of the health risk assessment estimated the probability of the occurrence of any given probable magnitude of adverse health effects over a specified time period. The health risk assessment of each heavy metal was based on the quantification of parameters in risk calculation equation. The two principal toxicities are the different toxicities between the carcinogenic effect and non-carcinogenic effect. The first toxicity is the slope factor (SF) for evaluating the carcinogen risk characterization, while the second toxicity is a reference dose (RfD) for determining the non-carcinogen risk characterization (Lim et al., 2008). The toxicity indices of each potentially toxic metal are shown in Table 1. The estimations of the concentration, frequency and duration of human exposure to each potentially toxic metal in the environment are measured as the average daily dose (ADD) (USEPA, 1992, Siriwong, 2006), as shown in the fourth risk assessment step.

3.6.1) Human Health Risk Assessment

There were four steps for health risk assessment: (1) hazard identification, (2) dose-response assessment (3) exposure assessment and (4) risk characterization.

- **The First Step: Hazard Identification**

The first step is the identification of the potential health effects of a chemical, which are different depending on the type of heavy metal or its toxicity. In addition, the adverse effects can be divided into two types: carcinogens and non-carcinogens. The first step of hazard identification is the process of determining when exposure to a chemical can cause or increase adverse human health effects.

- **The Second Step: Dose-Response Assessment**

This step is to estimate the intake rate that can affect human health. The association between the level of exposure and health effects is classified in this step. In this step, non-carcinogens and carcinogens are clearly separated and calculated in different manners.

1) Reference Dose of Non-Carcinogens

Non-carcinogenic effects result from multiple exposure that occurs over a long period of time, which can lead to chronic effects. The EPA has developed reference doses (RfDs) and estimates daily exposure. RfDs are usually in the unit of mg of contaminant per kg of the consumer's body weight per day (mg/kg-day) (Table 3.2) (US EPA 2000b).

2) Slope Factor of Carcinogens

Carcinogenic effects or cancer risk is assumed to be proportional to cumulative exposure at low exposure levels, and may be very small or even zero. Any exposure to a carcinogen might pose some cancer risk even at a low quantity or low concentration. Carcinogenic risk is usually expressed as a slope factor (SF) value with the units of risk per mg/kg-day of exposure (Table 3.2).

Table 3. 2 Oral reference dose and slope factor of heavy metals

Heavy Metal	Oral RfD	Oral Slope Factor
As	2.3×10^{-6} mg/kg-day	1.5 per (mg/kg-day)
Cd	5.0×10^{-4} mg/kg-day	-
Hg	3.0×10^{-4} mg/kg-day	-
Pb	3.5×10^{-3} mg/kg-day	-

The relevant oral reference dose (RfD) and slope factor (SF) were obtained from the US EPA's Integrated Risk Information System (IRIS), available on their website, www.epa.gov/iris/.

- **The Third Step: Exposure Assessment**

This process measures or estimates the magnitude, frequency, and duration of human exposure to an agent in the environment. The exposure assessment includes some discussion of the size, nature, and types of human populations exposed to the agent. Exposure could be measured directly, but more commonly is estimated indirectly through a consideration of the measured concentrations in the environment, a consideration of the models of chemical transport and fate in the environment, and an estimate of human intake over time (USEPA, 1992).

Average Daily Dose (ADD)

This includes calculations for the intake process via the ingestion route to determine whether a chemical is cancerous or not. This risk assessment considers the period of time over which exposure appears. Average exposures or doses over the period of exposure are sufficient for making an assessment. These averages are often in the form of average daily doses (ADDs). ADDs can be calculated by averaging the potential dose over the body weight and the average period of exposure (USEPA, 1992), as show in the following equation:

$$ADDs = \frac{CxIRxEFxED}{BWxAT} \qquad (1)$$

ADDs = Exposure duration (mg/kg-day)
C = Concentration (mg/L)
IR = Intake rate (L/day)
EF = Exposure frequency (day/year)
ED = Exposure duration (year)
BW = Body weight (kg)
AT = Average time (day)
: for non-carcinogenic effects, AT = ED in days
: for cancinogenic effects, AT = 70 years or 25,550 days

- **The Final Step: Risk Characterization**

This step involves the risk assessor's judgment as to the nature and presence or absence of risks, along with information about how the risk is assessed, where assumptions and uncertainties still exist, and where policy choices will need to be made. In practice, each component of a health risk assessment (hazard assessment, dose-response assessment, exposure assessment) has an individual risk characterization written to carry forward the key findings, assumptions, limitations, and uncertainties. The set of these individual risk characterizations provide the information basis to write an integrative risk characterization analysis.

1) Non-Carcinogen Risk Estimation (Hazard Quotient)

The comparison of exposure to the RfD indicates the degree to which exposure is greater or less than the RfD. This relationship is shown in Eq. (4).

The RfD is defined as the daily oral dose of a chemical that is unlikely to cause adverse effects given a lifetime of exposure. An evaluation of non-carcinogenic toxicity of individual risks can be computed by using the hazard quotient (HQ) ratio. This value indicates the degree of exposure, greater or less than the RfD. When the ratio is equal to or greater than 1, when the exposure exceeds the RfD, the exposure population may be at risk (US EPA, 1999a).

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure}}{\text{RfD}} \tag{4}$$

where Exposure = the chemical exposure level or ADDs (mg/kg-day), and

RfD = reference dose (mg/kg-day).

If $HQ \geq 1$, the adverse non-carcinogenic effect is of concern.

$HQ < 1$, it is at an acceptable level (no concern).

For the risk assessment of chemical mixtures, the hazard quotients are combined to form the Hazard Index (HI) (Eq. 5), which assumes that the effects of the different compounds and effects are additives. The HI method is recommended for groups of toxicologically similar chemicals for which there is dose response data. When the hazard index exceeds unity ($HI > 1$), the exposure population may be at risk, whereas an HI less than or equal to 1 should be taken as the acceptable reference or standard (US EPA, 1989a).

$$\text{Hazard Index (HI)} = \sum (\text{HQ}) \tag{5}$$

If $HQ \geq 1$, it is has adverse non-carcinogenic effects of concern.

$HQ < 1$, it is at an acceptable level (no concern).

For a risk assessment of multiple heavy metals, the individual HQs are combined to represent the hazard index (HI), where an HI > 1 denotes an unacceptable risk of non-carcinogenic effects on health, while an HI < 1 denotes an acceptable level of risk (ECETOC, 2001).

2) Carcinogen Risk Estimation (Cancer Risk)

Using the cancer slope factor (SF) and exposure data in mg/kg-day, cancer risks are calculated using following equation:

$$\text{Cancer Risk} = \text{Exposure} \times \text{SF} \qquad (2)$$

where Exposure = the chemical exposure level or ADDs (mg/kg-day)
SF = Slope Factor (per mg/kg-day)

The carcinogenic risk can be calculated as the product of the ADD (mg/kg-day) multiplied by the SF (mg/kg/day). An acceptable level is $\leq 10^{-6}$, which means that, on average, the probability is that approximately 1 person per 1,000,000 will develop cancer as a consequence of the exposure (Lim et al. 2008). Meanwhile, values $\geq 10^{-6}$ are unacceptable risk levels. According to the cancer risk assessment process, the results denote the probability of the occurrence of adverse health effects in participants. As mentioned earlier, in summary, the risk results can be indicated by either the value of the hazard quotient (HQ) in terms of the non-carcinogenic risk or the value of the cancer risk in terms of the carcinogenic risk.

3.6.2) Statistical Analysis

To compare heavy metal concentrations in the biomarkers of the groundwater-drinking participants and non-groundwater-drinking participants, the Kolmogorov-Smirnov test (K-S test) was used to investigate the normal distribution, and then the Mann-Whitney U-test (2-tailed) was applied to investigate the difference between the two independent groups of continuous data.

Statistical parameters including the range, average, median, and standard deviation were investigated. The independent variables were obtained from the questionnaire, the data were both category data and continuous data. The dependent variable was the concentration of heavy metals in biomarkers, which were classified as continuous data, also this data were separated into two groups to be category data for calculation in the binary logistic regression. The relationship between heavy metals in the three biomarkers; urine, hair, nail, and heavy metals in the drinking water and the relationship between heavy metals in the biomarkers and the health risk assessment were established using Kendall and Spearman correlation tests. Since heavy metal concentrations in biomarkers and the health risk assessment results (HQ and cancer risk) are both continuous data, the Kendall and Spearman tests could be used to find out their correlations. In addition, binary logistic regression and odd ratio were used to identify the potential associated risk factors using the SPSS 16.0 software (IBM Corporation, New York, United States). Both cancer risk and non-cancer risk values were calculated using the cancer risk level and HQ values. Bråtveit et al. (2011) studied in Norway on heavy metals in urine occurrence. Urine was used as a cadmium biomarker of chronic exposure in a population residing in the vicinity of a zinc producing plant and reported the risk factors affecting heavy metals in the urine by regression statistics.

- **Categorical Data**: Gender, smoking, and alcohol drinking, for example.
- **Numerical Data**: Weight, age, and concentration, for example.
- **Independent Data**: General information and socio-demographic data.
- **Dependent Data**: Heavy metal concentrations in hair, nail, and urine.

Descriptive statistics (e.g., means, standard deviations) were reported. The normality of distribution of the experimental results was assessed by the Shapiro–Wilk test (suitable for n<50). On this basis, the statistical test was selected, which was used to investigate the significance of the differences between the groups. For normally distributed data results, the heavy metal concentrations' (numerical data and dependent data) parametric T-Test would be used to investigate the significance of the differences between the groups.

In the case of the non-parametric data, the Mann–Whitney *U* test was applied. The statistical significance between the groups was accepted at the $P<0.05$ level. An analysis of correlation was carried out by determining the Pearson or spearman correlation coefficient. The coefficient was used to analyze the correlation and was considered statistically significant at $P<0.05$ (Marcin et al., 2011, Michalak et al., 2012). Also, the Chi-square, odds ratio (OR), and logistic regression analysis were used to investigate the relationship between the associated factor and the biomarkers, was undertaken by the use of the *SPSS* program (IBM Corporation, New York, United States).

In conclusion, The Kolmogorov-Smirnov test (K-S test) was applied to investigate the normal distribution. Mann-Whitney U-test (2-tailed) was applied to investigate the difference between the two independent groups. Statistical parameters including the range, average and standard deviation were investigated. The relationship between heavy metals in biomarkers and heavy metals in drinking water were established using Kendall and Spearman tests. In addition, a Chi-square, Odd ratio, and binary logistic regression were used to identify the associated risk factors.

Part I

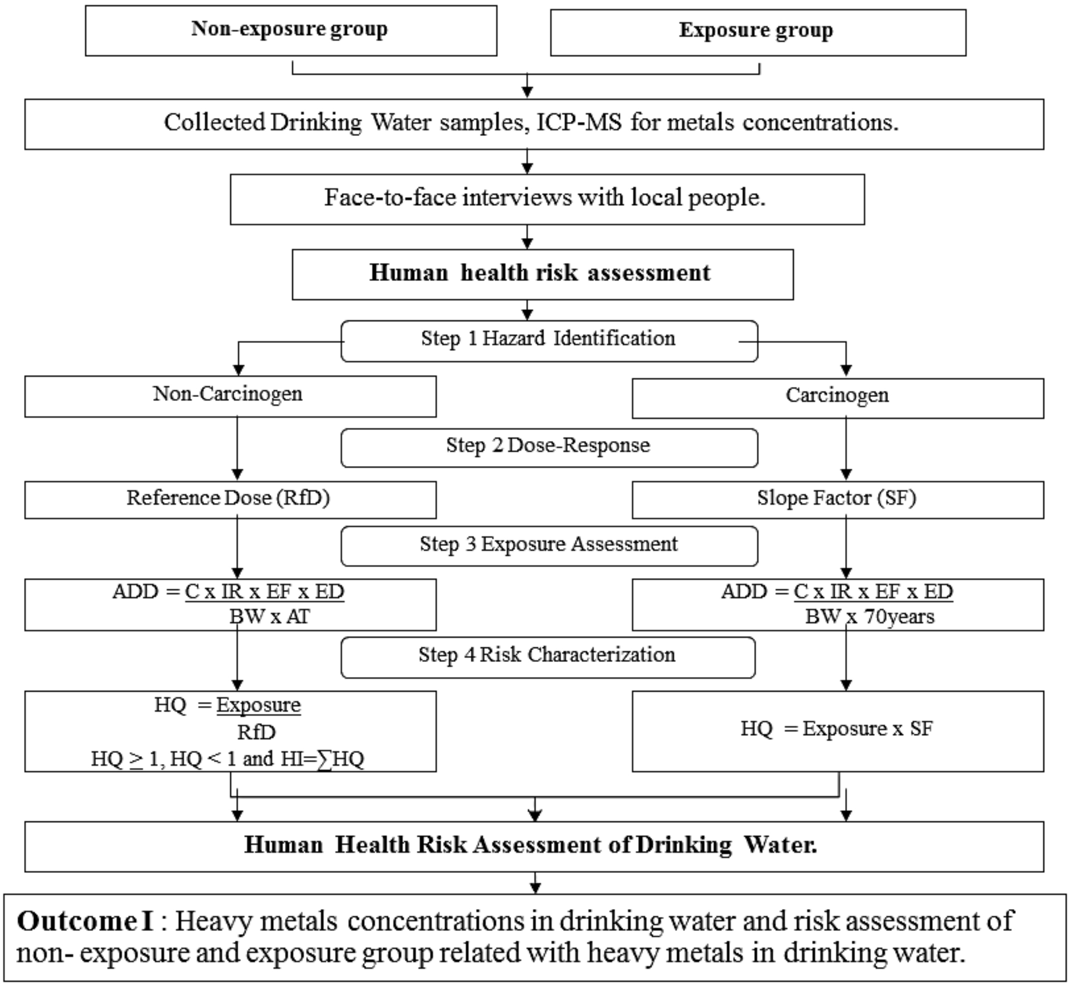
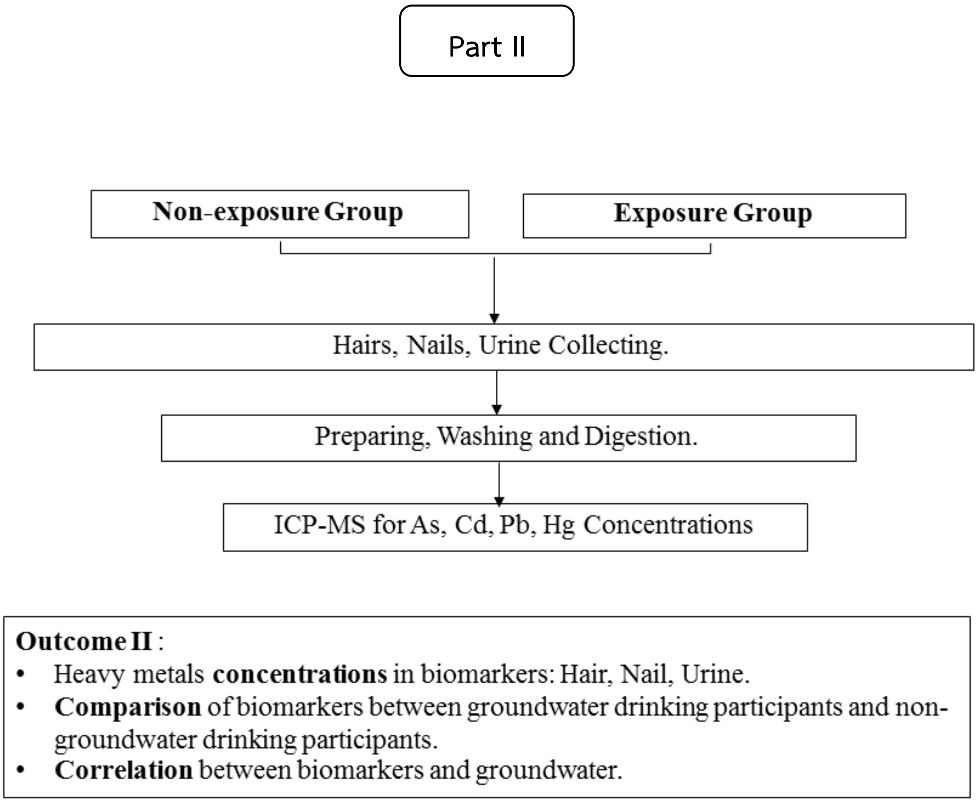


Figure 3. 6 Methodology Part 1



Outcome II :

- Heavy metals **concentrations** in biomarkers: Hair, Nail, Urine.
- **Comparison** of biomarkers between groundwater drinking participants and non-groundwater drinking participants.
- **Correlation** between biomarkers and groundwater.

Figure 3. 7 Methodology Part 2

Part III

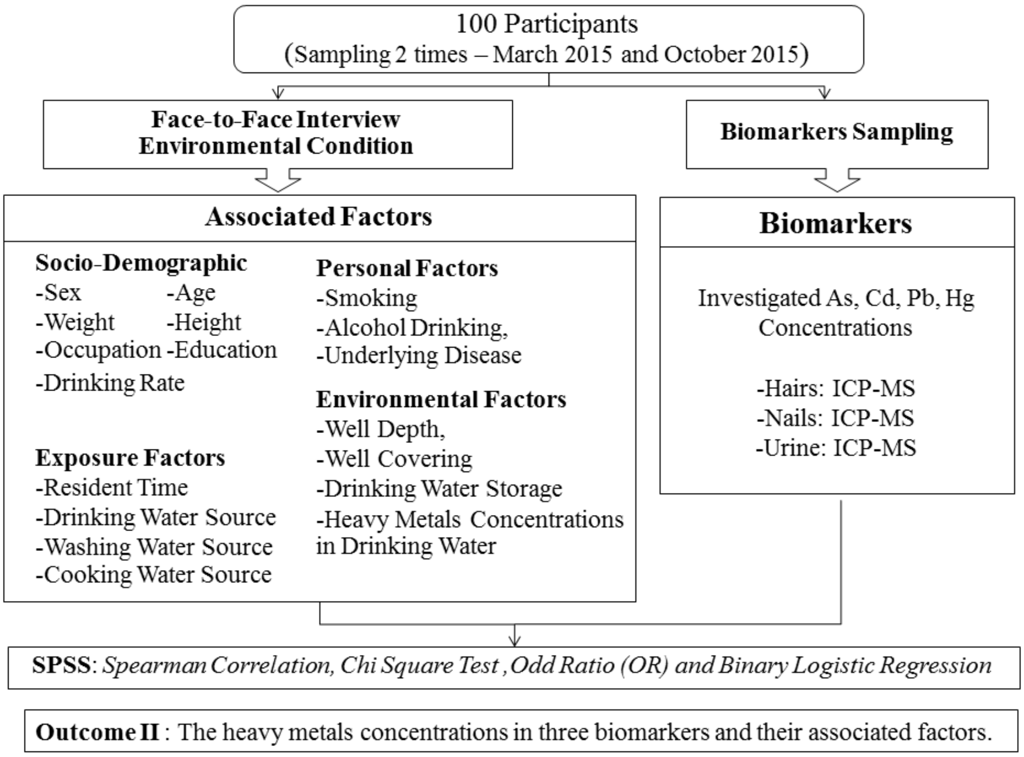


Figure 3. 8 Methodology Part 3

CHAPTER IV

PHYSICAL AND CHEMICAL PROPERTIES OF DRINKING WATER AND CHARACTERISTICS OF PARTICIPANTS IN STUDY SITE

4.1 Physical and chemical properties of shallow groundwater wells and tap water

The agricultural study area has been continuous and intensive farming for a long period of time and remains one of the largest chili farms in Thailand, with other crops including rice, rubber trees, and corn. This site located in the Mueang district in the Ubon Ratchathani province in northeast Thailand between a longitude of 1695000-1704000 UTM and latitude of 479000-469000 UTM. The soil textures consist of 3 types: sandy loam, loamy sand and sand with an average hydraulic conductivity of between 3.43 to 49.03 cm/day (Chotpantarat et al., 2011; Masipan and Chotpantarat, 2016). Furthermore, according to our previous study (Wongsasuluk et al., 2014), these agricultural areas had acidity in the shallow groundwater, where the average pH was 4.72±1 (Wongsasuluk 2010; Wongsasuluk et al. 2014). As mentioned, in terms of the hydrogeological characteristics, this intensively farming appears to have the potential for the movement of agrochemicals from the ground surface to shallow groundwater systems.

This study focused on 25 random sampling stations. There are only 7 stations where only shallow groundwater (no tap water) was found as follows: station nos. 3, 4, 9, 12, 18, and 22, with station no. 25 which is the reservoir. All shallow groundwater samples in this study were built by local people and located in their farm closed to their residents. Some of residents in these agricultural areas had no tap water and electricity, so the major source of water consumption was the shallow groundwater.

The average water level of groundwater during wet season was higher than dry season, which were 126.9±14.0 m. (asl) and 124.46±14.81 m. (asl) in wet and dry seasons, respectively (Figure 4.1). The average pH values of groundwater were 5.28±1.15 and 5.16±4.19 in dry and wet seasons, respectively (Figure 4.2). There were 19 of 25 wells in dry season, found pH were lower than standard (std.6.5-7.5), and 20 of 25 in wet season. They were similarly pattern of pH level of groundwater in both seasons as showed in Figure 4.3. The characteristics of drinking water during dry and wet season showed in Tables 4.1 and 4.2. In this study area, NH_4NO_3-based fertilizers have been mostly used, which leads to the groundwater becoming slightly acidic because the ammonium nitrate in water will produce hydronium ions (H_3O^+) and so lower pH of the soil and groundwater.

Tap water in this study area came from deep groundwater (more than 40 m underground) and would be refinement before approach resident. The filtration of tap water was managed by local government or village headman. The water cleaning process was in pressure sand filter tank, diameter 1.15 m and height 1.20 m. The layer of filter materials in filter tank were glass sand or silica sand (grain size 0.125-0.25 mm) 240 L, coke 40 L, fine sand (grain size 0.5-1.0 mm) 120 L, sand (grain size 1.0-2.0 mm) 80 L, fine gravel (grain size 4.0-8.0 mm) 80 L, small gravel (grain size 8.0-16.0 mm) 80 L, and gravel (grain size 16-30 mm) 160 L, respectively (Department of groundwater resources, 2008).

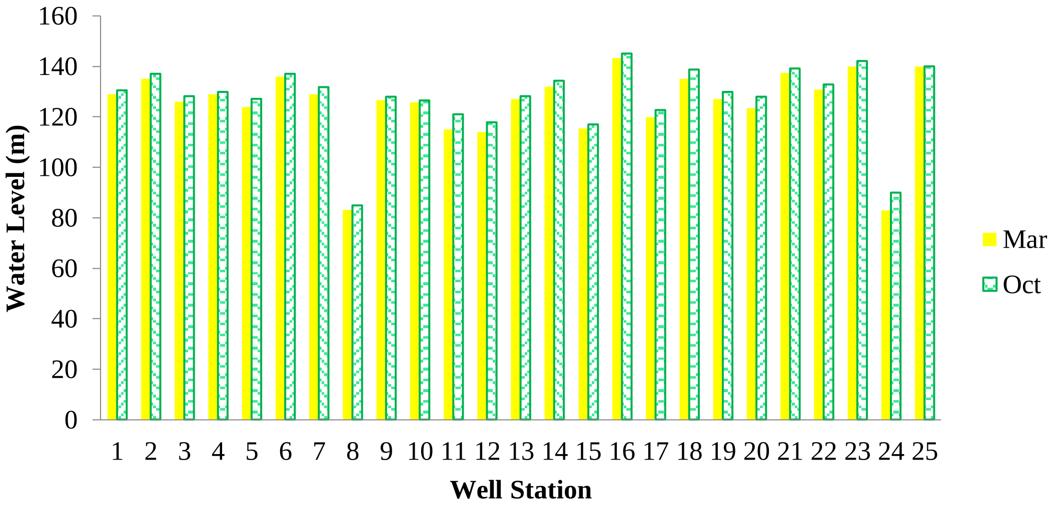


Figure 4. 1 The comparison of water level in dry and wet season

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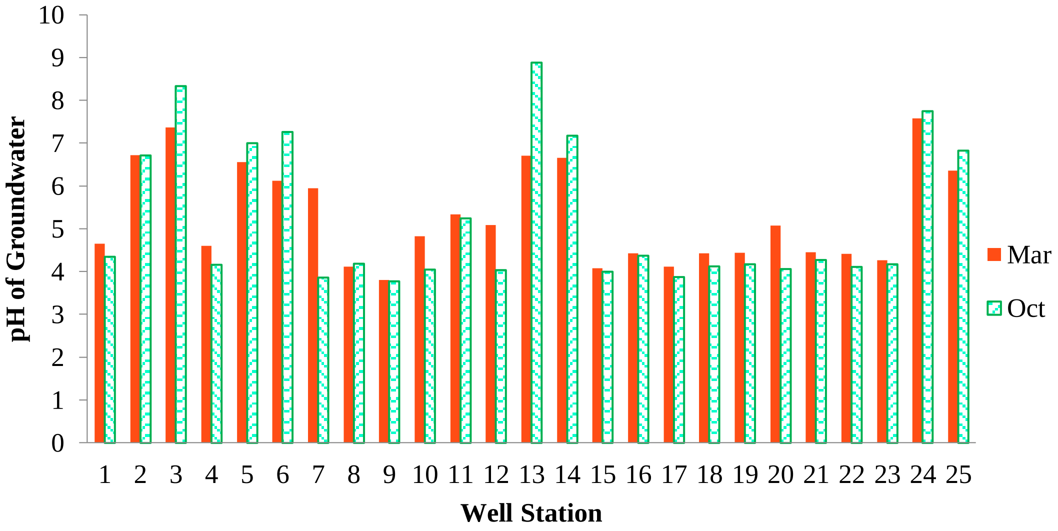
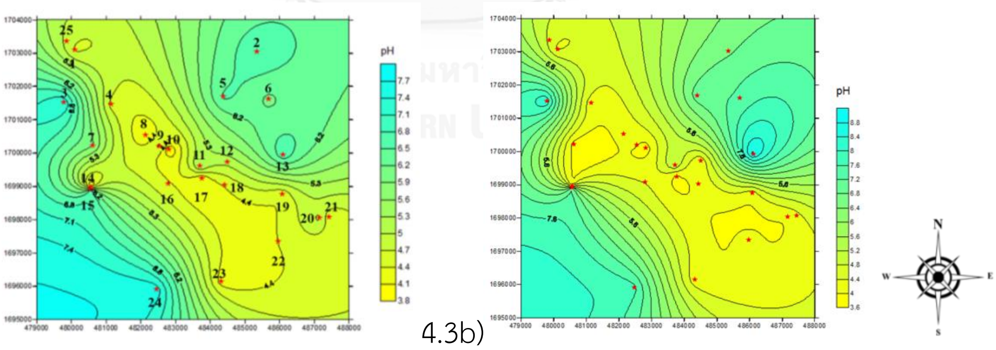


Figure 4. 2 The comparison of groundwater pH in dry and wet season



4.3a) 4.3b)

Figure 4. 3 a) The pH contour map of study area in dry season b) The pH contour map of study area in wet season

Table 4. 1 The characteristics of drinking groundwater and tap water during dry season

St.	Altitude (m)	Water Level (m, msl.)	Groundwater pH	Tap Water pH	Groundwater Conductivity (μS/cm)	Tap Water Conductivity (μS/cm)
1	133	129.00	4.65	7.52	53.0	1849.0
2	139	135.00	6.72	7.34	212.0	333.0
3	130	126.00	7.37	None*	358.5	None*
4	132	129.00	4.60	None*	223.0	None*
5	129	124.00	6.55	6.83	202.0	302.0
6	140	136.00	6.12	7.15	83.1	328.0
7	133	129.00	5.94	6.40	79.0	103.8
8	87	83.10	4.11	7.22	66.9	429.0
9	130	126.60	3.80	None*	108.1	None*
10	127	125.75	4.82	6.98	67.9	325.0
11	127	115.00	5.33	6.69	149.3	122.8
12	120	114.00	5.08	None*	58.1	None*
13	129	127.00	6.71	6.83	717.0	333.0
14	136	132.00	6.65	7.32	176.9	246.0
15	120	115.50	4.07	8.05	159.0	245.0
16	147	143.30	4.42	9.67	66.3	363.0
17	124	119.86	4.11	6.68	86.4	121.8
18	141	135.00	4.43	None*	183.8	None*
19	132	127.00	4.44	6.47	209.0	131.4
20	132	123.50	5.07	7.20	71.9	22.2
21	140	137.50	4.45	7.50	74.6	298.0
22	134	130.85	4.41	None*	25.9	None*
23	144	140.05	4.26	7.30	82.5	215.0
24	98	83.00	7.58	7.25	732.0	845.0
25	140	140.00	6.36	None*	48.2	None*
Avg.	129.76	124.46	5.28	7.24	171.8	367.4
SD		14.81	1.15	0.73	183.2	410.11
Min		83	3.8	6.4	25.9	22.2
Max		143.3	7.58	9.7	732	1849

None* = No tap water.

Table 4. 2 The characteristics of drinking groundwater and tap water during dry season

St.	Water Level (m, msl.)	Groundwater pH	Tap Water pH	Groundwater Conductivity (µS/cm)	Tap Water Conductivity (µS/cm)
1	130.5	4.35	7.9	1200	5560
2	136.9	6.72	7.79	337	519
3	128.1	8.34	None*	98	None*
4	129.8	4.16	None*	296	None*
5	127.1	7	7.05	322.5	724
6	136.9	7.26	7.92	73.9	5880
7	131.8	3.86	7.69	159	556
8	84.9	4.19	7.77	192.4	1167
9	128	3.77	None*	382.5	None*
10	126.4	4.05	7.62	192.8	708
11	121	5.25	7.62	161.1	710
12	117.7	4.04	None*	197.2	None*
13	128.1	8.89	7.16	983	199.8
14	134.3	7.18	7.4	306	523
15	116.9	4	8.6	42.1	529
16	145.1	4.38	7.5	63.3	602
17	122.7	3.88	7.37	232.5	416
18	138.7	4.13	None*	332	None*
19	129.8	4.17	6.55	149.8	406
20	128	4.06	7.85	156.6	211
21	139.1	4.27	6.93	66.3	1050
22	132.7	4.11	None*	153.4	None*
23	142.1	4.17	6.71	437.5	1045
24	90	7.75	7.42	1223	338
25	140	6.83	None*	151.7	None*
Avg.	126.94	5.16	7.49	316.4	1174.7
SD	14.03	1.66	0.49	328.1	1676.3
Min	84.9	3.77	6.55	42.1	199.8
Max	145.1	8.89	8.6	1223	5880

None* = No tap water.

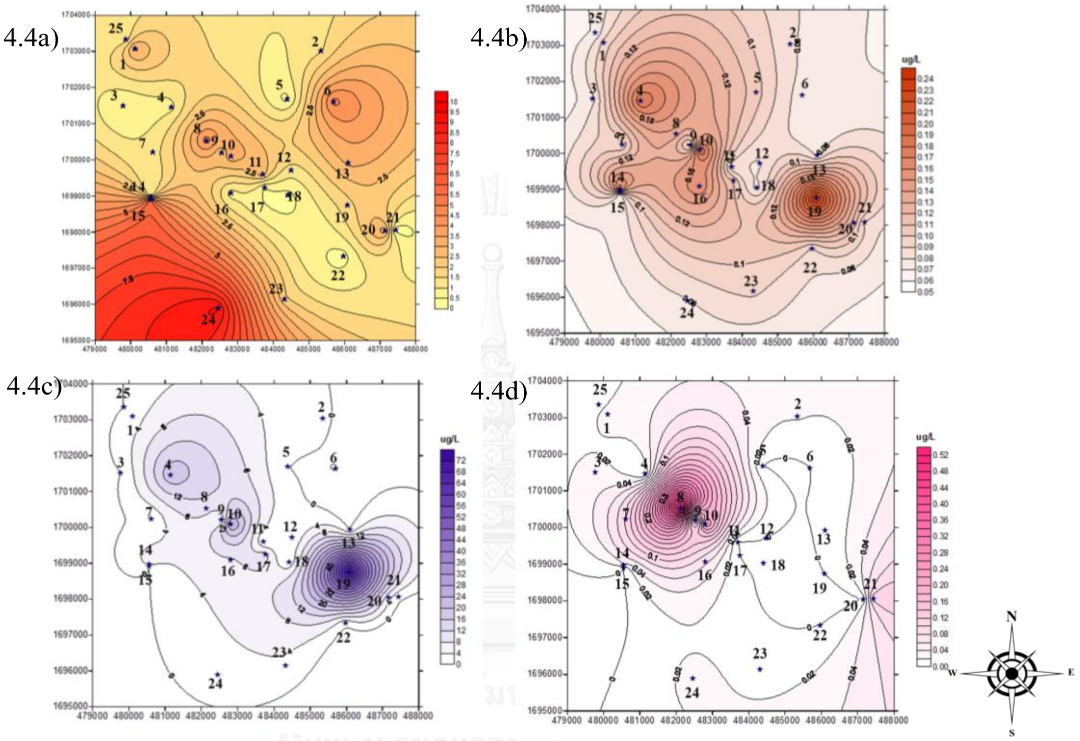
4.2 The concentrations of heavy metals in groundwater and tap water

Dry Season

This study found two main drinking water sources: groundwater and tap water. The average concentrations of As in shallow groundwater were 1.584±0.031 μg/L and 0.109±0.02 μg/L, 6.902±0.08 μg/L, 0.05±0.006 μg/L for Cd, Pb, and Hg, respectively, whereas the average concentrations of As in tap water were 2.185±0.033 μg/L and 0.002±0.002 μg//L, 0.11±0.003 μg//L, 0.022±0.005 μg//L for Cd, Pb, and Hg in tap water, respectively (Table 4.3 and 4.4). Moreover, there were 4 samples of shallow groundwater in which concentrations of Pb higher than standard were found, which were at stations 4, 8, 9 and 19. The concentrations of three heavy metals (i.e., Cd, Pb, Hg) had a similar pattern, which was relatively high levels in the central area and apparently lower levels in the surrounding areas, while As showed a different distribution pattern, which were fairly low in the central areas and appeared to be higher in the south-western part of the area, probably affecting the pH of groundwater (Figure 4.4). Generally, As becomes highly mobilized in high pH or alkaline groundwater and becomes less mobilized in low pH or acidic groundwater. The adsorption of As decreases as the pH increases over the pH 6-9 range (USGS, 2016). Therefore, the stations that had a high pH had higher concentrations of As compared to those with a low pH. In addition, the pH was slightly elevated in the groundwater due to the dissolution of carbonates and silicates and from the cation exchange process, promoting the release of As from iron and manganese oxides into the groundwater (Smedley et al., 2002, Bhattacharya et al., 2006). Elementary As is fairly insoluble, whereas arsenic compounds may readily dissolve. As is generally present in groundwater systems as $HAsO_4^{2-}$(aq) and $H_2AsO_4^{-}$ (aq) and most likely partially as H_3AsO_4 (aq), AsO_4^{3-}(aq) or $H_2AsO_3^{-}$(aq), as shown in the following equation (Lenntech, 2016):

$$As_2S_3 + 6\ H_2O \rightarrow 2\ H_3AsO_3 + 3\ H_2S$$

In this study area, a slightly basic pH and a relatively high As concentration was found, such as at well no.24 (pH 7.58 and As 9.081 μg As/L) compared to well no. 23, where the water was found to be acidic and had an As concentration that was 34 times lower (pH 4.41, As 0.27 μg/L).



★: Sampling stations of shallow groundwater wells

Figure 4. 4 a) Concentration (μg/L) contour map of As in shallow groundwater. b) Concentration (μg/L) contour map of Cd in shallow groundwater. c) Concentration (μg/L) contour map of Pb in shallow groundwater. d) Concentration (μg/L) contour map of Hg in shallow

Table 4. 3 The concentrations of heavy metals in shallow groundwater

st.	As (µg//L)	Cd (µg//L)	Pb (µg//L)	Hg (µg//L)
1	0.259±0.017*	0.094±0.004	2.048±0.042	0.030±0.009
2	1.368±0.009	0.082±0.002	0.363±0.019	0.022±0.009
3	1.591±0.096	0.076±0.002	0.003±0.015	0.031±0.008
4	0.811±0.045	0.181±0.005	**19.290±0.230**	0.000±0.000
5	1.166±0.055	0.105±0.003	0.069±0.010	0.000±0.000
6	1.051±0.037	0.075±0.002	0.133±0.003	0.000±0.000
7	5.753±0.081	0.092±0.001	0.122±0.012	0.102±0.008
8	0.481±0.013	0.136±0.006	**10.480±0.122**	0.525±0.022
9	0.811±0.046	0.184±0.003	**25.910±0.295**	0.233±0.010
10	0.324±0.019	0.091±0.004	2.920±0.085	0.040±0.012
11	1.336±0.013	0.082±0.000	0.194±0.023	0.000±0.000
12	1.422±0.028	0.079±0.002	0.528±0.014	0.000±0.000
13	4.683±0.032	0.079±0.002	0.000±0.000	0.005±0.006
14	2.997±0.057	0.073±0.001	0.000±0.000	0.000±0.000
15	0.322±0.007	0.171±0.002	6.959±0.079	0.042±0.012
16	0.503±0.010	0.160±0.005	7.370±0.149	0.066±0.009
17	0.366±0.011	0.110±0.001	9.897±0.088	0.000±0.000
18	1.227±0.030	0.077±0.001	0.268±0.028	0.000±0.000
19	1.795±0.042	0.237±0.007	**72.240±0.643**	0.000±0.000
20	0.356±0.009	0.116±0.002	5.814±0.020	0.000±0.000
21	0.286±0.012	0.089±0.001	1.561±0.007	0.114±0.007
22	0.267±0.012	0.088±0.001	0.979±0.030	0.000±0.000
23	0.258±0.003	0.092±0.003	3.280±0.058	0.013±0.009
24	9.081±0.073	0.079±0.001	2.054±0.018	0.026±0.010
25**	1.085±0.012	0.072±0.001	0.072±0.007	0.011±0.005
Avg.±SD	1.584±0.031	0.109±0.002	6.902±0.080	0.050±0.006
Min	0.258	0.072	ND	ND
Max	9.081	0.237	72.240	0.525
Std.	10.00	5.000	10.000	1.000
IDL	0.001	0.001	0.001	0.001

*Average Concentration±SD.

**Station number 25 was reservoir water.

Table 4. 4 The concentrations of heavy metals in tap water

st.**	As (µg/L)	Cd (µg/L)	Pb (µg/L)	Hg (µg/L)
1	4.306±0.048*	0.076±0.002	ND	ND
2	2.216±0.068	0.102±0.002	0.006±0.004	ND
5	1.428±0.025	0.106±0.007	0.049±0.028	ND
6	2.102±0.029	0.090±0.001	ND	0.005±0.006
7	1.113±0.034	0.096±0.002	ND	ND
8	2.527±0.052	0.073±0.001	ND	0.027±0.017
10	1.574±0.032	0.074±0.002	ND	ND
11	2.769±0.028	0.082±0.004	ND	ND
13	1.488±0.031	0.085±0.001	ND	ND
14	3.137±0.020	0.078±0.001	ND	0.025±0.009
15	3.307±0.031	0.074±0.000	0.142±0.024	0.000±0.000
16	1.979±0.027	0.073±0.001	ND	0.006±0.009
17	2.832±0.059	0.078±0.003	ND	ND
19	1.823±0.035	0.081±0.002	ND	ND
20	0.443±0.003	0.094±0.003	ND	0.024±0.012
21	1.715±0.016	0.077±0.002	ND	0.193±0.012
23	2.120±0.043	0.076±0.001	ND	0.060±0.016
24	2.454±0.006	0.075±0.002	ND	0.058±0.007
Avg.±SD	2.185±0.033	0.083±0.002	0.011±0.003	0.022±0.005
Min	0.443	0.073	ND	ND
Max	4.306	0.106	0.142	0.193

*Average Concentration±SD.

**Station numbers 3, 4, 9, 12, 18, and 22 do not have tap water.

Wet Season

The average As in groundwater during wet season was 1.311±2.179 μg/L and 0.020±0.035 μg/L, 6.882±10.858 μg/L, 0.000±0.000 μg/L for Cd, Pb, and Hg, respectively (Table 4.5), whereas the average concentrations of As in tap water were 0.765±0.662 μg/L and 0.000±0.000 μg//L, 0.004±0.017 μg//L, 0.000±0.000 μg//L for Cd, Pb, and Hg in tap water, respectively (Table 4.6). The results showed all 4 heavy metals in both groundwater and tap water during the dry season were higher than those in the wet season while Cd and Hg were not found in groundwater in the wet season (Figure 4.5-4.6). From these results, drinking groundwater in the wet season seemed to be safer than dry season in case of Cd and Hg were none also As and Pb were lower than drinking water standard (the drinking water standard were 10, 5, 10, 1 μg/L for As, Cd, Pb, and Hg respectively), but the result of risk assessment showed non-carcinogenic risk from Pb even low concentration as following ***Chapter V.***

The previous study in year 2011 (Wongsasuluk et al., 2014), All year results found average As was 1.06±1.74, Cd was 0.15±0.03, Pb was 16.7±18.5, Hg was 0.10±0.13 μg/L. For all year results of this study, average As was 1.45±2.11, Cd was 0.06±0.06, Pb was 6.89±13.00, Hg was 0.03±0.08 μg/L. The previous study found that concentrations of Cd, Pb, Hg were higher than those in this study because this recent study area was larger than the previous study area, focusing only 12 stations in the vicinity of intensively chilli farming areas. This study had a larger area consisting of 25 stations and the additional groundwater wells extended in the downstream areas which most wells had low Cd, Pb, Hg. This study found higher As concentration than the previous study because groundwater wells are located in the upstream areas, where close to the well found the highest As concentration from the previous study.

Table 4. 5 The concentrations of heavy metals in shallow groundwater wells in the wet season

St.	As (μg//L)	Cd (μg//L)	Pb (μg//L)	Hg (μg//L)
1	6.181	nd.	0.018	nd.
2	1.295	nd.	0.111	nd.
3	0.021	0.019	11.020	nd.
4	0.005	0.118	5.726	nd.
5	1.291	nd.	nd.	nd.
6	1.081	0.012	nd.	nd.
7	0.126	0.041	15.790	nd.
8	0.249	0.098	13.120	nd.
9	0.341	0.031	37.110	nd.
10	0.154	0.091	21.310	nd.
11	0.089	0.000	0.532	nd.
12	nd.	0.036	18.170	nd.
13	4.176	nd.	0.379	nd.
14	2.786	nd.	nd.	nd.
15	0.297	nd.	0.050	nd.
16	nd.	nd.	2.517	nd.
17	1.168	nd.	nd.	nd.
18	0.835	nd.	nd.	nd.
19	0.201	0.051	33.170	nd.
20	nd.	0.005	13.020	nd.
21	nd.	nd.	nd.	nd.
22	nd.	nd.	nd.	nd.
23	0.987	nd.	nd.	nd.
24	8.874	nd.	nd.	nd.
25	2.629	nd.	nd.	nd.
Ave.±SD	1.311±2.179	0.020±0.035	6.882±10.858	0.000±0.000
Med.	0.297	nd.	0.111	nd.
Min	nd.	nd.	nd.	nd.
Max	8.874	0.118	37.110	nd.
LOD	0.001	0.001	0.001	0.001

Table 4. 6 The concentrations of heavy metals in tap water in the wet season

St.	As (μg//L)	Cd (μg//L)	Pb (μg//L)	Hg (μg//L)
1	1.024	nd.	nd.	nd.
2	0.892	nd.	nd.	nd.
5	nd.	nd.	nd.	nd.
6	1.268	nd.	nd.	nd.
7	1.165	nd.	nd.	nd.
8	1.629	nd.	nd.	nd.
10	0.065	nd.	nd.	nd.
11	nd.	nd.	nd.	nd.
13	1.597	nd.	nd.	nd.
14	1.662	nd.	nd.	nd.
15	1.460	nd.	nd.	nd.
16	1.466	nd.	nd.	nd.
17	0.244	nd.	nd.	nd.
19	nd.	nd.	nd.	nd.
20	nd.	nd.	nd.	nd.
21	0.545	nd.	nd.	nd.
23	0.762	nd.	nd.	nd.
24	nd.	nd.	0.073±0.020	nd.
Ave.±SD	0.765±0.662	nd.	0.004±0.017	nd.
Med.	0.827	nd.	nd.	nd.
Min	nd.	nd.	nd.	nd.
Max	1.662	nd.	0.073	nd.

*Average Concentration±SD.

**Station numbers 3, 4, 9, 12, 18, and 22 do not have tap water.

U-test was used to investigate the different between heavy metals in groundwater and tap water, also different between dry and wet season. The U-test results showed As, Cd, Hg in groundwater were significant different between dry and wet season (Figure 4.5), also As, Cd, Hg in tap water were significant different between dry and wet season (U-test: sig<0.05). The comparison between dry and wet season, heavy metals in dry season showed higher than wet season. As, Cd, Pb were significantly different between groundwater and tap water in dry season while Cd, Pb were significant different between groundwater and tap water in wet season (U-test: sig<0.05) (Figure 4.5-4.6).

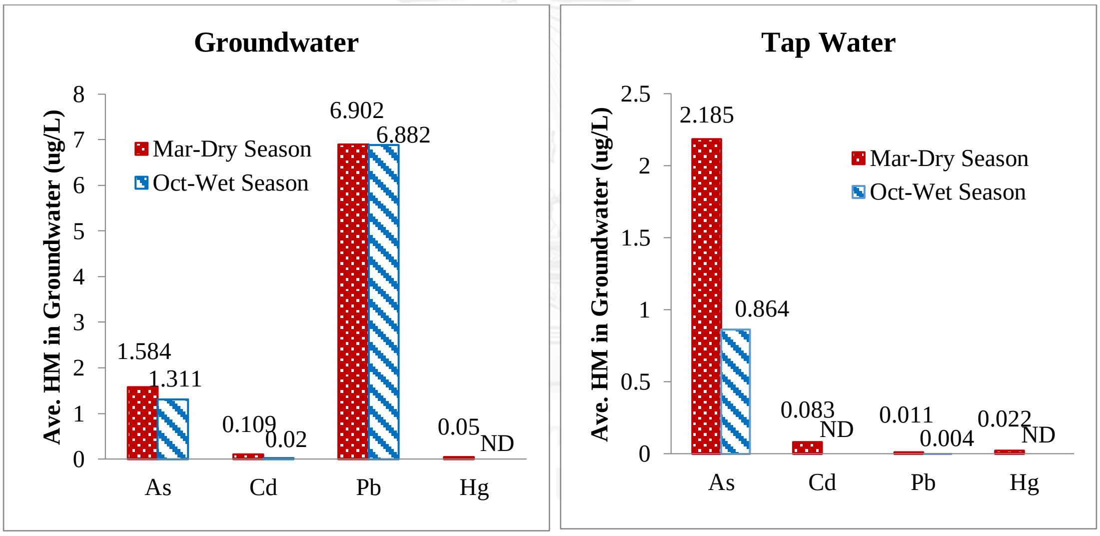


Figure 4. 5 The comparison of average concentrations heavy metals in drinking water between wet and dry seasons

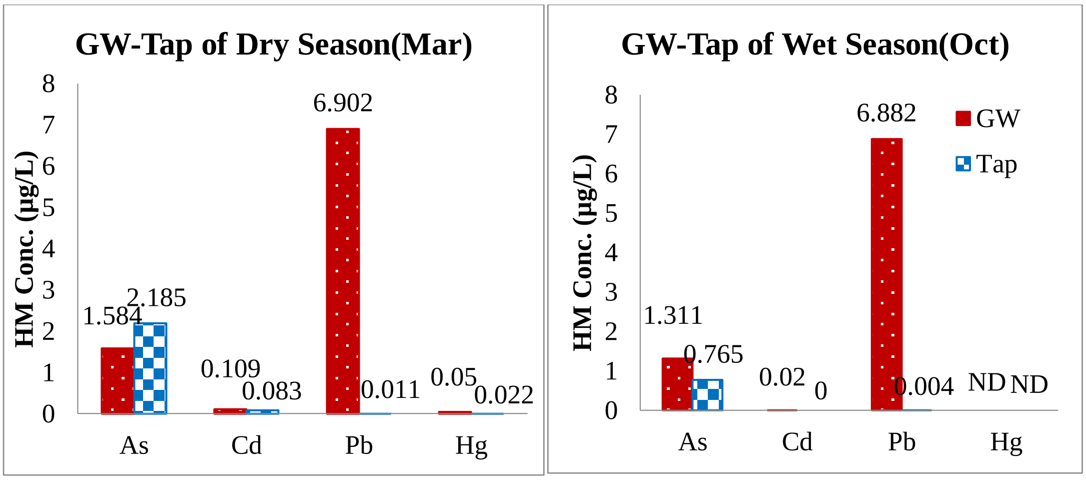


Figure 4. 6 The comparison of average concentrations heavy metals in drinking water between groundwater and tap water

The results of heavy metals in groundwater found that 4 wells of 25 found Pb higher than standard in dry season, and 8 wells found in wet season. The comparisons at each station were showed in Figure 4.7.

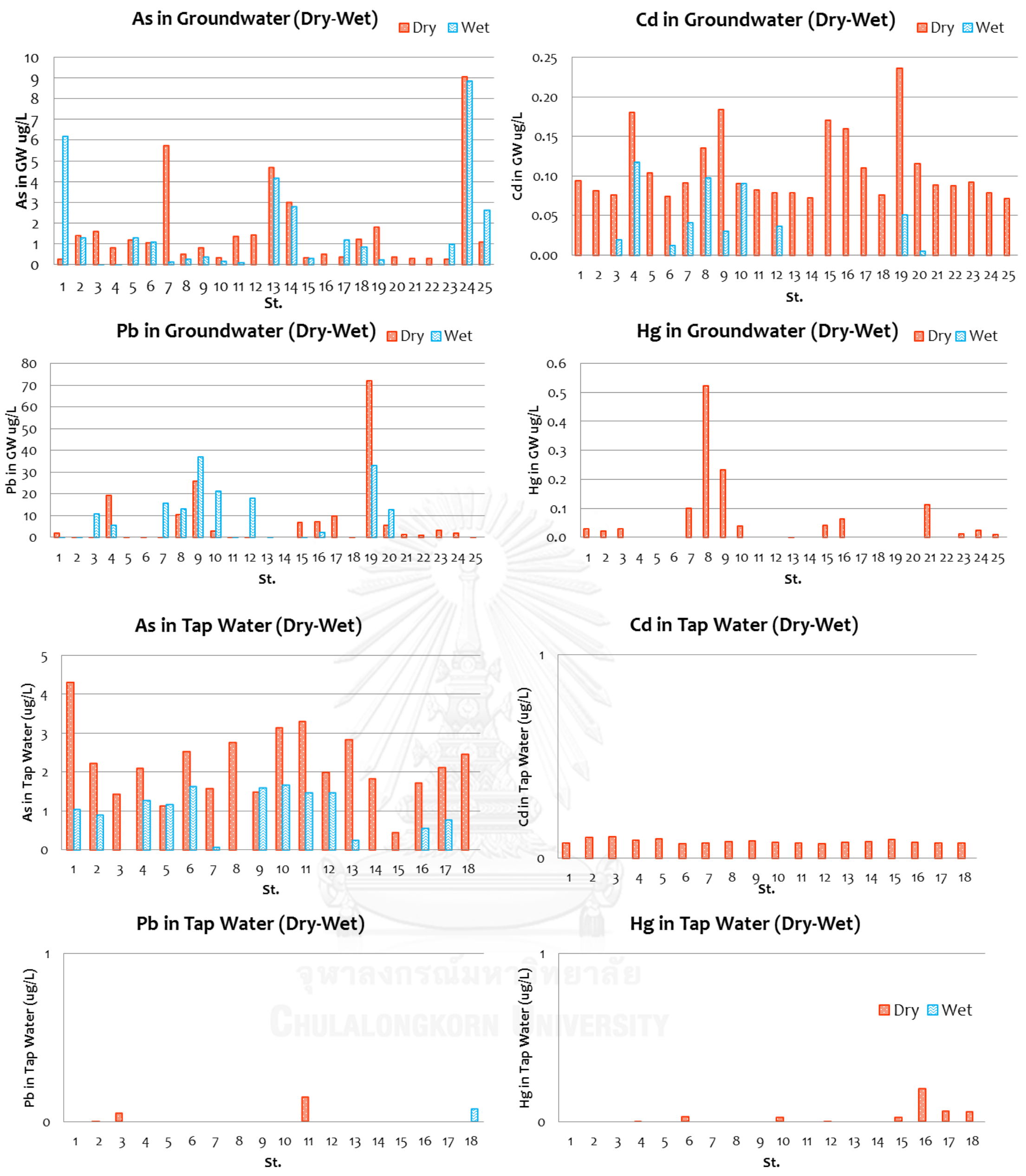


Figure 4. 7 The comparison of groundwater and tap water between wet and dry season at each station

4.3 The characteristics of 100 local participants

This study involved 100 participants, consisting of 58 people in the groundwater-drinking group and 42 people in the non-groundwater-drinking groups. The participants comprised 28 males and 72 females. Their average weight was 59.9±12.8 kg, ranging from 30.0 to 110.0 kg. Their average height was 157.6±7.31 cm, ranging from 140.0 to 176.0 cm. The average age of the participants, excluding children, was 45.8±13.8, ranging from 18 to 78 years of age. The USEPA normal standard consumption rate of adults ranged from 1.5 to 2.0 L/d/person (USEPA, 1980), but the locals in this study area had a drinking intake rate that was twice as high that the standard of 4.21±2.73 L/day, with the minimum and maximum drinking rate of 1.25 L/day and up to 12.5 L/day, respectively. This research found that the causes of health risks were that most were agriculturalists (78%) who had long working hours every (average 10.02±2.51 hours/day; maximum was 14 hours/day), implying intensive farm work in the sunshine and in a hot climate. As a result, they consumed a large amount of water while working, which was one of parameters in risk calculation (average daily dose, ADD), which significantly affected the risk assessment results. In Table 4.7, this study found a drinking intake rate that was higher than in other studies and differed from other countries, such as 1.5 L/day reported in Canada (Krishnan and R., 2008) and 1.8±0.6 L/day reported in France (Marion et al., 2015). Similarly, according to our previous research in 2010 in the Ubon Ratchathani province in Thailand, we found that average groundwater consumption was relatively high at 3.6±2.1 L/day/person for adults and the drinking intake rate for the elderly was 2.6±1.0 L/day/person (Chotpantarat et al., 2014). The interesting part of the study results identified the determinant, which was the relatively high intake rate of drinking water compared to participants in other studies. Thus, this study can be used as a representative of risk of low concentrations of heavy metals contaminating the groundwater in tropical areas, where there should be considerable concern and the risk assessment was report in the next chapter (***Chapter V Risk Assessment***). Moreover, the study used urine, hair, nail as biomarkers to confirm the accumulation of heavy metals in humans who had been consuming groundwater at low concentrations, the biomarkers were informed in ***Chapter VI***.

Table 4. 7 The Characteristics of 100 Participants

Factors	**Group Criterion**		
Gender	Male		28 %
	Female		72 %
Weight (kg)	> Median 60.0 <	Avg. 59.9±12.8	Range 30.0-110.0
Height (cm)	> Median 159.0 <	Avg. 157.6±7.31	Range 140.0-176.0
Age (years)	> Median 46.0 <	Avg. 45.8±13.8	Range 18-78
Drinking Rate (L)	> Std. 2 <	Avg. 4.21±2.73	Range 1.25-12.5
Drinking Source	Groundwater or Non-Groundwater	Tap Water	33 %
		Groundwater	58 %
		Buying Bottles (Retail Tap Water)	9 %
Drinking Water Container	Closed or open	Closed Storage	6 %
		Open-Air Storage	84 %
Drinking Water Cleaning Method	Done or none	Boiled	15 %
		Filtered	32 %
		None	53 %
Bath Water Source	Groundwater or	Tap Water	24 %
	Non-Groundwater	Groundwater	76 %

Washing Water Source	Groundwater or Non-Groundwater	Tap Water	23 %
		Groundwater	77 %
Cooking Water Source	Groundwater or Non-Groundwater	Tap Water	24 %
		Groundwater	75 %
		Buying Bottles (Retail Tap Water)	1 %
Education	> Primary School <l	Median=Primary School	
		Lower than Primary School	2 %
		Primary School	62 %
		Secondary School	16 %
		High School/Vocational Certificate	14 %
		Diploma/High Vocational Certificate	2 %
		Bachelor or Higher	4 %
Occupation	Agriculturalist or others	Median=Agriculturalist	
		Student	1 %
		Officer	2 %
		Merchant	11 %
		Agriculturalist	78 %
		Unemployed	8 %
Family Occupation	Agriculturalist or others	Median=Agriculturalist	
		Merchant	7 %
		Agriculturalist	89 %
		Officer	4 %

Family Members (persons)	>median 4 <	Avg. 4±2	Range 1-10
Work Rate	>Std. 8 hr/d<	Work Hours per Day (hrs.)	
		Avg. 10.02±2.51	Range 4-14
		Work Days per Week (Day)	
		Avg. 6.65±0.83	Range 4-7
Smoking Behavior	Smoking or none	Smoking	26 %
		Avg. 7±7 units/day	Range 2-13 units/day
		Non-Smoking	74 %
		Family Smoking	45 %
		Family Non-Smoking	65 %
Alcohol Drinking	Drinking or none	Alcohol Drinking	25 %
		Median= 1 meal/week	Median=1 Bottle/meal/person
		No-Alcohol Drinking	75 %
Underlying Diseases	Yes or no	Yes	64 %
		No	36 %
Use of Pesticide	Yes or no	Yes	69 %
		Indoor/House Using	23 %
		Use for Agriculture	56 %
		Both	21 %
		Pesticide Using Sequence	Median= 1 time/week
		No	31 %

Chemical Fertilizer Contact	Yes or no	Yes	79 %
		Fertilizer Using Sequence	Median= 1 time/month
		No	21 %
Washing Hands Before Meals	Always or Sometimes	Always	89 %
		Sometimes	11 %
Personal Protective Equipment Use	>Median 4 pieces<	Bamboo/Palm Leaf Hat	72 %
		Fabric Mask	42 %
		Short Rubber Gloves	48 %
		Long Rubber Gloves	11 %
		Long-Sleeved Shirt	75 %
		Long Pants	74 %
		Short Rubber Boots	48 %
		Long Rubber Boots	22 %

CHAPTER V

HEALTH RISK ASSESSMENT RELATED WITH AS, CD, PB, HG CONTAMINATED IN DRINKING WATER

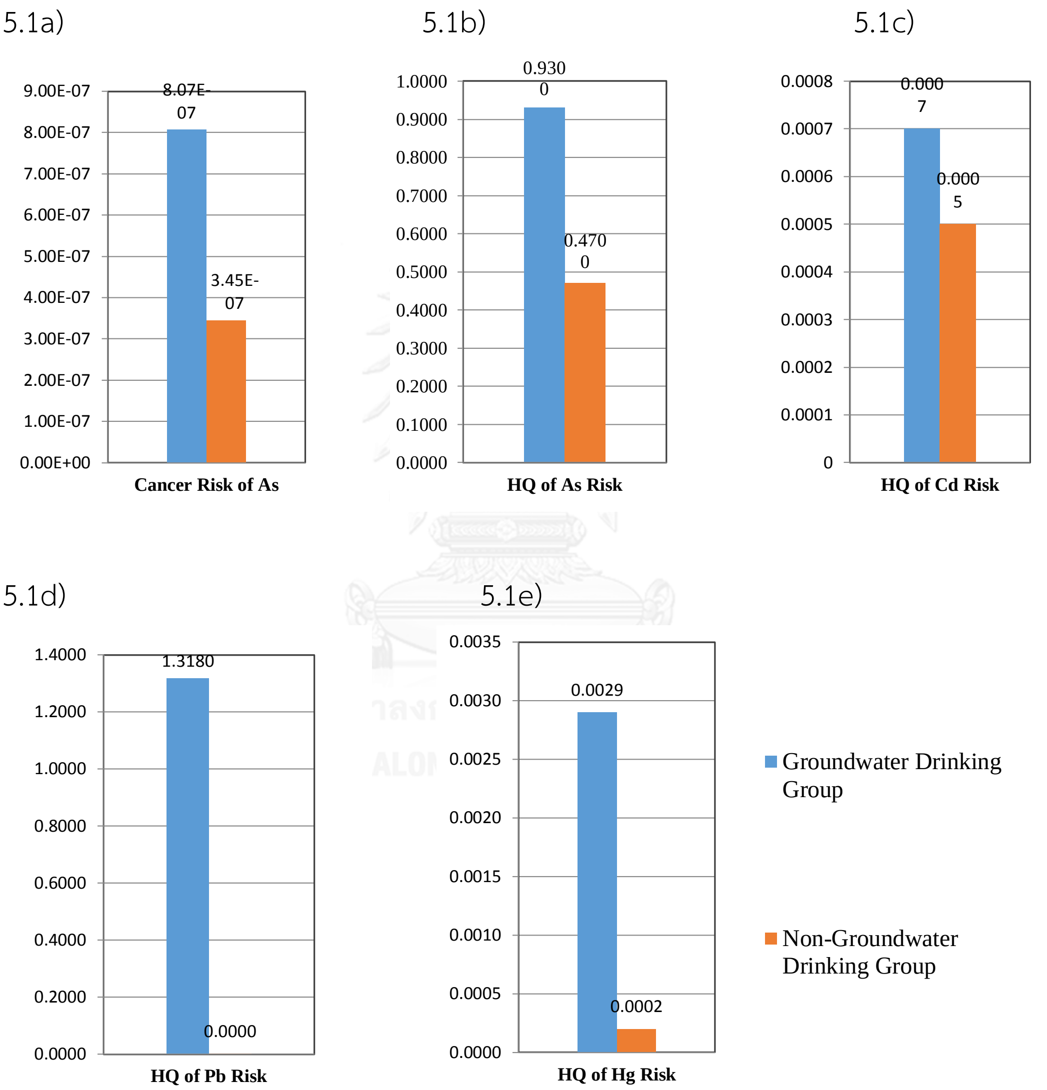
5.1 Health risk assessment in study area in the dry season

All heavy metals (As, Cd, Pb, and Hg) found in the groundwater-drinking group had higher non-carcinogenic risk (HQ) than those in the non-groundwater-drinking group. To compare the health risk assessment between the groundwater-drinking participants and the non-groundwater-drinking participants, the Mann-Whitney U-test (2-tailed) showed the significant difference of Pb non-carcinogenic risk ($p<0.05$, $p=0.000$) and Hg non-carcinogenic risk ($p<0.05$, $p=0.013$) between these two participants, while the As cancer risk, As non-carcinogenic risk and Cd non-carcinogenic risk did not show a statistically significant difference ($p>0.05$, $p=0.645$, $p=0.511$ and $p=0.453$, respectively). Although their risks were not significantly different, the results of the groundwater-drinking group were still higher than those of the non-groundwater-drinking group, even for low cancer risk or HQ. In the groundwater drinking group risk assessment associated with As, 24.14 % of the participants had a carcinogenic risk and 27.59 % had non-carcinogenic risks and may see adverse health effects from As-contaminated groundwater. Moreover, in the results of the Pb risk assessment, 13.79 % of the participants had a non-carcinogenic risk. For the health risk assessment of the non-groundwater-drinking group, As carcinogenic risk were found in only 11.90 % of participants, and only 9.52 % had As non-carcinogenic risks. Although the non-groundwater-drinking participants did not see adverse health effects from the groundwater, they may be affected by other factors involving accidental exposure (locals unintentionally exposed or unaware of exposure to heavy metals). Similarly, Soma and Abhay (2015) found that the largest chronic risk was contributed by As from the drinking water pathway. Locals can also accumulate toxic metals in their bodies via three exposure pathways: ingestion via the mouth, inhalation via breathing, and dermal contact via the skin. For the groundwater-drinking participants, the most important route was ingestion because the locals believe that groundwater tastes good and contains beneficial minerals since their ancestors had long drunk groundwater in the field.

For non-groundwater-drinking participants, the most important route in this study is dermal because most locals used groundwater for washing and cleaning, not for drinking. Moreover, they are generally exposed to agricultural chemicals without use of proper PPE, and most directly touch such chemicals with their hands without gloves since they feel comfortable in the tropical weather.

The human health risk assessment associated with As contaminating the drinking water of groundwater-drinking participants found that the average cancer risk was $8.07x10^{-7}$, ranging from $7.92\ x10^{-10}$ to $8.07\ x10^{-6}$ (see Figure 5.3 and Table 5.1), while the non-groundwater-drinking participants revealed that the average cancer risk was $3.45\ x10^{-7}$, ranging from $2.77\ x10^{-16}$to $1.54\ x10^{-6}$ (see Figure 5.1a and Table 5.1).

For the non-carcinogenic risk, the HQ of As contaminating the drinking water for the groundwater-drinking participants was 0.93, ranging from 0.00 to 8.65 (see Figure 5.1b and Table 5.1), compared to 0.47, ranging from 0.00 to 4.51 for the non-groundwater drinking participants (see Figure 5.1b and Table 5.1). For Cd-contaminated drinking water the average HQ was 0.0007, ranging from 0.0001 to 0.0028 in the groundwater-drinking participants (see Figure 5.1c and Table 5.1), compared to 0.0005, ranging from 0.0000 to 0.0022 in the non-groundwater-drinking participants (see Figure 5.1c and Table 5.1). For Pb-contaminated drinking water, the average HQ was 1.318, ranging from 0.00 to 25.67 for the groundwater-drinking participants (see Figure 7d and Table 8), compared to 0.00002, ranging from 0.0000 to 0.0005 for the non-groundwater-drinking participants (see Figure 5.1d and Table 5.1). Finally, For Hg-contaminated drinking water, the average HQ was 0.0029, ranging from 0.000 to 0.057 for the groundwater-drinking participants (see Figure 5.1e and Table 5.1), and 0.0002, ranging from 0.000 to 0.002 for the non-groundwater-drinking participants, as shown in Table 5.2. The non-carcinogenic contour map of heavy metals in groundwater showed in Figure 5.2. The main reasons for concomitantly affecting the risk level are much different between groundwater-drinking participants and non-groundwater-drinking participant: the concentration of heavy metals in the drinking water and intake amounts of drinking water consumption. Therefore, the participants who drink groundwater generally have a higher risk than those who drink tap water.



5.1a)

5.1b)

5.1c)

5.1d)

5.1e)

Figure 5. 1 The comparison of HQ and cancer risk of heavy metals in groundwater-drinking participants and non-groundwater-drinking participants; a) Cancer risk of As. b) HQ of As non-carcinogenic risk. c) HQ of Cd non-carcinogenic risk. d) HQ of Pb non-carcinogenic risk. e) HQ of Hg non-carcinogenic risk.

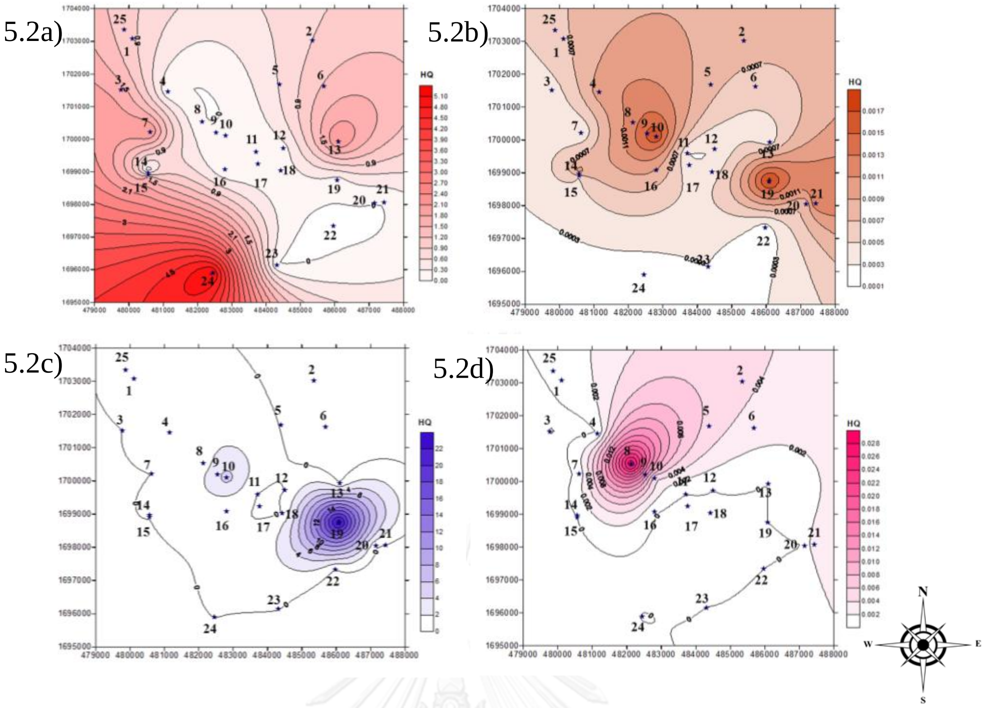
The HQ of the As risk in groundwater-drinking participants was higher than the acceptable level (HQ<1) at well nos. 7, 13, 14, and 24, with HQs of 2.172, 2.148, 1.447, and 5.149, respectively. The cancer risk of As is at a higher than acceptable level: 2.14×10^{-6}, 1.62×10^{-6}, 1.15×10^{-6}, and 4.47×10^{-6} for well nos. 7, 13, 14, and 24 (cancer risk > 1×10^{-6} meant concern risk, cancer risk < 1×10^{-6} meant acceptable level). Similarly, the HQ of Pb from groundwater-drinking participants found 3 stations had higher than the acceptable levels: well nos. 4, 9, and 19, with HQs of 1.62, 4.93, and 23.80, respectively, as shown in Tables 5.1 and 5.2 and Figures 5.1 to 5.3. As-contaminated water that caused adverse health effects in humans has been found in Thailand and in different countries around the world. For example, Muhammad et al. (2009) reported that in Southeast Asia, more than 100 million people were estimated to be at risk from As-contaminated groundwater and that 700,000 people were affected by As-related diseases. Research in the Baja Peninsula of Mexico reported that urinary samples contained a total arsenic concentration (sum of arsenical species) that ranged from 1.3 to 398.7 µg/L. These areas had reported As measurements in drinking water above the national standard (25 ng As/ mL) and that 40.5 % of the wells contain 0.35–10 µg As/L, 21.5 % contain 10.1–25 µg As/L, 31 % contain 25.1–200 µg As/L, and 7 % contain 200.1–2,270 µg As/L. They showed that the risk from drinking water was seen at an intake rate of 1.6 L/day, which contains a level of 2.5 µg/L or 1 in 1,000 persons, and 50 µg/L (1.6 L per day) being 21 in 1,000 persons (Carlos et al. 2014).

Table 5. 1 Health risk assessment associated with heavy metals of groundwater-drinking participants at each well

	Average Risk Assessment of Groundwater-drinking participants (Dry season)				
Station	**As-Cancer Risk**	**As-HQ**	**Cd-HQ**	**Pb-HQ**	**Hg-HQ**
well 3	2.43x10^{-7}	0.275	0.0004	8.38E-08	1.04E-04
well 4	2.06x10^{-8}	0.033	0.0010	1.62E+00	5.07E-10
well 7	2.14x10^{-6}	2.172	0.0003	8.37E-05	6.83E-04
well 8	1.64x10^{-8}	0.024	0.0012	9.77E-01	2.86E-02
well 9	5.14x10^{-8}	0.056	0.0017	4.93E+00	4.65E-03
well11	1.02x10^{-7}	0.117	0.0003	2.12E-04	6.57E-10
well 12	1.54x10^{-7}	0.166	0.0003	1.96E-03	8.22E-10
well 13	1.62x10^{-6}	2.148	0.0004	8.39E-11	2.45E-06
well 14	1.15x10^{-6}	1.447	0.0005	1.38E-10	1.61E-09
well 15	4.92x10^{-9}	0.006	0.0010	2.40E-01	1.02E-04
well16	7.85x10^{-9}	0.014	0.0009	2.65E-01	2.48E-04
well 18	1.02x10^{-7}	0.153	0.0004	6.24E-04	1.01E-09
well 19	1.51x10^{-7}	0.171	0.0018	2.38E+01	5.32E-10
well 20	9.57x10^{-9}	0.012	0.0007	2.64E-01	9.13E-10
well 21	1.52x10^{-8}	0.022	0.0013	5.52E-02	3.44E-03
well 22	2.07x10^{-9}	0.004	0.0002	4.10E-03	4.99E-10
well 23	5.03x10^{-9}	0.004	0.0003	6.23E-02	1.14E-05
well 24	4.47x10^{-6}	5.149	0.0002	2.26E-02	4.22E-05
Avg.±SD	8.07x10^{-7}±1.5 x10^{-6}	0.93±1.67	0.0007±0.0006	1.3180±4.5212	2.94E-03±9.15E-03
Min	7.92 x10^{-10}	0.00	0.0001	4.35E-11	2.67E-10
Max	8.07 x10^{-6}	8.65	0.0028	25.6711	5.68E-02

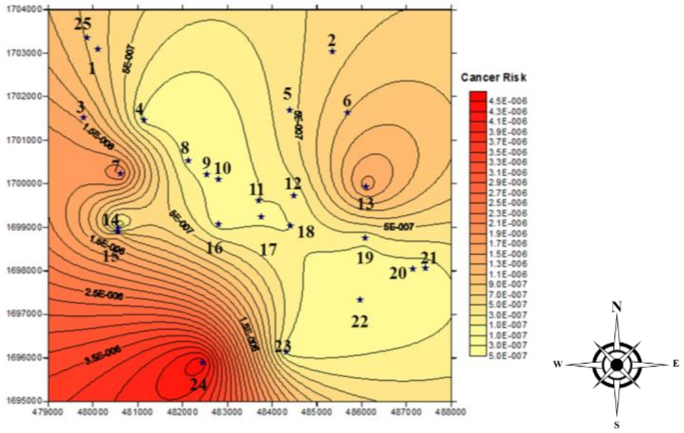
Table 5. 2 Health risk assessment of non-groundwater-drinking participants at each well

	Average Risk Assessment of Non-Groundwater-drinking participants (Dry season)				
Station	**As-Cancer Risk**	**As-HQ**	**Cd-HQ**	**Pb-HQ**	**Hg-HQ**
well 1	$1.17x10^{-6}$	2.09	6.51E-04	1.13E-09	1.13E-09
well 2	$2.93x10^{-7}$	0.29	6.15E-04	2.13E-06	5.91E-10
well 4	$7.75x10^{-16}$	0.00	9.68E-10	9.68E-10	9.68E-10
well 5	$1.88x10^{-7}$	0.18	1.01E-03	2.15E-04	8.94E-10
well 6	$3.25x10^{-7}$	0.41	7.58E-04	9.35E-10	2.34E-06
well 9	$5.70x10^{-16}$	0.00	7.07E-10	7.07E-10	7.07E-10
well 10	$1.50x10^{-7}$	0.10	2.17E-04	5.38E-10	5.38E-10
well 11	$1.12x10^{-6}$	0.65	5.68E-04	8.45E-10	8.45E-10
well 17	$7.02x10^{-7}$	0.98	7.46E-04	1.23E-09	1.23E-09
well 18	$5.64x10^{-16}$	0.00	1.22E-09	1.22E-09	1.22E-09
well 19	$1.34Ex10^{-7}$	0.23	4.50E-04	6.85E-10	6.85E-10
well 20	$2.14x10^{-8}$	0.03	1.23E-03	1.39E-09	8.00E-05
well 21	$3.57x10^{-7}$	0.18	3.69E-04	6.22E-10	2.32E-03
well 22	$1.25x10^{-7}$	0.45	5.77E-04	9.98E-10	3.59E-04
well 23	$1.35x10^{-7}$	0.38	3.55E-04	6.32E-10	2.13E-04
Avg.±SD	$3.45x10^{-7} \pm 4.21x10^{-7}$	0.47±0.80	0.0005±0.0005	0.00002±0.00008	0.0002±0.0006
Min	$2.77 \ x10^{-16}$	0.00	4.68E-10	2.53E-10	2.34E-10
Max	$1.54 \ x10^{-6}$	4.51	2.19E-03	4.68E-04	3.33E-03



★: Sampling stations of shallow groundwater wells

Figure 5. 2 a) Non-carcinogenic risk map of As in groundwater-drinking participants. b) Non-carcinogenic risk map of Cd in groundwater-drinking participants. c) Non-carcinogenic risk map of Pb in groundwater-drinking participants. d) Non-carcinogenic risk map of Hg in groundwater-drinking participants.



★: Sampling stations of shallow groundwater wells

Figure 5. 3 Carcinogenic risk map of As in groundwater-drinking participants

Interestingly, for the overall risk of 100 participants, the average hazard index (HI) was calculated, and the results found that average HI of 100 participants was higher than the acceptable level. The HI of the 100 participants ranged from 0.00 to 25.86 with an average of 1.51±3.63, revealing that 28 people appeared to have non-carcinogenic risk. To compare the two groups of participants, the HI of the groundwater-drinking participants showed that 24 people had non-carcinogenic risk, with an average HI being higher than the acceptable level at 2.25±4.59 (ranging from 0.01-25.86), while the HI of the non-groundwater-drinking participants showed that only 4 people had non-carcinogenic risk of 5 times lower than the average HI of 0.47±0.80 (ranging from 0.00-4.51). In addition, the U-test results showed HQ-Pb, HQ-Hg, and HI were significant different between the groundwater drinking and non-the groundwater drinking participants in dry season.

5.2 Health risk assessment in study area in the wet season

During the wet season for the groundwater drinking group, 11 of 58 groundwater drinking persons (18.97%) found As-non-carcinogenic risk and 10 of 58 persons (17.24%) found As cancer risk. Moreover, 21 of 58 persons (36.21%) found Pb non-carcinogenic risk, while there was no Pb non-carcinogenic risk for the non-groundwater participants. As a result, this study investigated that even low concentrations of heavy metals in groundwater can cause adverse health effects to humans by a high intake rate and HI. The human health risk assessment associated with As in groundwater found the average cancer risk was 5.30×10^{-7}, ranging from 0.00 to 7.71×10^{-7}, while the non-groundwater-drinking participants revealed that the average cancer risk was 2.95×10^{-8}, ranging from 2.06×10^{-16} to 3.15E-07.

For the non-carcinogenic risk, the HQ of As contaminating the drinking water for the groundwater-drinking participants was 0.63, ranging from 0.00 to 8.26, compared to 0.04, ranging from 0.00 to 0.26 for the non-groundwater drinking participants. For Cd-contaminated drinking water, the average HQ of Cd was 0.0001, ranging from 0.0001 to 0.0012 in the groundwater-drinking participants, but there is no HQ of Cd in the non-groundwater-drinking participants. For Pb-contaminated drinking water, the average HQ was 1.41, ranging from 0.00 to 16.32 for the groundwater-drinking participants, but there is no HQ of Pb in the non-groundwater-drinking participants. Finally, there is no HQ of Hg in both groups. The hazard index (HI) of 100 participants related with drinking water during the dry season ranged from 0.00 to 25.86, with an average of 1.51±3.63, which is higher than the acceptable level. The HI values in the wet season was lower than those in the dry season, but it was still greater than the acceptable level with an average of 1.20±2.50, ranged from 0.00 to 16.34. In addition, the U-test showed HQ-Cd, HQ-Pb, and HI were significant different between the groundwater drinking participants and the other participants in the wet season.

Table 5. 3 Health risk assessment associated with heavy metals of the groundwater-drinking participants at each well in the wet season

Average Risk Assessment of Groundwater-drinking participants (Wet Season)					
Station	As-Cancer Risk	As-HQ	Cd-HQ	Pb-HQ	Hg-HQ
well 3	4.24x10^{-11}	0.000	2.35E-05	1.131	1.09E-07
well 4	7.81x10^{-13}	0.000	4.24E-04	0.142	5.07E-08
well 7	1.03x10^{-9}	0.001	6.62E-05	1.403	6.56E-08
well 8	4.39x10^{-9}	0.007	5.98E-04	1.531	1.04E-07
well 9	9.08x10^{-9}	0.010	4.94E-05	10.116	8.57E-08
well11	4.53x10^{-10}	0.001	3.94E-08	0.002	6.57E-08
well 12	0.00	0.000	6.39E-05	2.325	8.22E-08
well 13	1.29x10^{-6}	1.708	5.88E-08	0.001	9.79E-08
well 14	9.91x10^{-7}	1.250	9.66E-08	0.000	1.61E-07
well 15	4.19x10^{-9}	0.007	3.47E-08	0.000	5.78E-08
well16	0.00	0.000	3.42E-08	0.031	5.69E-08
well 18	4.73x10^{-8}	0.071	6.08E-08	0.000	1.01E-07
well 19	1.90x10^{-9}	0.002	8.31E-05	5.019	5.32E-08
well 20	0.00	0.000	1.37E-06	1.326	9.13E-08
well 21	0.00	0.000	1.59E-07	0.000	2.64E-07
well 22	0.00	0.000	2.99E-08	0.000	4.99E-08
well 23	7.36x10^{-8}	0.076	4.06E-08	0.000	6.76E-08
well 24	4.27x10^{-6}	4.917	3.75E-08	0.000	6.24E-08
Avg.±SD	5.30E-07±1.42E-06	0.63±1.53	0.0001±0.0002	1.41±2.93	8.44E-08±5.08E-08
Min	0.00	0.00	1.60E-08	0.00	1.75E-08
Max	7.71E-06	8.26	1.19E-03	16.32	2.64E-07

Table 5. 4 Health risk assessment associated with heavy metals of the non-groundwater drinking participants at each well in the wet season

	Average Risk Assessment of Non-Groundwater-drinking participants (Wet Season)				
Station	**As-Cancer Risk**	**As-HQ**	**Cd-HQ**	**Pb-HQ**	**Hg-HQ**
well 1	$6.64x10^{-8}$	0.12	1.13E-09	1.13E-07	1.13E-09
well 2	$4.75x10^{-8}$	0.05	5.91E-10	5.91E-08	5.91E-10
well 4	$7.75x10^{-16}$	0.00	9.68E-10	9.68E-08	9.68E-10
well 5	$9.24x10^{-16}$	0.00	8.94E-10	8.94E-08	8.94E-10
well 6	$1.18x10^{-7}$	0.15	9.35E-10	9.35E-08	9.35E-10
well 9	$5.70x10^{-16}$	0.00	7.07E-10	7.07E-08	7.07E-10
well 10	$2.56x10^{-10}$	0.00	5.38E-10	5.38E-08	5.38E-10
well 11	$1.46x10^{-15}$	0.00	8.45E-10	8.45E-08	8.45E-10
well 17	$5.20x10^{-9}$	0.01	1.23E-09	1.23E-07	1.23E-09
well 18	$5.64x10^{-16}$	0.00	1.22E-09	1.22E-07	1.22E-09
well 19	$7.79x10^{-8}$	0.07	6.85E-10	6.85E-08	6.85E-10
well 20	$1.09x10^{-15}$	0.00	1.39E-09	1.39E-07	1.39E-09
well 21	$3.61x10^{-8}$	0.02	6.22E-10	6.22E-08	6.22E-10
well 22	$2.78x10^{-16}$	0.00	9.98E-10	9.98E-08	9.98E-10
well 23	$1.30x10^{-8}$	0.04	6.32E-10	6.32E-08	6.32E-10
Avg.±SD	$2.95x10^{-8}$ $\pm5.76x10^{-8}$	0.04±0.06	8.87E-10 ±2.34E-10	8.87E-08 ±5.83E-08	8.87E-10 ±5.83E-10
Min	$2.06\ x10^{-16}$	0	2.34E-10	2.34E-08	2.34E-10
Max	$3.15\ x10^{-7}$	0.26	2.59E-09	2.59E-07	2.59E-09

5.3 The average health risk level and seasonal variation on risk level

The risk assessment results showed HI during dry season was higher value than wet season, the effect from large amount of rain caused heavy metals concentrations in drinking water decreasing. Although the HI value of dry season was higher than wet season but in wet season, effect of heavy metals was spread in larger area than dry season caused of groundwater flow. The HI data found 8 wells points had HI>1 (ave. 2.46 ranged 0.01-23.97) in dry season and 1 tap point found HI>1 (ave. 0.40 ranged 0.00-2.49), while wet season found 10 wells points had HI>1 (ave. 1.73 ranged 0.00-10.13) and no risk at any tap point (ave. 0.03 ranged 0.00-0.15) (see Figure 5.4 and 5.5).

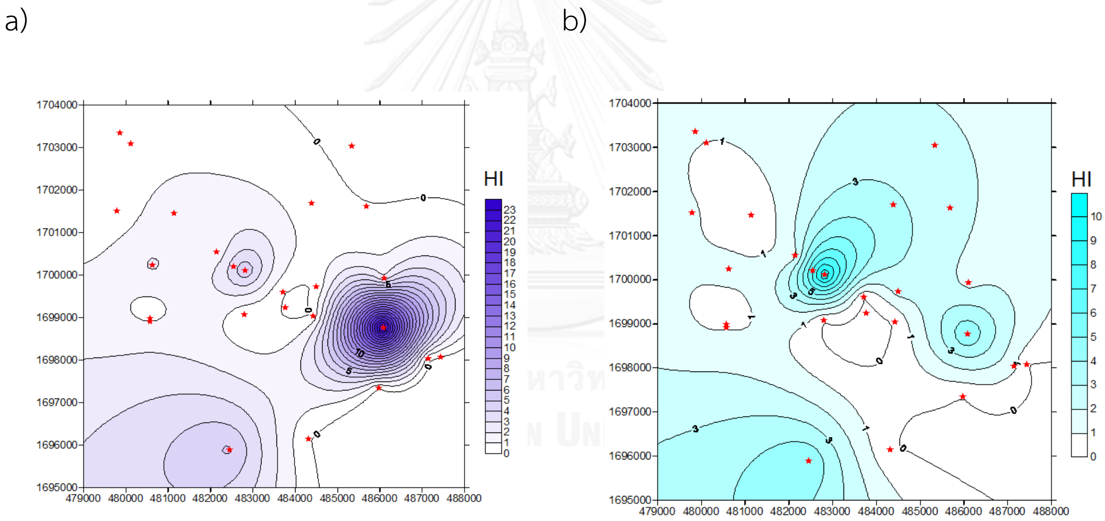


Figure 5. 4 The contour map of hazard index (HI) in a) dry season and b) wet season

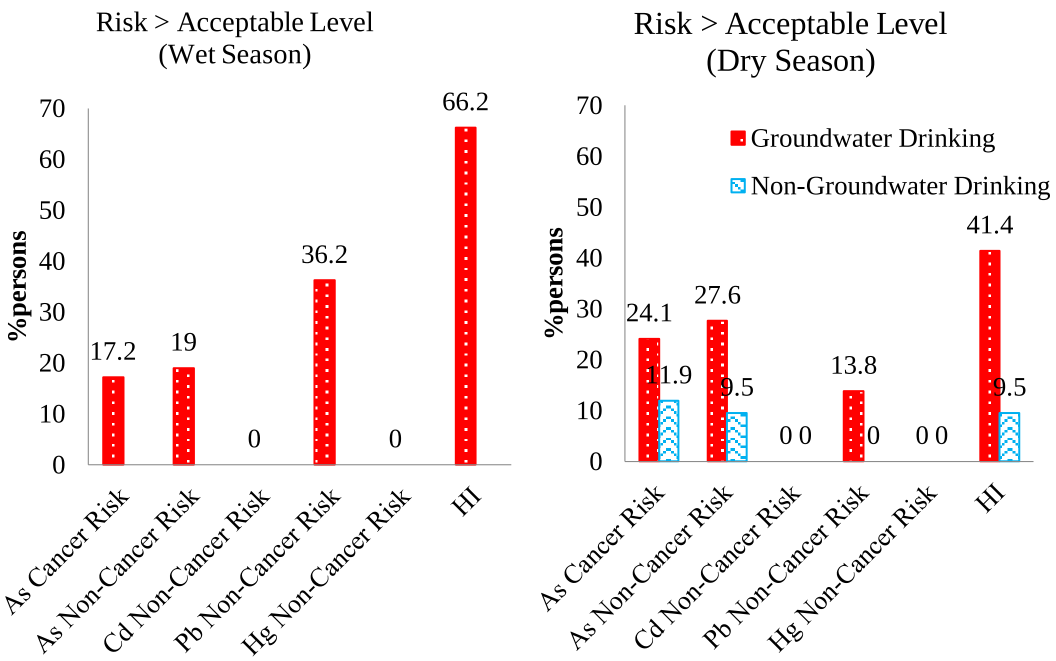


Figure 5. 5 The percentage of groundwater drinking and non-groundwater drinking participants those who found cancer and non-cancer risks higher than acceptable

The risk assessment of heavy metals contaminated in drinking water for whole year round found that 8 groundwater well stations had risk exposed to the groundwater drinking participants, and there is no risk found in the other group. The average HI of groundwater was 2.09 in the ranged from 0.01 to 14.50, while the average HI of tap water was 0.03, in the range from 0.00 to 0.15 (see Table 5.5).

Compare to the previous study in year 2011 (Wongsasuluk et al., 2014), HQ results found As was 35.571, Cd was 0.001, Pb was 3.548, Hg was 0.002 μg/L and HI was 39.112. For this study, average HQ of As was 0.556, Cd was 0.0004, Pb was 1.535, Hg was 0.001 μg/L, and HI was 2.093. Previous study found higher HQ and HI than this study because previous study area was small area where focused on center of high concentrations of heavy metals in groundwater as mention zone as mentioned in Chapter 4.

Table 5. 5 The Hazard Quotient (HQ) of drinking water all year round

Station	Year Round HI of Groundwater	Station	Year Round HI of Tap water
well 3	0.71	well 1	0.12
well 4	0.90	well 2	0.05
well 7	1.79	well 4	0.00
well 8	1.29	well 5	0.00
well 9	7.56	well 6	0.15
well11	0.06	well 9	0.00
well 12	1.25	well 10	0.00
well 13	1.93	well 11	0.00
well 14	1.35	well 17	0.01
well 15	0.13	well 18	0.00
well16	0.16	well 19	0.07
well 18	0.11	well 20	0.00
well 19	14.50	well 21	0.02
well 20	0.81	well 22	0.00
well 21	0.04	well 23	0.04
well 22	0.01		
well 23	0.08		
well 24	5.05		
Ave.	2.09		0.03
SD	3.66		0.05
Min	0.01		0.00
Max	14.50		0.15

CHAPTER VI

BIOMARKERS OF HUMAN EXPOSURE RELATED WITH AS, CD, PB, HG CONTAMINATED IN DRINKING WATER SOURCES

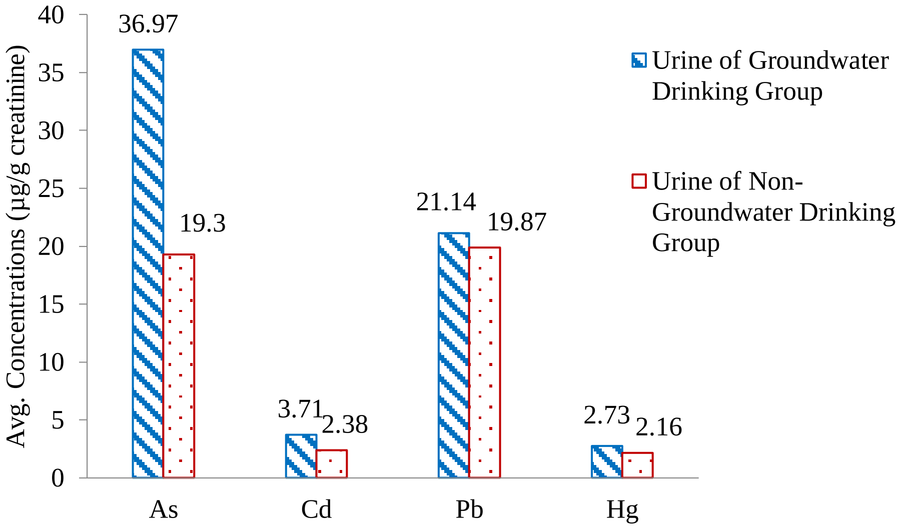
6.1 Urine as the biomarker of daily exposure

6.1.1 In the dry season

This study found that the average concentrations of all heavy metals in urine from participants in the groundwater-drinking group were higher than those in non-groundwater-drinking group. In addition, the average of As in urine from the participants in groundwater-drinking group were also greater than the ACGIH standard of 35 µg/L (the association advancing occupational and environmental health). The average As concentration in the urine of groundwater-drinking participants was 36.97 µg/L, ranging from 2.80 to 119.60 µg/L, while the non-groundwater-drinking participants' concentration was 19.30 µg/L, ranging from 2.20 to 34.10 µg/L, since the major factor is the average drinking rate of groundwater-drinking participants of 4.44 L/day, which is relatively higher than the other group at 3.88 L/day. The average Cd concentration in the urine of groundwater-drinking participants was 3.71 µg/g of creatinine, ranging from 0.30 to 8.66 µg/g of creatinine (the standard of Cd in the urine was 4 µg/g of creatinine), while the non-groundwater-drinking participants was 2.38 µg/g of creatinine, ranging from 0.64 to 4.22 µg/g of creatinine. The average concentration of Pb in the urine of groundwater-drinking participants was 21.14 µg/g of creatinine, ranging from 4.99 to 58.82 µg/g of creatinine (the standard of Pb in urine was 50 µg/g of creatinine), while the average was 19.87 µg/g of creatinine, ranging from 6.25 to 45.45 µg/g of creatinine for non-groundwater-drinking participants. The average concentration of Hg in the urine of groundwater-drinking participants was 2.73 µg/g of creatinine, ranging from 0.35 to 9.26 µg/g of creatinine (the standard of Hg in urine was 5 µg/g of creatinine), while the average concentration of non-groundwater-drinking participants was 2.16 µg/g of creatinine, ranging from 0.46 to 5.25 µg/g of creatinine (see Table 6.1 and 6.2).

The urine results of Cd, Hg, and Pb were standardized to the creatinine level in the urine because these heavy metals cause adverse effects on the kidney (Gil et al., 2011, Li et al., 2011, Ivanenko et al., 2013), while creatinine is not a suitable correction factor for As in the urine, so the As level were, in contrast, reported as µg As/L urine. However, many studies did not use creatinine as a correction factor anymore because of the effect from the hydration status and variable protein intake (Knudsen et al., 2000, Nermell et al., 2008, Jooste and Strydom, 2010). Carlos et al. (2014) found that chronic exposure to inorganic As and high urinary As levels had been linked to an increased creatinine concentration in urine. Furthermore, the urine results found that 30 of the 58 participants in the groundwater drinking group had As in their urine at a higher level than the standard (51.72 %) and 26, 2 and 9 participants had higher than standard levels for Cd (44.83 %), Pb (3.45 %), and Hg (15.52 %). On the other hand, only 3 participants in the non-groundwater-drinking group had Cd levels higher than the standard (7.14 %) and only 1 participant for Hg (2.38 %), as shown in Figure 6.1.

The Mann-Whitney U-test comparison results found a statistically significant difference between As and Cd in the urine of these two groups ($p<0.05$, $p=0.002$), whereas there was no such difference for Pb and Hg. Although they were not significantly different, the results of the groundwater-drinking group seemed to be higher than those of the non-groundwater-drinking group, even in low concentrations.



*Unit of As in urine is µg /L while Cd, Pb, and Hg is µg/g creatinine.

**Standard of As in urine is 35, Cd is 4, Pb is 50, Hg is 5 µg/g creatinine.

Figure 6. 1 Heavy metals in the urine of 100 participants, in which 58 were groundwater-drinking participants and 42 were non-groundwater-drinking participants

There were 7 stations that caused average As concentrations in the urine of groundwater-drinking group to be higher than the standard (35 µg/g of creatinine) at well as at stations 3, 7, 11-14, and 24, with a range from 36.70 to 80.90 µg/L (see Tables 6.1 and 6.2). For Cd concentration in the urine of the groundwater-drinking group, there were 10 stations at which the average Cd concentration was greater than the standard (4 µg/g of creatinine) at well nos. 4, 8, 9, 15, 16, and 19-23, with a range from 4.69 to 8.66 µg/g of creatinine (see Tables 6. and 6.2). There was only one station at well no. 19 that showed an average Pb concentration of 53.23 µg/g of creatinine that was higher than the standard (50 µg/g of creatinine). Furthermore, there were 3 stations that found an average Hg concentration in the urine of groundwater-drinking group that was larger than the standard (5 µg/g of creatinine) at well nos. 8, 18, and 19, with a range from 5.62 to 9.26 µg/g of creatinine (Tables 6.1 and 6.2). Figure 5 shows the distribution of concentrations of heavy metals in the urine of the groundwater-drinking group that conformed to those found in shallow groundwater, while Figure 6 shows the distribution of concentrations of heavy metals in the urine of the non-groundwater-drinking group.

The groundwater-drinking participants at the well stations were located at the center of the study area and mostly found Cd, Pb, Hg in the urine that was higher than those of non-groundwater-drinking participants, corresponding to the distributions of Cd, Pb, and Hg concentrations in the groundwater that was higher in the central area than in the surrounding areas. On the other hand, the concentration of As groundwater in the north-eastern and south-western parts of the study area were relatively higher than those in the central area, conforming to the distribution of As in the urine of groundwater-drinking participants (see Figures 6.2 and 6.3).

In general, As has high mobility in shallow aquifer, depending on the oxidation-reduction potential (ORP) where high oxygen levels in shallow groundwater affect high dissolution levels in water. Arsenic adsorb onto the surfaces of aquifer media, including iron oxides. Then, the oxidation reduction between arsenate and iron-oxide surfaces are very crucial since iron oxides are common in the subsurface environment as coatings on other solids and the desorption of arsenate from iron-oxide surfaces becomes higher as pH values become alkaline (USGS, 2016). As a result, the well sites where high As concentrations in the water were found also found high As in the urine of the participants. A few stations did not find an obvious difference between the heavy metals in the urine of the groundwater-drinking participants and non-groundwater-drinking participant which is probably due to other associated factors such as body weight, smoking, sex and using PPE. Groundwater was not only one source of heavy metals accumulated in human body, therefore some heavy metals was affected by other associated factors. The associated factors were presented in ***Chapter VII***.

Table 6. 1 Heavy metals in the urine of participants in the groundwater-drinking group at each station

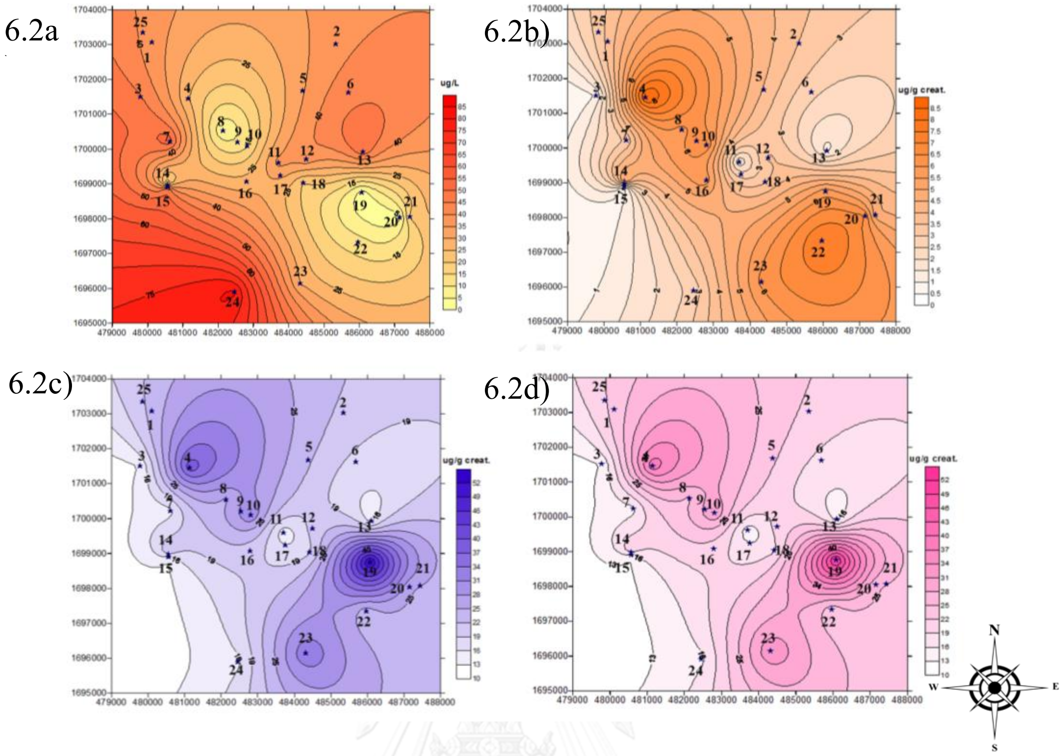
	Heavy Metals in the urine of Groundwater-drinking participants				
St.	**Number of participants** (persons)	**Avg.As** (μg/L)	**Avg.Cd** (μg/g of creatinine)	**Avg.Pb** (μg/g of creatinine)	**Avg.Hg** (μg/g of creatinine)
well 3	3	45.53±11.55*	1.03±0.64	13.24±3.55	0.99±0.20
well 4	1	25.60±0.00	8.66±0.00	38.98±0.00	2.46±0.00
well 7	5	52.98±24.44	3.06±2.37	15.21±9.37	1.92±1.85
well 8	5	5.50±17.47	5.83±1.97	24.60±6.15	8.67±1.79
well 9	4	13.90±6.52	6.49±1.48	30.91±6.78	3.60±1.10
well 11	4	36.70±1.41	1.55±1.27	14.22±5.36	1.03±1.91
well 12	5	38.50±2.16	3.61±0.68	20.09±3.54	0.59±1.24
well 13	4	48.90±12.53	1.34±0.72	14.81±3.18	4.92±1.55
well 14	4	47.90±6.22	0.85±0.63	13.19±2.41	3.31±0.67
well 15	4	15.50±8.34	5.28±0.90	21.88±6.95	0.88±1.03
well 16	4	26.70±9.44	5.79±0.63	20.63±3.76	1.90±0.86
well 18	1	19.70±0.00	3.42±0.00	18.50±0.00	5.62±0.00
well 19	2	2.80±5.44	5.77±0.84	53.23±3.95	9.26±2.35
well 20	1	2.80±0.00	6.53±0.00	25.81±0.00	1.72±0.00
well 21	1	21.60±0.00	4.69±0.00	25.15±0.00	1.13±0.00
well 22	3	12.20±3.23	7.49±1.80	23.08±6.81	1.11±0.95
well 23	2	29.90±6.43	6.14±1.48	33.59±7.51	1.29±0.48
well 24	5	80.90±19.96	2.71±1.94	15.27±4.56	3.38±1.01
Avg.		36.89	3.71	21.14	2.73
Median		36.40	3.52	20.05	2.10
Min-Max		2.80-119.60	0.30-8.66	4.99-58.82	0.35-9.26
Std.		35	4	50	5

*Average Concentration±SD (calculated from 3 repetitions at the point of analysis)

Table 6. 2 Heavy metals in the urine of participants in the non-groundwater-drinking group at each station

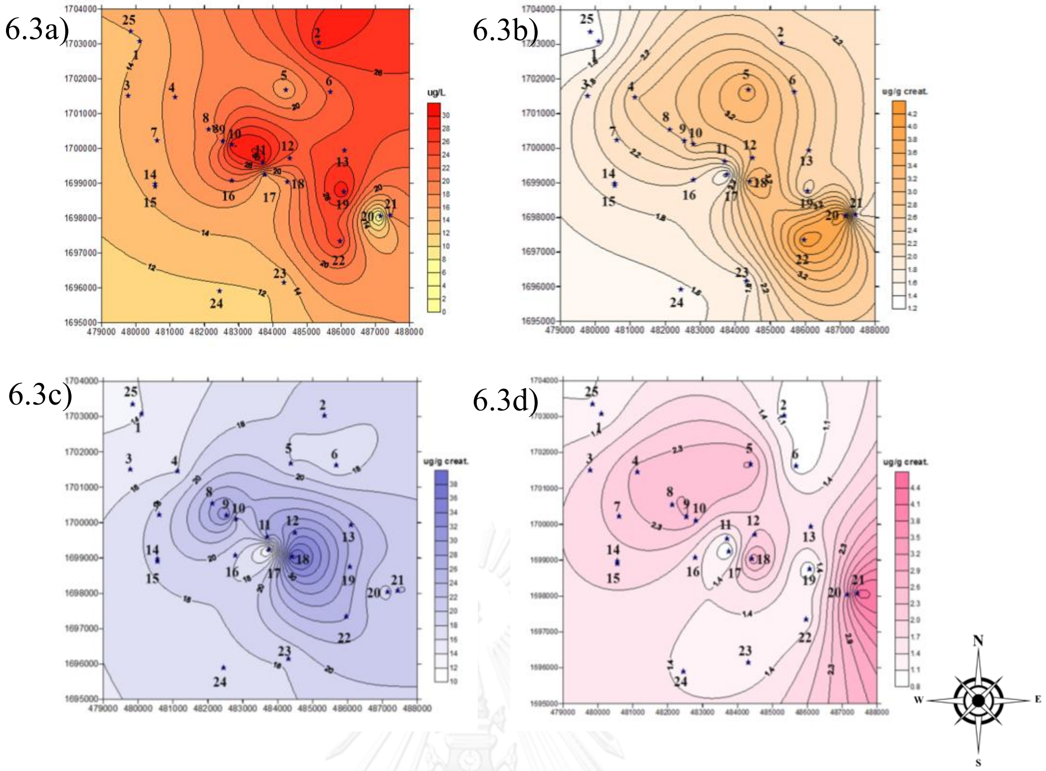
	Heavy Metals in the urine of Non-Groundwater-drinking participants				
St.	**Number of participants** (persons)	**Avg.As** (μg/L)	**Avg.Cd** (μg/g of creatinine)	**Avg.Pb** (μg/g of creatinine)	**Avg.Hg** (μg/g of creatinine)
well 1	4	13.88±13.56	1.34±0.62	13.97±4.24	1.14±0.84
well 2	4	29.10±5.32	2.37±0.45	19.21±1.81	0.98±1.19
well 4	3	16.80±1.06	2.59±0.97	15.92±4.38	2.41±1.61
well 5	4	16.00±1.76	3.51±0.60	18.06±2.51	2.65±1.18
well 6	4	22.30±3.97	2.79±0.32	16.36±13.75	0.94±1.79
well 9	1	30.20±0.00	2.85±0.00	21.55±0.00	2.36±0.00
well 10	4	20.60±11.01	2.59±0.90	30.36±7.82	2.61±0.57
well 11	1	30.80±0.00	2.37±0.00	26.36±0.00	1.22±0.00
well 17	4	15.10±6.98	1.48±1.59	10.15±13.10	0.80±1.32
well 18	2	17.10±9.05	3.54±1.60	39.13±12.87	2.85±0.95
well 19	2	29.70±0.35	2.27±0.40	25.00±7.04	0.88±0.30
well 20	2	5.00±2.97	4.22±0.45	18.84±1.33	3.04±1.56
well 21	3	18.20±2.36	2.00±0.83	22.22±16.55	4.55±2.00
well 22	2	25.60±0.14	4.15±1.00	24.20±4.90	1.17±0.13
well 23	2	12.50±12.66	1.72±0.42	18.28±12.21	1.23±0.28
Avg.		19.30	2.38	19.87	2.16
Median		18.20	2.42	18.18	1.97
Min-Max		2.20-34.10	0.64-4.22	6.25-45.45	0.46-5.25
Std.		35	4	50	5

*Average Concentration±SD (calculated from 3 repetitions at the point of analysis.)



★: Sampling stations of shallow groundwater wells

Figure 6. 2 a) Contour map of the As concentration in the urine of groundwater-drinking participants. b) Contour map of the Cd concentration in the urine of groundwater-drinking participants. c) Contour map of the Pb concentration in the urine of groundwater-drinking participants. d) Contour map of the Hg concentration in the urine of groundwater-drinking participants.



★: Sampling stations of shallow groundwater wells

Figure 6. 3 a) Contour map of the As concentration in the urine of non-groundwater-drinking participants. b) Contour map of the Cd concentration in the urine of non-groundwater-drinking participants. c) Contour map of the Pb concentration in the urine of non-groundwater-drinking participants. d) Contour map of the Hg concentration in the urine of non-groundwater-drinking participants.

6.1.2 In the wet season

The average As concentration in the urine of groundwater-drinking participants was 47.74±40.76 μg/L, ranging from 3.30 to 166.60 μg/L, while the non-groundwater-drinking participants' concentration was 12.99±6.76 μg/L, ranging from 1.60 to 28.80 μg/L. The average Cd concentration in the urine of groundwater-drinking participants was 1.31±0.75 μg/g of creatinine, ranging from 0.31 to 4.61 μg/g of creatinine, while the non-groundwater-drinking participants was 1.06±0.57 μg/g of creatinine, ranging from 0.41 to 3.24 μg/g of creatinine. The average concentration of Pb in the urine of groundwater-drinking participants was 35.17±19.84 μg/g of creatinine, ranging from 1.87 to 79.73 μg/g of creatinine, while the average was 23.37±9.14 μg/g of creatinine, ranging from 6.42 to 38.42 μg/g of creatinine for non-groundwater-drinking participants. The average concentration of Hg in the urine of groundwater-drinking participants was 2.35±2.13 μg/g of creatinine, ranging from 0.53 to 12.23 μg/g of creatinine, while the average concentration of non-groundwater-drinking participants was 1.46±0.77 μg/g of creatinine, ranging from 0.47 to 3.56 μg/g of creatinine (see Tables 6.3 and 6.4).

Table 6. 3 The concentrations of heavy metals in urine of the groundwater drinking participants.

	Heavy Metals in the Urine of Groundwater-drinking Participants				
St.	**Number of participants** (persons)	**Avg.As** (µg/L)	**Avg.Cd** (µg/g of creatinine)	**Avg.Pb** (µg/g of creatinine)	**Avg.Hg** (µg/g of creatinine)
well 3	3	22.90	1.20	38.50	2.60
well 4	1	33.10	1.05	6.88	0.71
well 7	5	22.70	1.70	51.90	2.00
well 8	5	31.60	1.40	42.90	2.10
well 9	4	11.80	3.00	51.60	2.60
well 11	4	34.70	0.70	22.70	1.50
well 12	5	16.70	2.10	60.30	4.90
well 13	4	94.10	0.90	22.80	2.30
well 14	4	65.20	0.90	24.30	1.90
well 15	4	45.20	0.80	10.30	2.10
well 16	4	35.10	1.10	30.50	3.90
well 18	1	52.30	1.15	69.74	1.57
well 19	2	51.60	0.70	20.00	0.70
well 20	1	27.40	0.70	32.92	0.71
well 21	1	24.60	0.74	2.71	0.58
well 22	3	22.40	0.80	25.70	2.40
well 23	2	58.30	0.50	21.70	0.70
well 24	5	155.40	1.70	45.30	2.60
Avg.		47.74	1.31	35.17	2.35
SD		40.76	0.75	19.84	2.13
Min		3.30	0.31	1.87	0.53
Max		166.60	4.61	79.73	12.23

*Avg, SD, Min, Max were from urine raw data.

Table 6. 4 The concentrations of heavy metals in urine of the non-groundwater drinking participants.

	Heavy Metals in the Urine of Non-Groundwater-drinking Participants				
St.	**Number of participants** (persons)	**Avg.As** (µg/L)	**Avg.Cd** (µg/g of creatinine)	**Avg.Pb** (µg/g of creatinine)	**Avg.Hg** (µg/g of creatinine)
well 1	4	13.90	0.80	25.56	0.76
well 2	4	13.05	1.02	22.12	1.19
well 4	3	8.43	0.67	18.55	2.19
well 5	4	15.28	0.72	17.11	1.45
well 6	4	13.25	0.95	18.71	1.21
well 9	1	7.70	0.53	37.94	1.79
well 10	4	12.03	1.08	27.72	2.00
well 11	1	21.10	1.11	19.80	1.16
well 17	4	12.13	1.08	29.31	1.81
well 18	2	9.75	1.03	24.68	1.64
well 19	2	2.20	2.53	27.19	0.93
well 20	2	15.75	1.17	28.51	1.44
well 21	3	19.20	1.62	22.20	1.73
well 22	2	10.80	1.00	16.25	1.22
well 23	2	19.25	0.94	23.02	1.28
Avg.		12.99	1.06	23.37	1.46
SD		6.76	0.57	9.14	0.77
Min		1.6	0.41	6.42	0.47
Max		28.8	3.24	38.42	3.56

*Avg, SD, Min, Max were from urine raw data.

Based on groundwater drinking participants, the urine results showed dry season had many participants who drink shallow groundwater found heavy metals in urine higher than standard (51.72% for As, 44.82% for Cd, 3.45% for Pb, and 15.52 for Hg), compared with As, Cd, Hg in urine of wet season (50% for As, 1.72% for Cd, 24.14% for Pb, and 6.90% for Hg) (Table 6.5). On the other hand, no one in non-groundwater drinking participants were found heavy metals in urine greater than standard during wet season.

Table 6. 5 The comparison of groundwater drinking participants between dry and wet season

Urine in GW drinking participants	**As (ug/L)**		**Cd (ug/g creat.)**		**Pb (ug/g creat.)**		**Hg (ug/g. creat.)**	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
>Std. (persons)	30	29	26	1	2	14	9	4
>Std.(%)	51.72%	50%	44.82%	1.72%	3.45%	24.14%	15.52%	6.90%
Med.	36.40	35.95	3.52	1.09	20.05	30.97	2.10	1.59
Ave.	36.89	47.74	3.71	1.31	21.14	35.17	2.73	2.35
SD	25.04	40.76	2.06	0.75	9.83	19.84	2.03	2.13
Min	2.80	3.30	0.30	0.31	4.99	1.87	0.35	0.53
Max	119.60	166.60	8.66	4.61	58.82	79.73	9.26	12.23

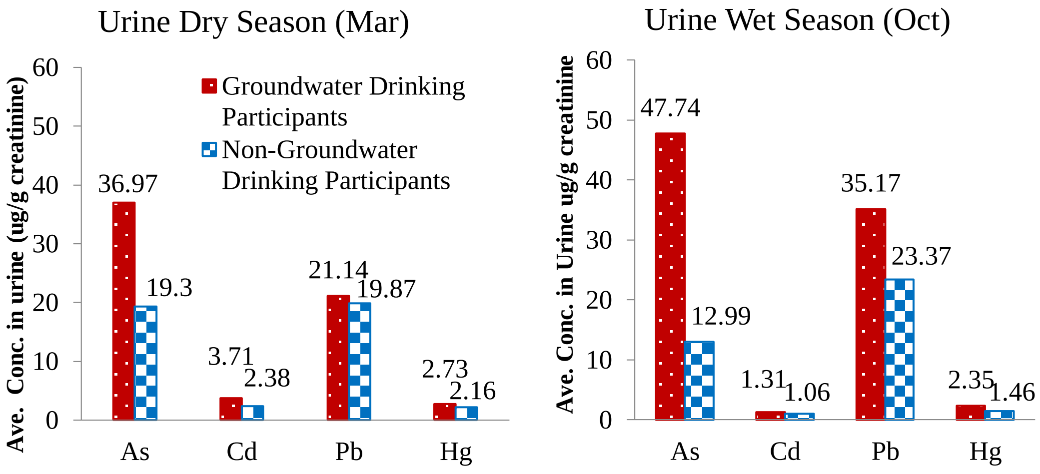


Figure 6. 4 The urine comparison between the groundwater drinking group and the non-groundwater drinking group in dry and wet seasons.

From Figure 6.4 the results of heavy metals in urine of 100 participants, 58 was groundwater drinking and 42 was non-groundwater drinking. In the dry season the urine results found 51.72% of participants in the groundwater-drinking group had As in their urine higher level than the standard, and 44.83% for Cd, 3.45% for Pb, and 15.52 % for Hg. As and Cd found significant different between groundwater drinking and non-groundwater drinking participants (U-test<0.05).

The results of the wet season, heavy metals in urine of 100 participants found 50.00% of participants in the groundwater-drinking group had As in their urine higher level than the standard, and 1.72% for Cd, 24.14% for Pb, and 6.90% for Hg. As and Pb found significant different between groundwater drinking and non-groundwater drinking participants (U-test<0.05).

6.2 Hair and Nail as the biomarkers of long-term exposure

6.2.1 In the dry season

All four heavy metals found in both biomarkers (hairs and nails) of groundwater drinking participants were greater than non-groundwater drinking participants. The average concentrations of As, Cd, Pb and Hg in hairs of the groundwater drinking group were 0.121, 0.123, 28.657, and 166.605 ug /gH, respectively, while for nails, the average concentrations of As, Cd, Pb and Hg were 0.309 ug/gN, 0.093 ug/gN, 100.006 ug/gN, and 2.948 ug/gN, respectively.

For non-groundwater drinking group, the average concentrations of As, Cd, Pb and Hg in hairs of the groundwater drinking group were 0.097 ug/gH, 0.075 ug/gH, 25.917 ug/gH, and 29.652 ug/gH, while for nails for the average concentrations of As, Cd, Pb and Hg were 0.198 ug/gN, 0.048 ug/gN, 30.916 ug/gN, and 1.635 ug/gN, respectively. The heavy metals concentrations in hairs and nails of groundwater drinking participants at each station were shown in Tables 6.6-6.9.

Table 6. 6 Heavy metals in the hairs of groundwater drinking participants at each station in the dry season

St. (Dry Season)	Number of GW participants (persons)	Avg.As (µg/g Hairs)	Avg.Cd (µg/g Hairs)	Avg.Pb (µg/g Hairs)	Avg.Hg (µg/g Hairs)
well 3	3	0.086	0.097	6.795	1.836
well 4	1	0.146	0.146	0.146	0.146
well 7	5	0.144	0.127	3.203	0.653
well 8	5	0.202	0.187	106.537	891.604
well 9	4	0.135	0.065	20.659	1.436
well 11	4	0.148	0.208	26.960	1.180
well 12	5	0.107	0.154	52.264	1.256
well 13	4	0.155	0.123	43.210	3.117
well 14	4	0.102	0.057	5.914	1.459
well 15	4	0.072	0.080	3.192	0.611
well 16	4	0.063	0.043	4.998	9.048
well 18	1	0.057	0.057	0.057	0.057
well 19	2	0.145	0.212	106.535	985.714
well 20	1	0.045	0.045	0.045	0.045
well 21	1	0.122	0.122	0.122	0.122
well 22	3	0.127	0.088	2.193	1.190
well 23	2	0.064	0.071	2.260	0.705
well 24	5	0.126	0.185	34.829	137.103
Avg.		**0.121**	**0.123**	**28.657**	**166.605**
SD.		**0.084**	**0.113**	**67.950**	**681.014**
Median		**0.107**	**0.088**	**3.930**	**0.974**
Min		**0.020**	**0.009**	**0.655**	**0.349**
Max		**0.571**	**0.575**	**396.880**	**4404.156**

*The detection limits were 0.001 µg/L for As. Cd, Pb, Hg.

Table 6. 7 Heavy metals in the hairs of non-groundwater drinking participants at each station in the dry season

St. (Dry Season)	**Number of NGW participants** (persons)	**Avg.As** (µg/g Hairs)	**Avg.Cd** (µg/g Hairs)	**Avg.Pb** (µg/g Hairs)	**Avg.Hg** (µg/g Hairs)
well 1	4	0.068	0.063	43.934	2.410
well 2	4	0.109	0.095	40.714	1.982
well 4	3	0.155	0.086	66.063	1.177
well 5	4	0.127	0.071	37.331	6.397
well 6	4	0.102	0.111	4.913	0.718
well 9	1	0.080	0.080	0.080	0.080
well 10	4	0.107	0.056	3.311	0.646
well 11	1	0.083	0.083	0.083	0.083
well 17	4	0.071	0.070	2.885	8.116
well 18	2	0.110	0.084	5.126	6.866
well 19	2	0.059	0.132	2.272	0.770
well 20	2	0.032	0.039	2.090	560.245
well 21	3	0.043	0.062	3.434	4.184
well 22	2	0.087	0.051	3.050	1.923
well 23	2	0.187	0.097	107.344	0.966
Avg.		**0.097**	**0.075**	**25.917**	**29.652**
SD.		**0.07**	**0.052**	**41.85**	**172.103**
Median		**0.082**	**0.063**	**3.383**	**0.923**
Min		**0.024**	**0.013**	**0.893**	**0.309**
Max		**0.359**	**0.230**	**148.471**	**1118.009**

*The detection limits were 0.001 µg/L for As. Cd, Pb, Hg.

Table 6. 8 Heavy metals in the nails of groundwater drinking participants at each station in the dry season

St. **(Dry Season)**	**Number of GW participants** (persons)	**Avg.As** (μg/g Nails)	**Avg.Cd** (μg/g Nails)	**Avg.Pb** (μg/g Nails)	**Avg.Hg** (μg/g Nails)
well 3	3	0.301	0.727	1657.894	0.191
well 4	1	0.146	0.146	0.146	0.146
well 7	5	0.409	0.172	23.903	1.375
well 8	5	0.231	0.040	7.514	17.403
well 9	4	0.207	0.070	8.347	1.405
well 11	4	0.207	0.039	8.577	0.366
well 12	5	0.186	0.041	8.883	1.859
well 13	4	0.305	0.058	16.716	0.197
well 14	4	0.836	0.101	25.492	0.643
well 15	4	0.273	0.064	16.362	0.258
well 16	4	0.273	0.010	16.494	0.122
well 18	1	0.057	0.057	0.057	0.057
well 19	2	0.145	0.006	5.181	0.055
well 20	1	0.045	0.045	0.045	0.045
well 21	1	0.122	0.122	0.122	0.122
well 22	3	0.250	0.046	8.434	1.518
well 23	2	0.325	0.024	17.026	4.400
well 24	5	0.209	0.017	14.654	5.206
Avg.		**0.309**	**0.093**	**100.006**	**2.948**
SD		**0.333**	**0.291**	**649.023**	**10.549**
Median		**0.242**	**0.030**	**10.037**	**0.103**
Min		**0.039**	**nd.**	**1.713**	**nd.**
Max		**2.440**	**2.125**	**4956.471**	**75.235**

*The detection limits were 0.001 μg/L for As, Cd, Pb, Hg.

Table 6. 9 Heavy metals in the nails of non-groundwater drinking participants at each station in the dry season

St. (Dry Season)	Number of NGW participants (persons)	Avg.As (µg/g Nails)	Avg.Cd (µg/g Nails)	Avg.Pb (µg/g Nails)	Avg.Hg (µg/g Nails)
well 1	4	0.121	0.004	9.238	0.095
well 2	4	0.314	0.042	21.117	0.159
well 4	3	0.166	0.179	6.601	0.077
well 5	4	0.253	0.128	219.208	10.652
well 6	4	0.225	0.059	11.062	0.058
well 9	1	0.276	0.167	10.313	nd.
well 10	4	0.136	0.017	6.490	0.032
well 11	1	0.164	0.010	6.093	0.082
well 17	4	0.227	0.031	10.323	0.541
well 18	2	0.169	0.032	11.806	0.138
well 19	2	0.240	0.023	6.260	0.127
well 20	2	0.130	0.007	9.537	8.346
well 21	3	0.166	0.011	18.316	1.365
well 22	2	0.243	0.003	14.206	0.320
well 23	2	0.106	nd.	6.970	0.117
Avg.		**0.198**	**0.048**	**30.916**	**1.635**
SD		**0.124**	**0.087**	**127.934**	**6.519**
Median		**0.167**	**0.018**	**8.132**	**0.105**
Min		**0.049**	**nd.**	**2.595**	**nd.**
Max		**0.806**	**0.425**	**838.056**	**40.158**

*The detection limits were 0.001 µg/L for As, Cd, Pb, Hg.

From the concentrations data of heavy metals in hairs and nails, contour maps were created to present the concentrations of heavy metals in hairs and nails of the local people, who drink shallow groundwater located in each individual well. Arsenic, Cd, and Pb in hairs showed a similar distribution pattern, while the contour map of Hg showed the opposite result from those of others metals. For nails, contour maps of Cd and Pb had a similar pattern, while As and Hg were not. The comparison between hairs and nails, there was only As that had similar pattern, while Cd, Pb and Hg showed a different pattern between both hair and nails.

These different pattern contour maps for hair and nails may imply that As, Cd, Pb and Hg in hairs and nails do not come from only same sources, but may from other contributing factors, which affected to human biomarkers. For example, the associated factors affecting to human biomarkers in others studies were smoking, traditional medicine using, vapor atmosphere, alcohol, gender, occupation (Gil et al., 2011, Li et al., 2011, Jamal et al., 2013, Rebecca et al., 2013).

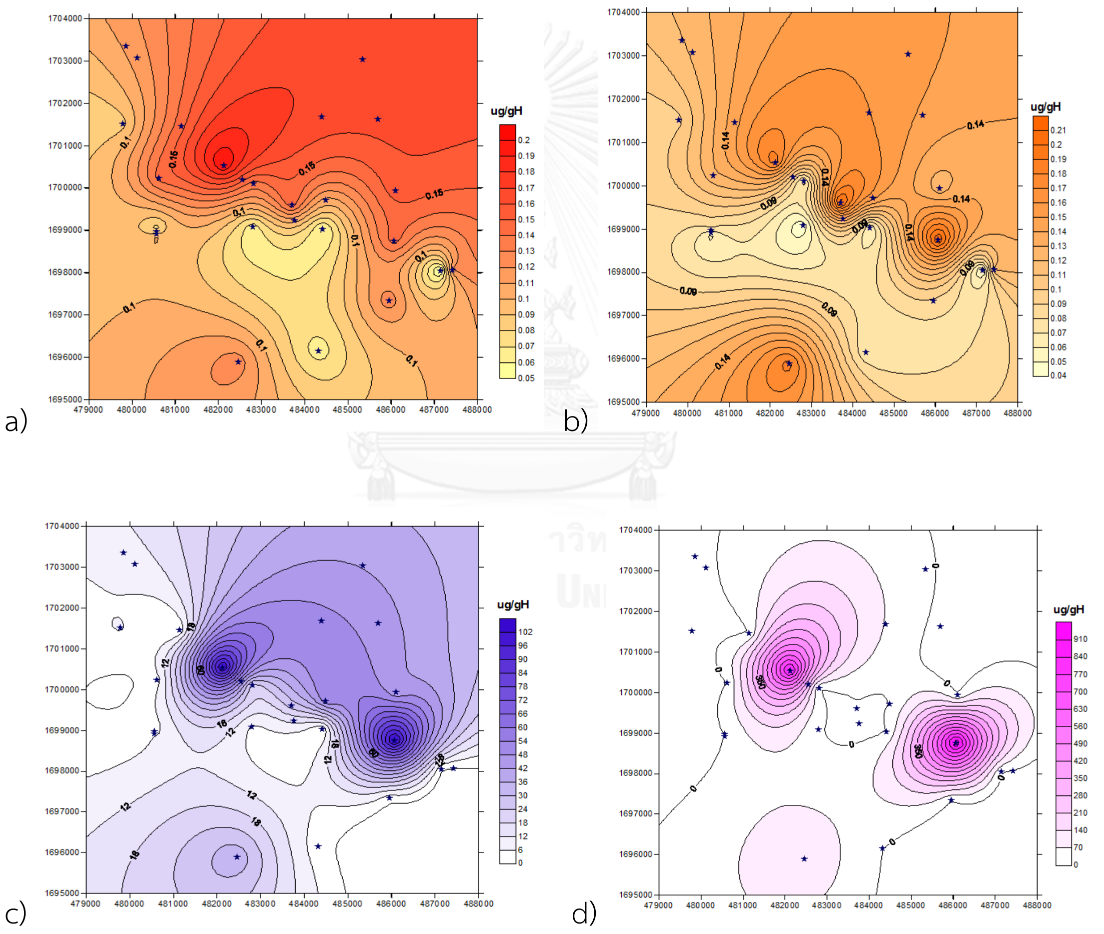


Figure 6. 5 a) Contour map of the As concentration in the hairs of groundwater-drinking participants. b) Contour map of the Cd concentration in the hairs of groundwater-drinking participants. c) Contour map of the Pb concentration in the hairs of groundwater-drinking participants. d) Contour map of the Hg concentration in the hairs of groundwater-drinking participants (dry season).

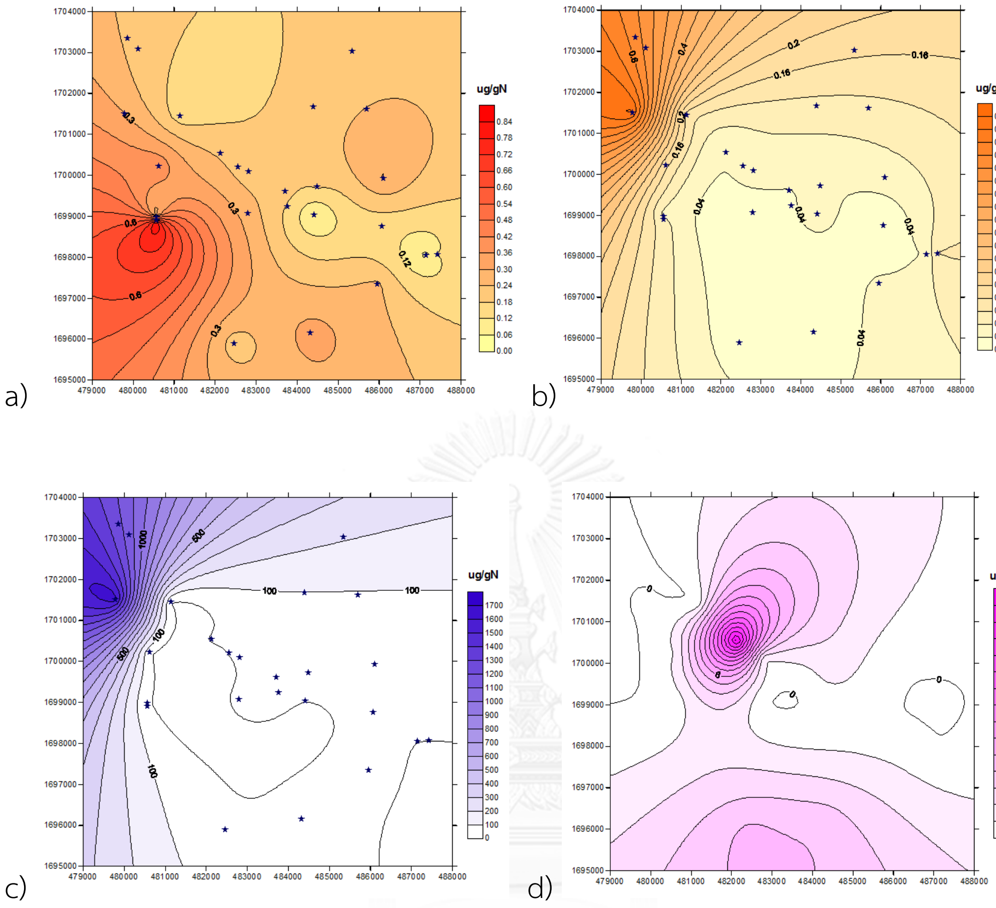


Figure 6. 6 a) Contour map of the As concentration in the nails of groundwater-drinking participants. b) Contour map of the Cd concentration in the nails of groundwater-drinking participants. c) Contour map of the Pb concentration in the nails of groundwater-drinking participants. d) Contour map of the Hg concentration in the nails of groundwater-drinking participants (dry season).

6.2.2 In wet season

The heavy metals concentrations found that As was in fingernails higher than those in hairs both wet and dry seasons, corresponding to Pb which is obvious higher in nails than in hairs. On the other hand, Hg was found in hair greatly higher than found in nails for both dry and wet season while Cd was not. For comparison of all 100 participants between dry and wet seasons, the results showed dry season during March had higher heavy metals concentrations in both biomarkers than those in the wet season during October. Moreover, the biomarkers results were in line with with the drinking water results that heavy metals in the dry season were greater than those in the wet season (Table 6.10-6.13 and figure 6.5-6.6). From this results, It could be suggested that biomarker-hairs was more proper for investigating Hg exposure than nails, and biomarker-nails was more proper to identifying As and Pb exposure than hairs.

Table 6. 10 Heavy metals in the hairs of the groundwater drinking participants at each station in the wet season

St. **(Wet Season)**	**Number of GW participants** (persons)	**Avg.As** (µg/g Hairs)	**Avg.Cd** (µg/g Hairs)	**Avg.Pb** (µg/g Hairs)	**Avg.Hg** (µg/g Hairs)
well 3	3	0.020	0.070	2.490	1.480
well 4	1	0.888	0.888	0.888	0.888
well 7	5	0.040	0.180	8.900	2.780
well 8	5	0.040	0.090	7.360	0.950
well 9	4	0.090	0.050	3.490	0.370
well 11	4	0.040	0.130	8.350	14.210
well 12	5	0.120	0.160	3.070	14.550
well 13	4	0.060	0.070	4.420	1.480
well 14	4	0.032	0.052	2.743	0.651
well 15	4	0.070	0.000	7.370	0.670
well 16	4	0.150	0.030	3.320	1.930
well 18	1	0.090	0.090	0.090	0.090
well 19	2	0.030	0.150	19.130	5.780
well 20	1	0.229	0.229	0.229	0.229
well 21	1	0.542	0.542	0.542	0.542
well 22	3	0.070	0.060	5.390	28.680
well 23	2	0.060	0.060	5.390	0.900
well 24	5	0.020	0.200	18.150	13.300
Avg.		0.060	0.102	7.853	7.932
SD.		0.079	0.123	9.195	18.592
Median		0.043	0.078	4.778	0.899
Min		0.000	0.000	0.801	0.157
Max		0.397	0.600	45.700	83.008

Table 6. 11 Heavy metals in the hairs of the non-groundwater drinking participants at each station in the wet season

St. **(Wet Season)**	**Number of NGW participants** (persons)	**Avg.As** (µg/g Hairs)	**Avg.Cd** (µg/g Hairs)	**Avg.Pb** (µg/g Hairs)	**Avg.Hg** (µg/g Hairs)
well 1	4	0.055	0.083	4.420	0.354
well 2	4	0.053	0.126	3.448	0.410
well 4	3	0.057	0.075	2.518	0.282
well 5	4	0.085	0.093	3.210	0.406
well 6	4	0.012	0.026	2.007	0.795
well 9	1	0.154	0.027	1.730	0.838
well 10	4	0.015	0.009	4.606	6.028
well 11	1	0.076	0.021	1.254	0.504
well 17	4	0.083	0.086	9.530	2.448
well 18	2	0.053	0.049	1.453	0.386
well 19	2	0.056	0.121	2.511	0.383
well 20	2	0.095	0.045	3.586	0.538
well 21	3	0.043	0.082	2.467	0.290
well 22	2	0.058	0.164	4.456	1.210
well 23	2	0.053	0.128	3.997	0.405
Avg.		0.056	0.077	3.782	1.206
SD.		0.048	0.079	4.270	2.458
Median		0.054	0.054	3.042	0.493
Min		0.000	0.000	0.645	0.075
Max		0.201	0.358	28.692	13.645

Table 6. 12 Heavy metals in the nails of groundwater drinking participants at each station in the wet season

St. **(Wet Season)**	**Number of GW participants** (persons)	**Avg.As** (µg/g Nails)	**Avg.Cd** (µg/g Nails)	**Avg.Pb** (µg/g Nails)	**Avg.Hg** (µg/g Nails)
well 3	3	0.020	0.070	2.490	1.480
well 4	1	0.888	0.888	0.888	0.888
well 7	5	0.040	0.180	8.900	2.780
well 8	5	0.040	0.090	7.360	0.950
well 9	4	0.090	0.050	3.490	0.370
well 11	4	0.040	0.130	8.350	14.210
well 12	5	0.120	0.160	3.070	14.550
well 13	4	0.060	0.070	4.420	1.480
well 14	4	0.032	0.052	2.743	0.651
well 15	4	0.070	0.000	7.370	0.670
well 16	4	0.150	0.030	3.320	1.930
well 18	1	0.090	0.090	0.090	0.090
well 19	2	0.030	0.150	19.130	5.780
well 20	1	0.229	0.229	0.229	0.229
well 21	1	0.542	0.542	0.542	0.542
well 22	3	0.070	0.060	5.390	28.680
well 23	2	0.060	0.060	5.390	0.900
well 24	5	0.020	0.200	18.150	13.300
Avg.		0.446	0.291	23.275	1.642
SD		0.33	0.528	20.756	3.831
Median		0.345	0.127	18.352	0.515
Min		0.029	0.009	1.192	0.022
Max		1.6	3.293	118.936	26.143

Table 6. 13 Heavy metals in the nails of non-groundwater drinking participants at each station in the wet season

St. (Wet Season)	**Number of NGW participants** (persons)	**Avg.As** (µg/g Nails)	**Avg.Cd** (µg/g Nails)	**Avg.Pb** (µg/g Nails)	**Avg.Hg** (µg/g Nails)
well 1	4	0.341	0.120	19.589	0.324
well 2	4	0.408	0.434	19.069	0.360
well 4	3	0.092	0.027	4.663	0.095
well 5	4	0.211	0.056	6.908	0.239
well 6	4	0.212	0.071	11.788	0.391
well 9	1	0.154	0.154	0.154	0.154
well 10	4	0.170	0.060	9.676	0.859
well 11	1	0.076	0.076	0.076	0.076
well 17	4	0.294	0.124	11.555	0.693
well 18	2	0.075	0.023	3.643	0.093
well 19	2	0.266	0.230	12.793	0.175
well 20	2	1.221	2.746	71.879	0.773
well 21	3	0.396	0.098	12.815	0.308
well 22	2	0.175	0.054	9.552	0.537
well 23	2	0.861	0.287	54.342	0.798
Avg.		0.316	0.251	16.080	0.421
SD		0.364	0.828	21.696	0.451
Median		0.236	0.077	10.628	0.289
Min		0.037	0.011	1.558	0.041
Max		1.928	5.336	111.420	2.387

The results of the wet season similarly found that the concentrations of biomarkers from groundwater drinking participants were mostly higher than the median of 100 participants than non-groundwater drinking group. The heavy metals in hairs of 58 groundwater drinking participants found 27 persons or 46.55% of groundwater drinking participants had As concentrations in hairs higher than the median of all 100 participants, 31 (53.45%), 38 (65.52%), and 39 (67.24%) persons for Cd, Pb, and Hg respectively. On the other hand, there were 22 (52.38%) persons of 42 non-groundwater drinking participants found As concentrations in hairs higher than median, 19 (45.24%), 12 (28.57%), 11 (18.97%) for Cd, Pb, and Hg, respectively.

For nails results, found 36 (62.07%) persons from 58 groundwater drinking participants had As concentrations in nails higher than the median, 35 (60.34%), 37 (63.79%), and 36 (62.07%) persons for Cd, Pb, and Hg respectively. On the contrary, there were 14 persons or 33.33% from non-groundwater drinking group had As concentrations in nails higher than the median, 14 (33.33%), 13 (30.95%), 42 (100%) for Cd, Pb, and Hg, respectively. From these results, it presented that mercury (Hg) which accumulated in nails of participants was not major from groundwater drinking because all of non-groundwater participants in the wet season found Hg greater than median of 100 participants. Therefore, there were other associated factors that affected heavy metals in human body. Furthermore this study used binary logistic regression to figure out the associated factors which presented in ***Chapter VII***.

Table 6. 14 The heavy metals concentrations in hairs and nails of 100 participants in dry and wet season

Dry season (March)				
µg Heavy Metal per g Hairs	As in Hairs	Cd in Hairs	Pb in Hairs	Hg in Hairs
Average±SD	0.111±0.079	0.103±0.095	27.506±58.185	109.085±532.829
Median	0.094	0.076	3.866	0.943
Min	0.02	0.009	0.655	0.309
Max	0.571	0.575	396.88	4404.156
µg Heavy Metal per g Nails	As in Nails	Cd in Nails	Pb in Nails	Hg in Nails
Average±SD	0.262±0.270	0.074±0.229	70.988±500.479	2.397±9.061
Median	0.212	0.024	9.574	0.103
Min	0.039	0	1.713	0
Max	2.44	2.125	4956.471	75.235

Wet season (October)				
µg Heavy Metal per g Hairs	As in Hairs	Cd in Hairs	Pb in Hairs	Hg in Hairs
Average±SD	0.058±0.068	0.091±0.107	6.143±7.766	5.107±14.582
Median	0.048	0.059	3.780	0.640
Min	0.000	0.000	0.645	0.075
Max	0.397	0.600	45.700	83.008
µg Heavy Metal per g Nails	As in Nails	Cd in Nails	Pb in Nails	Hg in Nails
Average±SD	0.392±0.349	0.274±0.667	20.253±21.348	1.129±2.983
Median	0.274	0.093	14.334	0.39
Min	0.029	0.009	1.192	0.022
Max	1.928	5.336	118.936	26.143

The hair and nail comparison between groundwater drinking and non-groundwater drinking participants.

Both average and median concentrations of As, Cd, Pb, and Hg in hairs of groundwater drinking participants were higher than those of non-groundwater drinking participants. Similarly, for nails results, the average concentrations of As, Cd, Pb, and Hg of groundwater drinking participants were higher than nails from non-groundwater drinking participants.

The statistical results were employed by using Mann-Whitney U-test (2-tailed) to investigate the difference of hairs concentrations between the groundwater drinking participants and non-groundwater drinking participants. Arsenic in hairs was found significantly different between two groups of participants ($p<0.05$, $p=0.031$) and also Cd ($p<0.05$, $p=0.022$), whereas there were no significant difference between two groups for Pb and Hg. For the nails, only As was showed significantly different between two groups ($p<0.05$, $p=0.003$). Similarly, some previous researches reported that groundwater consumption and tube-wells drinking water were major contributions to concentration of As in hairs and nails (Badal et al., 2003, Gautam et al., 2004, Blakely et al., 2006, Suthipong et al., 2010). Both hair and nails are composed of compact protein, hard keratin. Hair has a high affinity for metals, due mainly to the cystine that makes up approximately 14% of its total composition. Many metals found in hair are bound to sulphur atoms in cystine or to sulphydryl (SH) groups in other amino acids. Metals can bind to the hair structure through melanin. Melanins are polyanionic polymers containing negatively charged carboxyl groups and semiquinones at physiological pH. As a result, they can bind cations by ionic interactions. Organic amines and metal ions have a high melanin affinity, because they are positively charged at physiological pH and interact with the melanin polymer by electrostatic forces between their cationic groups and the negative charges in the melanin polymer. The ionic binding may also be enhanced by other forces such as van der Waals attraction. Uncharged metals, such as elemental Hg, may also bind to the hydrophobic core of the melanin polymer in hair. These are the human body excretion of heavy metals via hair and nail and heavy metals can be found permanent in hair and nails (Katz, 1988, Krystyna, 2006, Marcin et al., 2011).

From U-test results, As-Hair, As-Nail, Cd-Hair were significant different between groundwater drinking participants and non-groundwater drinking participants (U-test<0.05) in dry season. For wet season, As-Nail, Cd-Nail, Pb-Hair, Pb-Nail, Hg-Hairs, Hg-Nails were significant different between groundwater drinking participants and non-groundwater drinking participants. Although other heavy metals were not significantly different, but the results of both hairs and nails from the groundwater-drinking group seemed to be higher than those of the non-groundwater-drinking group as shown in Figures 6.7 and 6.8.

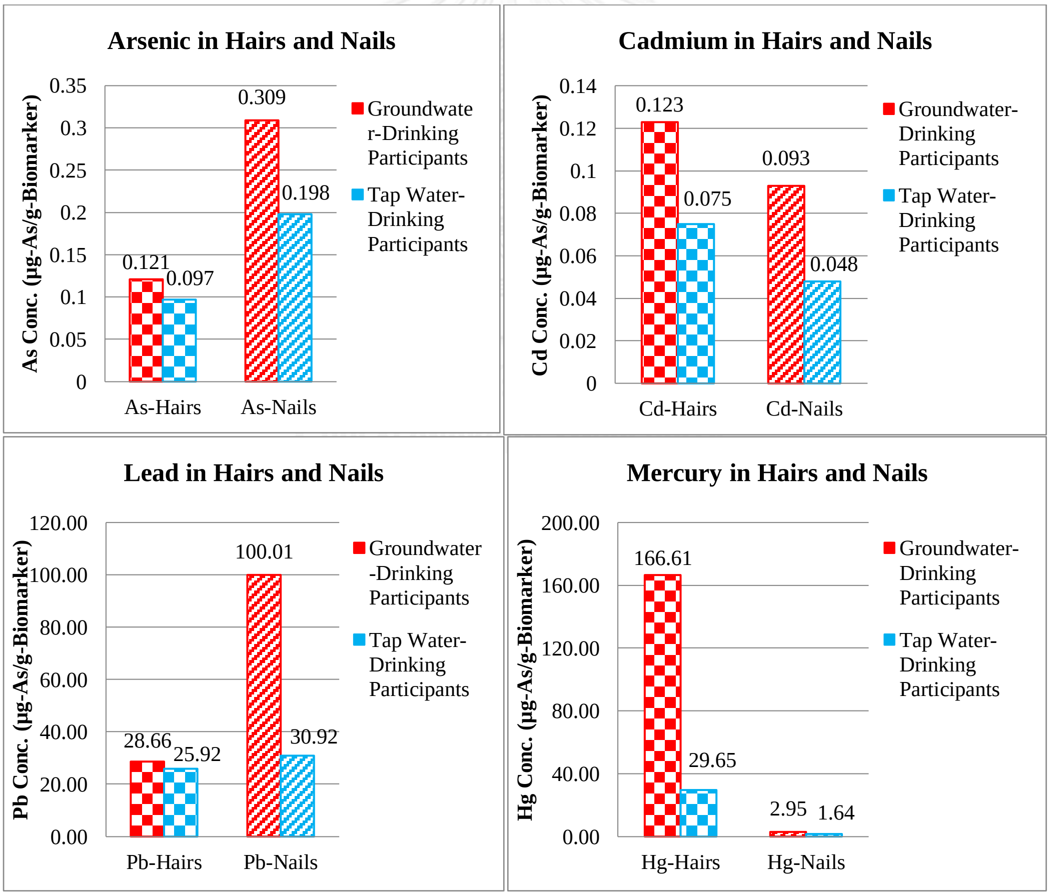


Figure 6. 7 The average concentrations of As, Cd, Pb and Hg of hairs and nails for both groundwater and non-groundwater drinking participants

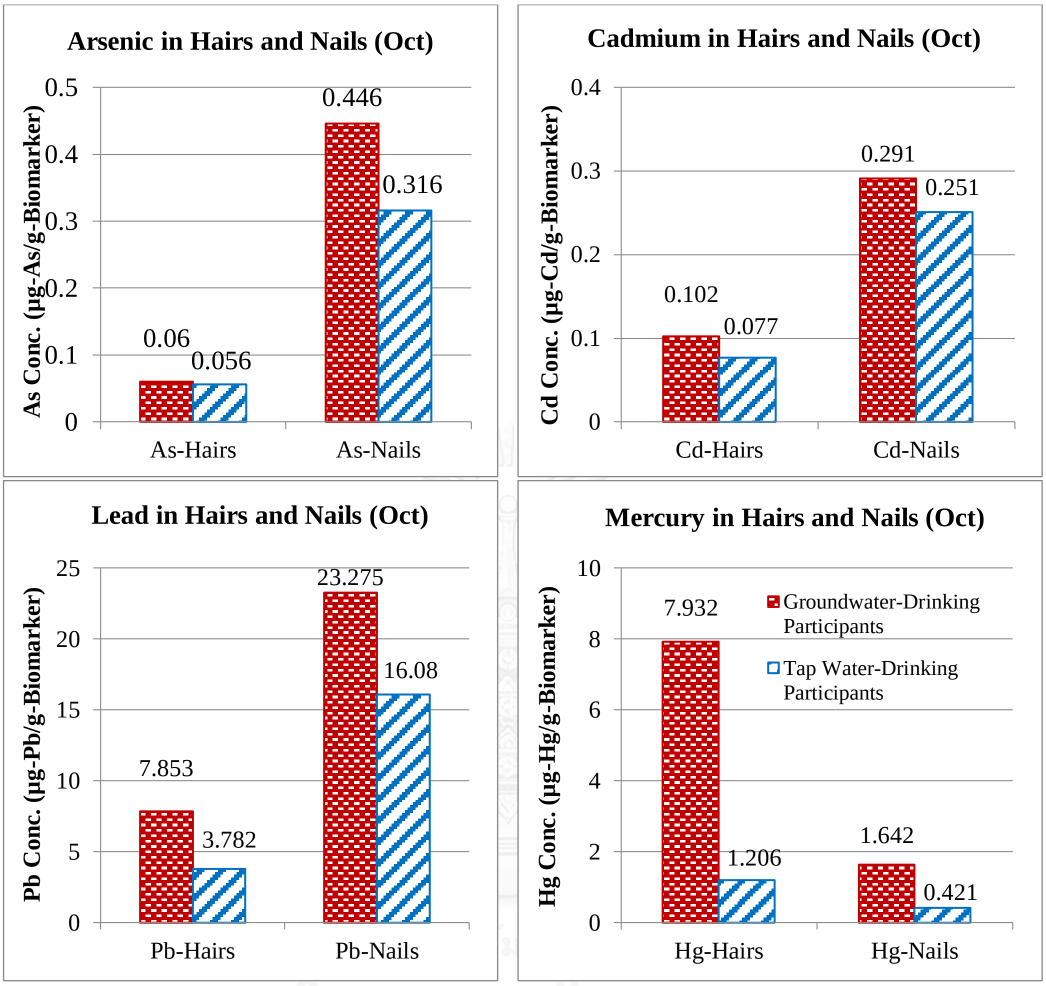


Figure 6. 8 The average concentrations of hairs and nails of groundwater and non-groundwater drinking participants during wet season

The average concentrations of As, Cd, Pb and Hg in hairs of the groundwater drinking group were 0.091, 0.613, 18.26, and 87.27 ug /gH, respectively, while non-groundwater drinking group were 0.077, 0.076, 14.851 and 15.43 ug/gH. For nails, the average concentrations of the groundwater drinking group of As, Cd, Pb and Hg were 0.378, 0.192, 61.640, and 2.281 ug/gN, while non-groundwater drinking group were 0.257, 0.150, 23.500, and 1.030 ug/gN, respectively.

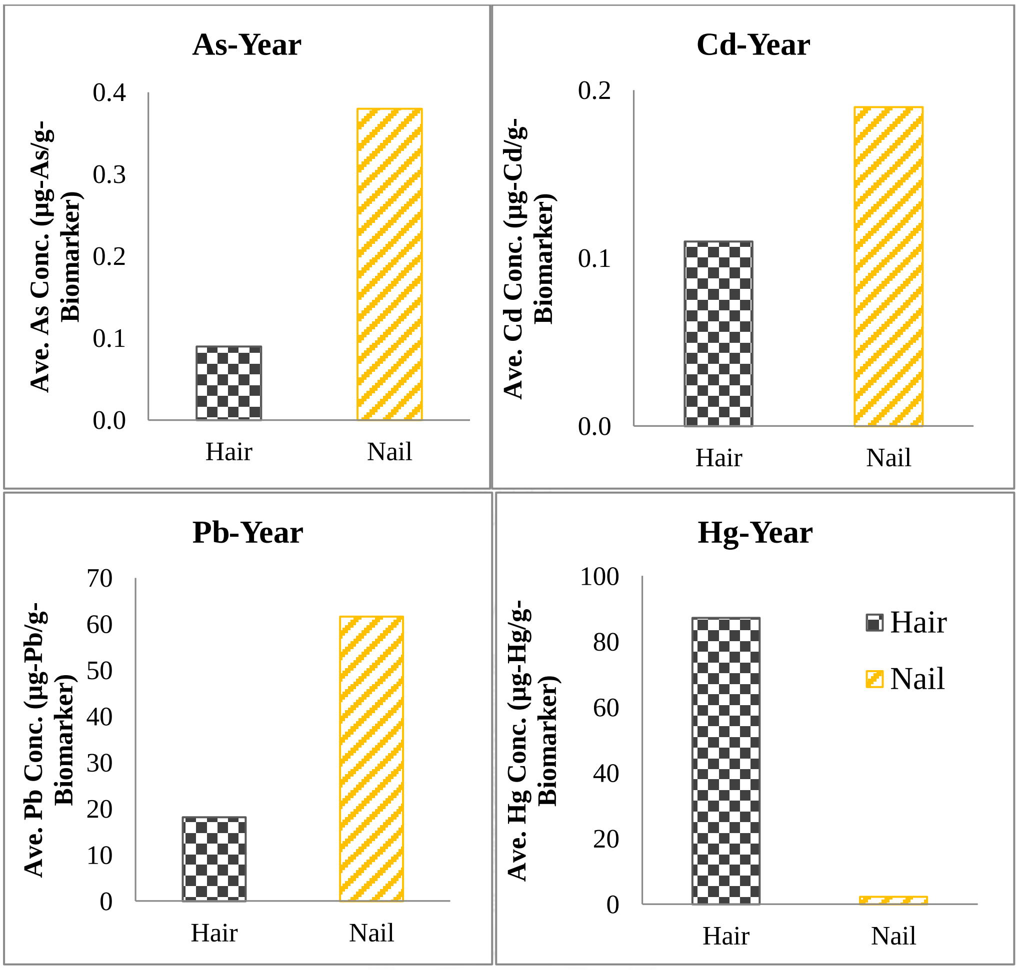


Figure 6. 9 The average concentrations of hairs and nails samples of groundwater drinking participants.

From figure 6.9, this study supported the risk awareness in the low concentration contamination site by using the appropriated biomarker, which is easier to sample and transport in a rural area for screening exposure of heavy metals especially by using hairs, nails and urine. Urine is the good biomarkers related daily exposure with all fours heavy metals, As, Cd, Pb ,Hg. On the other hands, long term exposure, nail is better to be used as biomarkers for As, Cd, and Pb than hairs, As, Cd, and Pb were excreted via nail greater than hair. On the other hand, hair is better to be used as biomarkers for Hg than nail.

Furthermore, to compare hairs concentrations results between two groups, there was no standard for heavy metal concentrations in hairs. In this study, the median of all 100 participants was used (Table 5). The heavy metals in hairs of 58 groundwater drinking participants found that 34 persons or 58.62% of groundwater drinking participants had As concentrations in hairs higher than the median of all 100 participants, as well as 35 (60.34%), 31 (53.45%), and 29 (50%) persons for Cd, Pb, and Hg respectively. On the other hand, there were 17 persons of 42 non-groundwater drinking participants (or 40.48%) found that As concentrations in hairs were higher than the median, 16 (38%), as well as 19 (45.24%), 20 (47.62%) for Cd, Pb, and Hg, respectively. Similarly, some previous researches reported groundwater consumption was one of the important associated factor of As in hairs and nails (Badal et al., 2003, Gautam et al., 2004, Blakely et al., 2006, Suthipong et al., 2010)

To compare heavy metal concentrations in nails between two groups, the median of all participants was also used to compare concentrations results between groundwater and non-groundwater drinking participants. The heavy metals in nails found 35 (60.34%) persons from 58 groundwater drinking participants had As concentrations higher than the median, 32 (55.17%), 32 (55.17%), and 28 (48.28%) persons for Cd, Pb, and Hg respectively. On the other hand, there were 15 persons or 35.71% from non-groundwater drinking group had As concentrations in nails higher than the median, 18 (42.86%), 18 (42.86%), 26 (61.90%) for Cd, Pb, and Hg, respectively.

From these results, could be presented that mercury (Hg), which accumulated in nails of participants was not mainly from groundwater drinking because nails of non-groundwater participants mostly found Hg greater than median of 100 participants. Therefore, there were other associated factors that affected heavy metals in human body. Not only drinking water, but many researchers found the other associated factors that affected to concentration of Hg in biomarker, such as smoking and food consumption also (Wael et al., 2002, Gautam et al., 2004, Tomoko et al., 2007, Catherine et al., 2014, Park and Seo, 2017). Furthermore, this study used the binary logistic regression to figure out the others associated factors. In addition, biomarkers were mostly used to evaluate the heavy metals exposure in areas that found high concentration contamination such as from industrial and mining areas as shown in Table 6.15

Table 6. 15 The heavy metals found in previous researches worldwide in hairs and nails

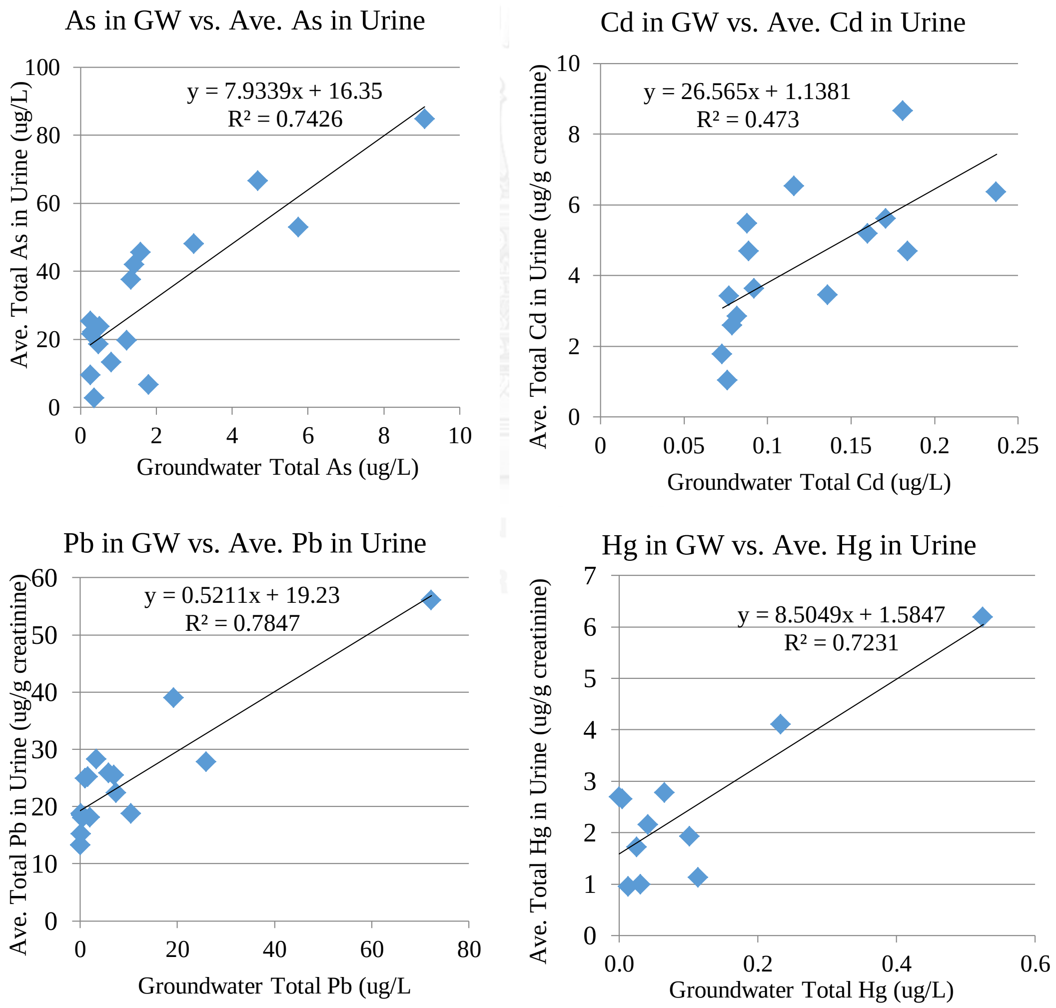
Ref. Study	Country	Site	Metals	Ave.Conc.±sd (Range)	
				Hairs (ug/g hair)	Nails (ug/g nail)
Gautam et al., 2004	India	As-Rich in Groundwater Area	As	3.43±0.73 (0.17-14.39)	7.24±1.28 (0.74-36.63)
			Cd	0.40±0.17 (0.00-2.14)	0.32±0.09 (0.02-1.93)
			Hg	0.88±0.08 (0.19-3.0)	0.45±0.04 (0.18-1.32)
			Pb	8.03±1.56 (0.57-41.71)	10.99±2.04 (1.19-52.56)
Eid et al., 2006	UK	Urban Area	As	0.12	0.18
Andrew et al., 2008	Cambodia	As-Rich in Groundwater Area	As	2.43±0.52 (0.26-7.95)	1.96±0.33 (0.53-4.95)
Thanh et al., 2009	China	Waste Recycling Area	Pb	85.3±96.4 (1.93-730)	
Katarzyna et al., 2010	Poland	Industrial Area	As	0.83±0.33 (0.65-3.96)	
			Cd	0.09±0.06 (0.05-0.49)	
			Hg	0.21±0.16 (0.03-0.80)	
			Pb	3.08±1.69 (0.00-10.89)	

Ref. Study	Country	Site	Metals	Hairs (ug/g hair)	Nails (ug/g nail)
Ping et al., 2011	China	Mining Area	Hg	43.5±47.2 (6.28-123.0)	
Catherine et al., 2014	Belgium	Urban Area	Cd	0.38	
Varica et al., 2014	Italy	Volcanic Area	As	0.03±0.02 (0.00-0.16)	
			Cd	0.02±0.02 (0.00-0.11)	
			Pb	0.84±0.8 (0.00-5.07)	
This study (2017)	Thailand	Agricultural area	As	0.111±0.079 (0.02-0.571)	0.262±0.270 (0.039-2.44)
			Cd	0.103±0.095 (0.009-0.575)	0.074±0.229 (0.00-2.125)
			Pb	27.506±58.185 (0.655-396.88)	70.988±500.479 (1.713-4956.471)
			Hg	109.085±532.829 (0.309-4404.156)	2.397±9.061 (0.00-75.235)

6.3 Correlation between biomarkers and heavy metals exposure

Urine Statistical Analysis: Spearman Correlation Tests

Dry season: the linear relationship between the concentrations of these four heavy metals in the groundwater and the average heavy metals in the urine of the participants who drink groundwater from each shallow groundwater well were shown in Figure 6.10. The r-square of the correlation between the As-groundwater and As-urine was 0.7426, and 0.473, 0.7847, 0.7231, for Cd, Pb and Hg, respectively. The spearman correlation results showed that As in the urine of the groundwater-drinking participants was significantly correlated with As concentrations in drinking groundwater at the 0.05 level. Moreover, the As concentration in the groundwater was significantly correlated with the As cancer risk and As non-carcinogenic risk also at the 0.05 level, while the As urine of non-groundwater-drinking participants was not significantly correlated. This relationship between As urine and As concentrations in groundwater were supported by a study from Normandin et al. (2014), who investigated the As concentration in the urine of participants in a rural region in Canada and found a significant relationship between biomarkers and As in drinking water intake (As in the groundwater ranged from 0.02–140 µg/L).



As in GW vs. Ave. As in Urine

Cd in GW vs. Ave. Cd in Urine

Pb in GW vs. Ave. Pb in Urine

Hg in GW vs. Ave. Hg in Urine

Figure 6. 10 The correlation of As, Cd, and Pb in groundwater and urine

For Cd in the urine of groundwater-drinking participants, the 0.01 level significantly correlated with Cd in the groundwater and the 0.05 level was significant with non-carcinogenic risk, while Cd in the urine of non-groundwater-drinking participants was not significantly correlated at the 0.05 level. Similarly, Pb in the urine of groundwater-drinking participants was significantly correlated at the 0.01 level with Pb concentration in both the groundwater and non-carcinogenic risk, while Pb in the urine of the non-groundwater-drinking participants was not significantly correlated. In addition, Hg in the urine of the groundwater-drinking participants had a 0.01 level that was significantly correlated with Hg non-carcinogenic risk, while Hg in the urine of non-groundwater-drinking participants was not significantly correlated. (see Figure 6.10) The correlation results showed that groundwater and biomarkers have a strong correlation, indicating that groundwater contamination results in the bio-accumulation of heavy metals in human. Moreover, there were no correlations between heavy metals and biomarkers in the non-groundwater-drinking group, supporting the fact that the groundwater considerably affected the locals who consumed it. According to a study in the US regarding the effect of As in populations exposed to As in water and the diet, they found that 76 % of total As in the urine was affected by drinking groundwater (Steven et al., 2012). Similarly, a study on urinary heavy metal levels and the relevant factors among exposed people reported that urinary heavy metal levels were related to the human health risk (Hongmei et al., 2011). The relationship between heavy metals in the urine and the drinking water in the population of West Bengal showed linear regressions with very good correlations between As concentrations in the water (ranging from 0.01 to 0.25 mg/L) and urine. This study in India found a moderately high concentration of As in the urine, approximately 83 % and 68 % of the urine samples (n = 250) containing As above 100 and 200 μg/L, respectively (Tarit 2010).

Table 6. 16 Urine Spearman Correlation Results in Dry season

Dry Season (Correlation Coefficient)	As_GW	As-Cancer Risk	As-Non-cancer Risk	Cd_GW	Cd-Non-cancer Risk	Pb_GW	Pb-Non-cancer Risk	Hg-Non-cancer Risk
As_Urine	737**	692**	708**					
Cd_Urine				.640**	.342**			
Pb_Urine						624**	.646**	
Hg_Urine								.318*

*Correlation significant at 0.05 level (2-tailed)

**Correlation significant at 0.01 level (2-tailed)

Wet season: Similarly with urine of dry season, the urine results from wet season found linear relationship between As in urine of groundwater drinking participants and As in drinking groundwater, R^2 was 0.9093. (Figure 6.11).

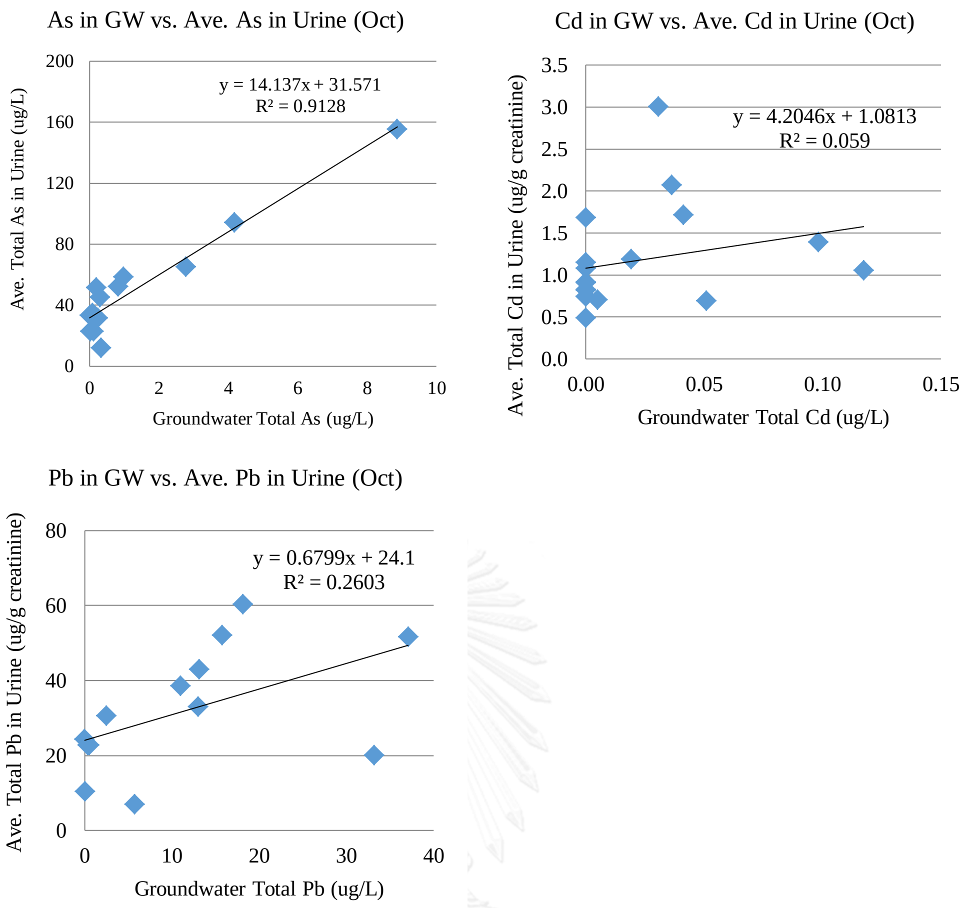


Figure 6. 11 The relationship between heavy metals in urine and groundwater during wet season

Table 6. 17 Urine Spearman Correlation Results in Wet season

Wet Season (Correlation Coefficient)	As_GW	Cd_GW	Pb_GW
As_Urine	.664**		
Cd_Urine		.527**	
Pb_Urine			.448**

**Correlation significant at 0.01 level (2-tailed)

Hairs and Nails Statistical Analysis: Spearman Correlation Tests

Dry season: the correlations between the average heavy metals in hairs and nails of the participants who drink groundwater from each shallow groundwater well and the concentrations of these four heavy metals in the groundwater were not correlated. The Kendall and Spearman correlation results showed that As, Cd, Pb, Hg in the hairs of the groundwater drinking participants was not significantly correlated with As, Cd, Pb, Hg concentrations in drinking groundwater at 0.05 level ($p>0.05$). Similarly, the correlation results of 4 heavy metals from nails of groundwater drinking participants were not significantly correlated with heavy metals in groundwater. However, Hg in hairs was significantly correlated with Hg in nails at 0.01 level ($p<0.01$).

Spearman correlation: Hair and Nail in Dry season

- As-Hair correlated with As-Urine (Coefficient .737**)
- Cd-Hair correlated with Cd-Urine (Coefficient .640**)
- Pb-Hair correlated with Pb-Urine (Coefficient .624**)
- Hg-Nail correlated with Hg-GW (Coefficient .409**)

**significant correlated at 0.01 level.

Wet Season: the correlations between the average heavy metals in hairs and nails of the participants who drink groundwater from each shallow groundwater well and the concentrations of these four heavy metals in the groundwater were not correlated for both dry and wet season. The Kendall and Spearman correlation results showed that As, Cd, Pb, Hg in the hairs of the groundwater drinking participants was not significantly correlated with As, Cd, Pb, Hg concentrations in drinking groundwater at 0.05 level ($p>0.05$). Similarly, the correlation results of 4 heavy metals from nails of groundwater drinking participants were not significantly correlated with heavy metals in groundwater for both dry and wet season.

Spearman correlation: Hair and Nail in Wet season

- Hg-Nail correlated with Hg-Urine (Coefficient .489**)

**significant correlated at 0.01 level.

CHAPTER VII

ASSOCIATED RISK FACTORS RELATED WITH AS, CD, PB, HG IN BIOMARKERS OF 100 LOCAL PARTICIPANTS

7.1 Associated risk factors of urine of 100 local participants

7.1.1 Binary Logistic Regression

For dry season, the chi square results showed that the significant associated factors of As in urine were gender, water drinking source, smoking, underlying disease, and PPE using. Chi square results of Cd were gender, weight, height, drinking water source, smoking, while Pb was only one factor which was underlying disease. For Hg, the associated factors were weight and PPE using (Table 7.1 and 7.2).

In case of wet season, the significant associated factors of As in urine were drinking water source and fertilizer using. For Pb found gender, drinking water source, and smoking. Cd and Hg did not show significant factors due to urine was representative of daily intake, during wet season, there were no Cd and Hg in both drinking groundwater and drinking tap water samples. The chi square results for urine during dry and wet season were shown in Table 7.1 and 7.3.

Table 7. 1 Factors showing significant from Chi2 results of urine in dry and wet season

	Dry Season				**Wet Season**			
Factors	As-U	Cd-U	Pb-U	Hg-U	As-U	Cd-U	Pb-U	Hg-U
Gender	0.002	0.041					0.058	
Weight (kg)		0.111		0.079				
Height (cm)		0.084						
Age (years)								
Drinking Rate (L)								
Drinking Source	0.000	0.000			0.000		0.000	
Drinking Water Container								
Drinking Water Cleaning Method								
Bath Water Source								
Washing Water Source								
Cooking Water Source								
Education								
Occupation								
Family Occupation								
Family Members (persons)								
Work Rate								
Smoking Behavior	0.048	0.198					0.098	
Alcohol Drinking Behavior								
Underlying Diseases	0.175		0.132					
Pesticide Use								
Chemical Fertilizer Contact					0.170			
Washing Hands Before Meals								
Personal Protective Equipment Use	0.049			0.076				

All urine associated factors of heavy metals in urine from chi square results were continue used in equation of the binary logistic regression. The associated factors results of urine during dry and wet season were show in Table 7.2 and 7.3.

Table 7. 2 Factors associated with concentration of heavy metals in urine in the dry season

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		70.625	7.857	634.832
Smoking		2.413	0.235	24.752
Underlying Diseases		3.153	0.857	11.593
PPE Using		0.168	0.043	0.655
Gender		0.061	0.005	0.718
Constant	-2.521	0.080		

Equation (1) As-Urine: $Y = -2.521 + 70.625X_1 + 2.413X_9 + 3.153X_{12} + 0.168X_{18} + 0.061X_5$

where Y = Probability that As concentration in urine higher than standard.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		14.358	3.526	58.464
Gender		10.066	1.094	92.632
Weight		0.903	0.259	3.151
Height		0.779	0.223	2.721
Smoking		1.646	0.198	13.705
Constant	-4.612			

Equation (2) Cd-Urine: $Y = -4.612 + 14.358X_1 + 10.066X_5 + 0.903X_{16} + 0.779X_6 + 1.646X_9$

Where Y = Probability that Cd concentration in urine higher than standard.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Underlying Diseases		1.800	0.109	29.673
Constant	-4.143	0.016		

Equation (3) Pb-Urine: $Y = -4.143 + 1.800X_1$

where Y = Probability that Pb concentration in urine higher than standard.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Weight		0.189	0.036	0.997
PPE Using		0.181	0.034	0.956
Constant	-1.012	0.363		

Equation (4) Hg-Urine: $Y = -1.012 + 0.189X_{16} + 0.181X_{18}$

where Y = Probability that Hg concentration in urine higher than standard.

Table 7. 3 Factors associated with concentration of heavy metals in urine in the wet season

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		3.704	1.258	10.902
Fertilizer Using		2.372	0.616	9.131
Constant	-2.537	0.079		

Equation (5) As-Urine: $Y = -2.537 + 3.704X_1 + 2.372X_{14}$

where Y = Probability that As concentration in urine higher than standard.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		6.275	1.693	23.254
Gender		0.553	0.105	2.917
Smoking		1.273	0.234	6.934
Constant	-2.220			

Equation (7) Pb-Urine: $Y = -2.220 + 6.275X_1 + 0.553X_5 + 1.273X_9$

where Y = Probability that Pb concentration in urine higher than standard.

*(X_1= Drinking Source, $X_{2=}$ Bath water source, X_3=Washing water Source, X_4=Drinking Rate, X_5=Gender, X_6=Height, $X_{7=}$ Education, X_8=Occupation, X_9=Smoking, X_{10}=Alcohol Drinking, X_{11}=Working Hour per day, $X_{12=}$Underlying Diseases, X_{13}=Pesticides Using, X_{14}=Fertilizer Using, X15=Age, X16=Weight, X17=Cooking Water Source, X18=PPE Using)

Although the concentrations of heavy metals in hair and nail samples were not correlated with heavy metals concentrations in groundwater, but the result of associated factors showed that groundwater consumption affected to hair and nail. Interesting, during wet season there were no Cd and Hg in any drinking water but the result showed concentrations Cd and Hg in hair and nail also related with groundwater consumption, conform with the duration time of hair and nail growth that they represented for long term exposure. On the other hand, urine represent for daily exposure, there were not Cd and Hg in water samples during wet season, conform with water consumption was not significant associated factors with Cd and Hg in urine.

7.1.2 Odd ratio (OR)

The results of odd ratio found significant risk factors related with As in urine were drinking water source, smoking, use of PPE and gender, significant risk factors related with Cd in urine were drinking water source and gender in dry season. Similarly in wet season, OR presented significant risk factors related with As in urine were drinking water source, for Pb were drinking water source and gender. Interesting, the significant risk factors results from OR greatly supported binary logistic regression results which found mostly associated factors related with groundwater drinking (see table 7.4).

Table 7. 4 Results of odd ratio of risk factors related with heavy metals in urine

Dry Season	**Urine-Risk Factors**		**95%CI**		
		OR	Lower	Upper	
As-U	Drinking Water Source	43.500	5.600	337.910	sig
	Smoking	2.667	1.046	6.801	sig
	Underlying Disease	1.909	0.794	4.589	
	Use of PPE	0.382	0.154	0.951	sig
	Gender	0.385	1.721	11.174	sig
Cd-U	Drinking Water Source	9.252	2.629	34.516	sig
	Gender	0.256	0.070	0.934	sig
	Weight	2.219	0.889	5.542	
	Height	2.100	0.842	5.239	
	Smoking	2.327	0.718	7.549	
Pb-U	Underlying Disease	1.800	0.109	29.673	
Hg-U	Weight	4.462	0.879	22.664	
	Use of PPE	0.214	0.042	1.089	
Wet Season	**Urine-Risk Factors**		**95%CI**		
		OR	Lower	Upper	
As-U	Drinking Water Source	2.63	1.954	3.539	sig
	Use of Fertilizers	2.728	0.734	10.148	
Pb-U	Drinking Water Source	7.018	1.92	25.654	sig
	Gender	2.778	1.031	7.482	sig
	Smoking	2.484	0.909	6.791	

7.2 Associated risk factors of hair and nail of 100 local participants

7.2.2 Binary Logistic Regression

Dry Season

This study used the binary logistic regression to find out the associated factors for each heavy metal in biomarker hairs and nails to predict the odds of being a case and non-case. In this method, participants would be divided into two groups, depended on the cut point of each factor criteria. The independent variables were As, Cd, Pb, Hg in hairs and nails samples from all 100 participants. Before running a binary logistic regression, chi square was used to screen the associated factors from all dependent factors by determining whether there were associated between the two variables. The chi square results showed that the significant associated factors of As in hair were follows: drinking source, drinking rate, gender, education and smoking. The associated factors of Cd in hair were follows: drinking source and bath water source, drinking source and underlying diseases for Pb in hairs, drinking rate and height for Hg in hairs.

In case of nails, the significant associated factors of As were drinking source, gender, washing source, and pesticides using. For Cd in nails were smoking, alcohol drinking, and work hour per day. Drinking source and pesticides using were associated factors for Pb in nails, similarly gender and pesticides using were Hg associated factors of nails. The chi square results of all factors were shown in Table 7.5.

Table 7. 5 Factors showing significant from Chi2 results (sig. <0.2)

Factors (Dry Season)	As-H	Cd-H	Pb-H	Hg-H	As- N	Cd- N	Pb- N	Hg- N
Gender	0.003				0.183			0.118
Weight (kg)								
Height (cm)				0.161				
Age (years)								
Drinking Rate (L)	0.192			0.192				
Drinking Source	0.106	0.020	0.158		0.006		0.106	
Drinking Water Container								
Bath Water Source		0.011						
Washing Water Source					0.091			
Cooking Water Source								
Education	0.097							
Occupation							0.140	
Family Occupation								
Family Members (persons)								
Work Rate						0.048		
Smoking Behavior	0.011					0.170		
Alcohol Drinking Behavior						0.001		
Underlying Diseases			0.144					
Pesticide Usage					0.198		0.194	0.194
Chemical Fertilizer Contact								
Washing Hands Before Meals								
Personal Protective Equipment Use								

All associated factors from chi square results were continue used in the binary logistic regression to predict the odds of being a case, based on the values of the independent variables. The odds were defined as the probability that a particular outcome was divided by the number of non-particular outcomes. From binary logistic regression results, probability Y and independent factors X were investigated, then eight probability equations were figured out (Table 7.6 and 7.7).

Table 7. 6 Factors associated with concentration of heavy metals in hairs in dry season

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.373	0.602	3.132
Drinking Rate		1.986	0.618	6.380
Gender		0.263	0.054	1.281
Education		1.716	0.689	4.273
Smoking		1.096	0.217	5.531
Constant	-0.178	0.837		

Equation (1) As-Hair: $Y = -0.187 + 1.373X_1 + 1.986X_4 + 0.263X_5 + 1.716X_7 + 1.096X_9$
where Y = Probability that As concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.594	0.560	4.538
Bath Water Source		2.700	0.743	9.811
Constant	-0.361	0.837		

Equation (2) Cd-Hair: $Y = -0.361 + 1.594X_1 + 2.700X_2$
Where Y = Probability that Cd concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.893	0.837	4.280
Underlying Diseases		1.972	0.844	4.608
Constant	-0.536	0.585		

Equation (3) Pb-Hair: $Y = -0.536 + 1.893X_1 + 1.972X_{12}$
where Y = Probability that Pb concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Rate		1.998	0.665	6.000
Height		0.584	0.258	1.322
Constant	0.159	1.172		

Equation (4) Hg-Hair: $Y = 0.159 + 1.998X_4 + 0.584X_6$
where Y = Probability that Hg concentration in hair higher than median.

Table 7. 7 Factors associated with concentration of heavy metals in nails in dry season

Independent Factors	B	Exp(B)	95%CI for EXP(B)	
			Lower	Upper
Drinking Water Source		2.758	0.940	8.092
Gender		0.662	0.255	1.714
Washing Water Source		1.099	0.301	4.015
Pesticides Using		1.955	0.776	4.929
Constant	-0.478	0.620		

Equation (5) As-Nail: $Y = -0.478 + 2.758X_1 + 0.662X_5 + 1.099X_3 + 1.955X_{13}$

where Y = Probability that As concentration in nail higher than median.

Independent Factors	B	Exp(B)	95%CI for EXP(B)	
			Lower	Upper
Smoking		1.152	0.360	3.689
Alcohol Drinking		6.899	1.924	24.734
Working Hour per Day		3.259	1.182	8.983
Constant	-1.315	0.268		

Equation (6) Cd-Nail: $Y = -1.315 + 1.152X_9 + 6.899X_{10} + 3.259X_{11}$

where Y = Probability that Cd concentration in nail higher than median.

Independent Factors	B	Exp(B)	95%CI for EXP(B)	
			Lower	Upper
Drinking Water Source		2.075	0.902	4.775
Occupation		1.903	0.652	5.558
Pesticides Using		1.668	0.653	4.260
Constant	-0.556	0.573		

Equation (7) Pb-Nail: $Y = -0.556 + 2.075X_1 + 1.903X_8 + 1.668X_{13}$

where Y = Probability that Pb concentration in nails higher than median.

Independent Factors	B	Exp(B)	95%CI for EXP(B)	
			Lower	Upper
Gender		2.196	0.883	5.461
Pesticides Using		1.887	0.785	4.537
Constant	-0.411	0.663		

Equation (8) Hg-Nail: $Y = -0.411 + 2.196X_5 + 1.887X_{13}$

where Y = Probability that Hg concentration in nails higher than median.

*(X_1= Drinking Source, $X_{2=}$ Bath water source, X_3=Washing water Source, X_4=Drinking Rate, X_5=Gender, X_6=Height, $X_{7=}$ Education, X_8=Occupation, X_9=Smoking, X_{10}=Alcohol Drinking, X_{11}=Working Hour per day, $X_{12=}$Underlying Diseases, X_{13}=Pesticides Using)

According to the binary logistic regression equations, both hairs and nails results showed that the drinking source was mostly presented in equations, indicating that the major source of heavy metals exposed to human in this study area was shallow groundwater. The local people who drink shallow groundwater had 1.9 times higher risk to present Pb in hairs (1.893X_1) than the median of local people. Likewise, the As equation of nails showed 2.758X_1 indicated that the local people who drink shallow groundwater had chance 2.76 times higher than people who drink tap water, implying that. So, As in nails of the local people who drink shallow groundwater was higher than median of local people. Interestingly, the associated factors showed underlying disease, the previous studies investigated that heavy metals had associated with type 2 diabetes. In addition, heavy metals could catalyze oxidative stress reactions by causing oxidative stress and decreasing insulin gene promoter activity. Many researchers reported heavy metals as the risk factors for diabetes (Nikhil et al., 2010, Khan and Awan, 2014, Jeon et al., 2015, Andy et al., 2016). The other concerned factors found in this study was pesticides usage, which related to heavy metals in biomarkers. The previous studies found As, Cd, Pb, Hg, Co, Cu, Ni, Zn, Fe and Mn in the herbicides and fungicides and they reported heavy metals as impurities that soil receives from agricultural practices (Gimeno-Garcia et al., 1996, Nema et al., 2016).

Wet Season

For wet season, the chi square results showed that the significant associated factors of As in hair were underlying diseases and fertilizers using. The associated factors of Cd in hair were drinking water source weight and age, while drinking water source, cooking water source, pesticides using and PPE using for Pb in hairs, drinking water source, bath water source, washing water source for Hg in hairs.

In case of nails, the significant associated factors of As were drinking water source, bath water source, washing water source, and alcohol drinking. For Cd in nails were bath water source, alcohol drinking and PPE using. Drinking water source, bath water source, and alcohol drinking were associated factors for Pb in nails, in addition, gender, age, and drinking water source were Hg associated factors of nails. The chi square results for hair and nail during wet season were shown in Table 7.8.

Table 7. 8 Factors showing significant from Chi2 results of hair and nail during wet season

Factors (Wet Season)	As-H	Cd-H	Pb-H	Hg-H	As- N	Cd- N	Pb- N	Hg- N
Gender								0.118
Weight (kg)		0.160						
Height (cm)								
Age (years)		0.109						0.071
Drinking Rate (L)								
Drinking Source		0.130	0.001	0.000	0.087		0.069	0.043
Drinking Water Container								
Bath Water Source				0.034	0.004	0.100	0.005	
Washing Water Source				0.028	0.015			
Cooking Water Source			0.165					
Education								
Occupation								
Family Occupation								
Family Members (persons)								
Work Rate								
Smoking Behavior								
Alcohol Drinking Behavior					0.065	0.063	0.168	
Underlying Diseases	0.096							
Pesticide Use			0.194					
Chemical Fertilizer Contact	0.132							
Washing Hands Before Meals								
Personal Protective Equipment Use			0.070			0.160		

Table 7. 9 Factors associated with concentration of heavy metals in hairs in wet season

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Underlying Disease		2.169	0.924	5.094
Fertilizer Using		2.436	0.863	6.876
Constant	-0.756	0.470		

Equation (1) As-Hair: $Y = -0.756 + 2.169X_{12} + 2.436X_{14}$

where Y = Probability that As concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		2.119	0.886	5.065
Age		2.233	0.976	5.108
Weight		0.422	0.178	1.001
Constant	-0.363	0.696		

Equation (2) Cd-Hair: $Y = -0.363 + 2.119X_{1} + 2.233X_{15} + 0.422\ X_{16}$

Where Y = Probability that Cd concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.331	0.455	3.895
Cooking Water Source		1.672	0.495	5.655
Pesticides Using		1.108	0.447	2.746
PPE Using		0.802	0.347	1.851
Constant	-0.515	0.598		

Equation (3) Pb-Hair: $Y = -0.515 + 1.331X_{1} + 1.672X_{17} + 1.108X_{13} + 0.802X_{18}$

where Y = Probability that Pb concentration in hair higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.939	0.789	4.767
Bath Water Source		2.005	0.758	5.303
Washing Water Source		1.218	0.450	3.295
Constant	-0.621	0.537		

Equation (4) Hg-Hair: $Y = -0.621 + 1.939X_{1} + 2.005X_{2} + 1.218X_{3}$

where Y = Probability that Hg concentration in hair higher than median.

Table 7. 10 Factors associated with concentration of heavy metals in nails in wet season

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		3.578	1.278	10.013
Alcohol Drinking		2.539	0.921	7.001
Bath Water Source		17.327	1.480	202.814
Washing Water Source		2.140	0.205	22.342
Constant	-3.325	0.036		

Equation (5) As-Nail: $Y = -3.325 + 3.578X_1 + 2.539X_{10} + 17.327\ X_2 + 2.140X_3$

where Y = Probability that As concentration in nail higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Bath Water Source		2.181	0.813	5.855
Alcohol Drinking		2.574	0.964	6.871
PPE Using		1.847	0.808	4.222
Constant	-1.108	0.330		

Equation (6) Cd-Nail: $Y = -1.108+ 2.181X_2 + 2.574X_{10} + 1.847X_{18}$

where Y = Probability that Cd concentration in nail higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Drinking Water Source		1.113	0.388	3.192
Bath Water Source		4.498	1.233	16.416
Alcohol Drinking		1.855	0.700	4.914
Constant	-1.322	0.267		

Equation (7) Pb-Nail: $Y = -1.322+ 1.113\ X_1 + 4.498X_2 + 1.855X_{10}$

where Y = Probability that Pb concentration in nails higher than median.

Independent Factors	**B**	**Exp(B)**	**95%CI for EXP(B)**	
			Lower	Upper
Water Drinking Source		2.185	0.931	5.127
Gender		0.418	0.158	1.107
Age		2.772	1.173	6.548
Constant	-0.318	0.728		

Equation (8) Hg-Nail: $Y = -0.318 + 2.185X_1 + 0.418X_5 + 2.772X_{15}$

where Y = Probability that Hg concentration in nails higher than median.

*(X_1= Drinking Source, $X_{2=}$ Bath water source, X_3=Washing water Source, X_4=Drinking Rate, X_5=Gender, X_6=Height, $X_{7=}$ Education, X_8=Occupation, X_9=Smoking, X_{10}=Alcohol Drinking, X_{11}=Working Hour per day, $X_{12=}$Underlying Diseases, X_{13}=Pesticides Using, X_{14}=Fertilizer Using, X15=Age, X16=Weight, X17=Cooking Water Source, X18=PPE Using)

Although the concentrations of heavy metals in hair and nail samples were not correlated with heavy metals concentrations in groundwater, but the result of associated factors showed that groundwater consumption affected to hair and nail. Interesting, during wet season there were no Cd and Hg in any drinking water but the result showed concentrations Cd and Hg in hair and nail also related with groundwater consumption, conform with the duration time of hair and nail growth that they represented for long term exposure. On the other hand, urine represent for daily exposure, there were not Cd and Hg in water samples during wet season, conform with water consumption was not significant associated factors with Cd and Hg in urine.

7.2.2 Odd ratio (OR)

The results of odd ratio found significant risk factors related with As in hair were smoking, and for Cd there were drinking water source and bath water source in the dry season. In the wet season, the significant risk factors related with Pb in hair were drinking water source, and significant risk factors related with Hg in hair there were drinking water source, bath water source, and washing water source (Table 7.11).

Table 7. 11 Results of odd ratio of risk factors related with heavy metals in hair

Dry Season	Hair-Risk Factors		95%CI		
		OR	Lower	Upper	
As-H	Drinking Water Source	1.926	0.865	4.290	
	Drinking Rate	2.316	0.793	6.764	
	Gender	0.225	0.085	0.597	
	Education	2.190	0.954	5.028	
	Smoking	3.765	1.41	10.051	sig
Cd-H	Drinking Water Source	2.774	1.222	6.297	sig
	Bath Water Source	3.706	1.325	10.366	sig
Pb-H	Drinking Water Source	1.926	0.865	4.29	
	Underlying Disease	2.02	0.879	4.645	
Hg-H	Drinking Rate	2.316	0.793	6.764	
	Height	1.909	0.862	4.227	

Wet Season	Hair-Risk Factors		95%CI		
		OR	Lower	Upper	
As-H	Underlying Disease	2.135	0.922	4.944	
	Use of Fertilizers	2.388	0.861	6.618	
Cd-H	Drinking Water Source	1.791	0.805	3.983	
	Age	2.071	0.933	4.597	
	Weight	1.902	0.859	4.215	
Pb-H	Drinking Water Source	3.857	1.67	8.911	sig
	Cooking Water Source	2.144	0.842	5.459	
	Use of Pesticides	1.941	0.818	4.607	
	Use of PPE	0.442	0.198	0.987	
Hg-H	Drinking Water Source	4.644	1.981	10.883	sig
	Bath Water Source	3.165	1.176	8.518	sig
	Washing Water Source	3.778	1.343	10.628	sig

*Water source = Tap water is reference and groundwater is risk factor.

The results in the dry season of odd ratio found that significant risk factors related with As in nail were drinking water source, and significant risk factors related with Cd in nail were alcohol drinking and working hour per day. For wet season, found significant risk factors related with As in nail were drinking water source, bath water source, and washing water source. Alcohol drinking was significant risk factors related with Cd in nail, and bath water source and alcohol drinking were significant risk factors for Pb in nail. Drinking water and age were significant risk factors related with Hg in nail (see Table 7.12)

Table 7. 12 Results of odd ratio of risk factors related with heavy metals in nail

Dry Season	**Nail-Risk Factors**		**95%CI**		
		OR	**Lower**	**Upper**	
As-N	Drinking Water Source	2.988	1.313	6.800	sig
	Gender	0.518	0.213	1.260	
	Washing Water Source	2.135	0.811	5.621	
	Use of Pesticides	1.831	0.772	4.343	
Cd-N	Smoking	2.105	0.832	5.324	
	Alcohol Drinking	5.375	1.823	15.852	sig
	Working hours per day	2.52	1.021	6.223	sig
Pb-N	Drinking Water Source	1.926	0.865	4.29	
	Occupation	2.042	0.769	5.419	
	Use of Pesticides	1.941	0.818	4.607	
Hg-N	Gender	2.25	0.913	5.545	
	Use of Pesticides	1.941	0.818	4.607	
Wet Season	**Nail-Risk Factors**		**95%CI**		
		OR	**Lower**	**Upper**	
As-N	Drinking Water Source	2.17	0.966	4.874	
	Alcohol Drinking	2.523	0.989	6.441	
	Bath Water Source	4.694	1.588	13.877	sig
	Washing Water Source	4.32	1.456	12.818	sig
Cd-N	Bath Water Source	2.471	0.944	6.463	
	Alcohol Drinking	2.705	1.04	7.036	sig
	Use of PPE	0.522	0.235	1.159	

Wet Season	Nail-Risk Factors	OR	Lower	Upper	
Pb-N	Drinking Water Source	2.072	0.929	4.626	
	Bath Water Source	4.355	1.553	12.21	sig
	Alcohol Drinking	2.032	7.798	5.171	sig
Hg-N	Drinking Water Source	2.279	1.017	5.108	sig
	Gender	2.25	0.913	5.545	
	Age	0.444	0.2	0.989	sig

*Water source = Tap water is reference and groundwater is risk factor.

The results from both binary logistic regression and odd ratio showed that the drinking water source, especially groundwater. Both bathing and washing with groundwater were impacted associated factors related with heavy metals accumulated in human body.

Furthermore, others report also revealed associated factors affecting heavy metals in hairs and nails. The study of Wael et al. (2002) in Egypt revealed that gender and smoking habit were associated factors of Cd, Pb, Hg in human blood, urine, hairs, and nails. In addition, the study of Rakib et al. (2013) in Bangladesh found the difference of gender that caused the significant different arsenic mass fraction between female and male biomarkers. They reported that As content in female hair was greater than that of male hair and As contents in female nails were two times higher than male nail. A significant difference of As arsenic content in both male and female hair was observed due to using of As-enriched groundwater. Similarly, Muhammad et al. (2009) studied investigated As level in hair of smoker and non-smoking group in Pakistan and found that As in hair of smoker (0.94+0.21 ugAs/g) were significantly higher than non-smoker (0.43+0.18 ugAs/g). Furthermore, there were others researches that found that one of the associated factors of heavy metals was from the water sources, which is similar to this study as shown in Table 7.13.

Table 7. 13 The associated factors of heavy metals related with biomarkers found in other previous studies

Reference Studies	Heavy Metals	Biomarkers	Associated Factors
Norwak and Chmielnicka, 2000	Cd, Pb	Hairs, Nails, Teeth	Gender, Age
Wael et al., 2002	Cd, Pb, Hg	Blood, Urine, Hair, Nail	Gender, Smoking habit
Badal et al., 2003	As	Finger nails, Hairs	Tube-wells water
Gautam et al., 2004	As, Se, Hg, Zn, Pb, Ni, Cd, Mn, Cu, and Fe	Hairs, Nail, Skin-scales	Drinking water, Food
Blakely et al., 2006	As	Toenails	Drinking water
Sukumar and Subramanian, 2007	Cd, Cr, Cu, Ni, Pb, Zn	Hairs, Finger nails	Smoking status, Place of residence (rural/urban)
Tomoko et al., 2007	Hg	Hairs, Toenails, Urine	Age, Smoking status
Suthipong et al., 2010	As	Hairs	Groundwater consumption period, Age and Gender
Gil et al., 2011	Cd, Cr, Mn, Ni, Pb	Blood, Urine, Hair, Saliva	Gender, Smoking, Alcohol, Place of residence, Occupation, Lifetime working experience
Li et al., 2011	Hg	Hairs	Mining work, Vapor atmosphere
Jamal et al., 2013	As	Hairs	Age, Smoking status, Chinese Traditional medicine using

Reference Studies	Heavy Metals	Biomarkers	Associated Factors
Rebecca et al., 2013	As	Toenails, Urine	Water consumption, Seafood consumption, Tobacco consumption. Gender, Smoking status
Catherine et al., 2014	Hg	Hairs	Seafood consumption
Denise et al., 2016	Se	Toenail	Gender, Cigarette smoking, Alcohol consumption.
Park and Seo, 2017	Hg	Toenail	Fish consumption, Seafood in dietary intakes

CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions and recommendations

The results of this study showed that shallow groundwater in intensively agricultural area at Muang district, UbonRatchathani province, Thailand can cause the human health risk. This research studied 25 groundwater wells and 17 tap water samples, 58 groundwater-drinking participants and 42 non-groundwater-drinking participants. This study investigated the average drinking rate of approximately 4.21±2.73 L/day, which is twice as high as the standard and caused health risk related with heavy metals contaminated in drinking water. The shallow groundwater showed acidity, average pH was 5.28±1.15 and 5.16±4.19 during dry and wet season respectively.

According to the heavy metals contaminated in groundwater data, the health risk assessment for 4 metals which were As, Cd, Pb and Hg, showed groundwater drinking group had both carcinogenic risk and non-cancer risk higher than non-groundwater drinking participants in both dry and wet season. The urine results showed significant correlation with cancer risk, non-carcinogenic risk, and concentration in drinking groundwater at 0.01 level. In the same way, the results of 4 heavy metals concentrations (As, Cd, Pb, Hg) in all biomarkers (hairs, nails, urine) from groundwater drinking participants were found greater than non-groundwater drinking participants for both wet and dry season. Interestingly, the As levels in the groundwater correlated with those in the urine of the groundwater-drinking participants, but not in the non-groundwater-drinking participants, as well as with the As-related cancer and non-carcinogenic risks. The hazard index (HI) of drinking water during dry season ranged from 0.00 to 25.86, with an average of 1.51±3.63 higher than the acceptable level. The HI during wet season was 1.20±2.50 ranged from 0.00 to 16.34.

The odd ratio and binary logistic regression investigated the associated factors of heavy metals in urine, hair, and nails which were similarly in both season, most of them were groundwater consumption including drinking, bath, and washing. Not only heavy metals in groundwater exposure, but this research also found others influence associated factors such as smoking, alcohol drinking, contact with fertilizers and pesticides.

Interesting, in case of agriculturalists, use of PPE covering whole body such as mask, hat, long-sleeve shirt, long-leg trousers, rubber gloves and boots, were protective factors that can prevent heavy metal exposure and accumulated in human body. In addition, avoid or decrease smoking and alcohol drinking can also decrease heavy metals exposure.

Therefore this study suggested that groundwater should be filtered with proper filtration before drinking or should not be directly used as major drinking water supply including bath and washing because of their long term exposure effects.

8.2 Recommendation

This study shows that shallow groundwater at Muang district, Ubon Ratchathani province, Thailand had heavy metals contamination to pose human health effect and had potential to accumulate in human body even low concentrations. Moreover, local people who generally drinking groundwater in this area could be get adverse health effect from heavy metals exposure.

Furthermore, this study supported the risk awareness in the low concentration contamination site by using the appropriated biomarker, which is easier to sample and transport in a rural area for screening exposure of heavy metals and metalloids, especially As, by using hairs. Urine is the good biomarkers related daily exposure with all fours heavy metals, As, Cd, Pb ,Hg. On the other hands, long term exposure, nail is better to be used as biomarkers for As, Cd, and Pb than hairs, As, Cd, and Pb were excreted via nail greater than hair. On the other hand, hair is better to be used as biomarkers for Hg than nail.

This study could serve as a database for using biomarker even at low concentrations of heavy metal contamination in sites such as agricultural areas. The results could serve as an informative database for groundwater drinking standards, especially for tropical zones or agricultural countries that were affected by high water intake rates. Furthermore, there is a greater need for risk awareness and communication with locals who live in farming area and use groundwater as a main water supply or with the village head and government about risks from heavy metal contamination in the groundwater even at low concentrations to prevent the adverse human health effect on local people who long-term generally consume groundwater as major fresh water supply.

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APPENDIX

VITA

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