UNIVERSITI PUTRA MALAYSIA

***TOENAIL METAL CONCENTRATION AS BIOMARKER OF EXPOSURE TO HEAVY METAL IN DRINKING WATER OF THE PASIR MAS DISTRICT, MALAYSIA***

**NURUL HAFIZA BINTI AB RAZAK**

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**By**

**NURUL HAFIZA BINTI AB RAZAK**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Doctor of Philosophy**

**August 2016**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Doctor of Philosophy

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**Chairman: Sarva Mangala Praveena, PhD**
**Faculty: Medicine and Health Sciences**

Drinking water consumed by population is generally from piped water to the house, public pipe or standpipes, boreholes, protected dug wells, protected springs and rainwater collection. Population in Pasir Mas use tap water as the main source of drinking water. Even the water sources has been treated before distributed to consumers, drinking water is still contaminated by heavy metal due to less efficient treatment system or leach out from plumbing systems. The first objective of this study was to ascertain the level of knowledge, attitude and practice (KAP) regarding heavy metal contamination of Pasir Mas drinking water. The second objective was to determine the concentration of heavy metals (Al, Cr, Cu, Fe, Ni, Pb, Zn, and Cd) in drinking water and toenail from Pasir Mas. The third objective was to identify potential sources of heavy metal in drinking water from Pasir Mas. The next objective was to investigate correlation between heavy metal (Fe, Cu and Pb) concentration in drinking water and toenail. This study also identified the associations of confounders with heavy metal in toenail. Finally, this study evaluated health risks for heavy metal exposure via drinking water among respondents from Pasir Mas District. Stratified random sampling was used to select 214 respondents in Pasir Mas. A set of modified questionnaire were administered to the respondents to achieve the first objective. Then, analysis of heavy metal in drinking water were carried out using Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS) and heavy metal in toenail were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analysis involved in this study were cluster analysis and Mann-Whitney U test for the third objective. Spearman correlation analysis was carried out to investigate correlation between heavy metal (Fe, Cu and Pb) concentration in drinking water and toenail. Lastly, Multiple Linear Regression (MLR) was carried out to identify the associations of confounders with heavy metal in toenail. Pasir Mas population has good knowledge (80%), less positive attitude (93%), and good practice (81%) towards heavy metal contamination in drinking water. Heavy metal (Al, Cr, Cu, Fe, Ni, Pb, Zn, and Cd) analysed in this study was found to be under the permissible limit of drinking water quality set by the Malaysian Ministry of Health. Concentrations of heavy metal in toenail from Pasir Mas population were 35.29 ± 39.22 µg/g (Fe), 3.89 ± 2.08 µg/g (Cu) and 0.30 ± 0.34 µg/g (Pb). Cluster analysis (CA) was performed to classify the heavy metal on the basis of the similarities of their physical and chemical properties into clusters for purposes of source apportionment. Cluster analysis of heavy metal in drinking water showed three clusters which were Ni, Pb and Cr (C1), Cu, Zn and Fe (C2) and Al (C3). Thus, the cluster analysis indicated three main possible sources of heavy metal in drinking water. Cluster 1 (Ni, Pb and Cr) might originate from heavy metal leaching from galvanised iron and mild steel pipeline. Cluster 2 (Cu, Zn and Fe) might originate from corrosion of pipeline and occurrence of the heavy metal in Kelantan river water which were affected by agricultural run-off. Cluster 3 (Al) was likely to originate from usage of alum in conventional water treatment system. Mann-Whitney U test has shown that heavy metal concentration of Pb, Fe, Cu and Zn were significantly ($p<0.05$) different at house and distribution tanks which indicated that plumbing material were the source of the heavy metal in drinking water sample from Pasir Mas District. This study also showed no significant correlation between heavy metal (Fe, Cu and Pb) in drinking water and toenail. This study found confounder associated with heavy metal in toenail is dietary intake (cockles, sandwich bread and marine fish). A total of 17.5% variation in Fe concentration in toenail was explained by Cu in toenail and dietary intakes of cockles and sandwich bread. Model for Cu showed Fe concentration in toenail and cockles intake had accounted to 13.5% variation in Cu concentration in toenail. Dietary intakes of sandwich bread and marine fishes had 14.2% of variance for Pb concentration in toenail. Health Risk Assessment conducted in this study showed no potential non-carcinogenic and carcinogenic risks from the intake of heavy metal through drinking water. In conclusion, this study provided information on KAP of the Pasir Mas population towards heavy metal in drinking water. Output of this study provided information that conventional treatment system is adequate to treat the Kelantan river water to be used as drinking water. This study also showed that pipeline material of galvanised pipes, mild steel and PVC could be the source of heavy metal in treated drinking water supplied to population in Pasir Mas. Output of this study provided evidence that toenail was a less efficient biomarker to reflect heavy metal (Fe, Cu and Pb) exposure through drinking water ingestion pathway. Lastly, this study provided information to fulfil the knowledge gap on health risk assessment (HRA) regarding heavy metal exposure through ingestion of drinking water. This information could be beneficial for decision makers and authorities to improve drinking water quality and management in the future.

**Keywords**: Drinking water, Heavy metal, Toenail, Health Risk Assessment, Confounder

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**KEPEKATAN LOGAM DALAM KUKU KAKI SEBAGAI PENANDA BIOLOGI TERHADAP PENDEDAHAN KEPADA LOGAM BERAT DALAM AIR MINUMAN DI DAERAH PASIR MAS, MALAYSIA.**

Oleh

**NURUL HAFIZA BINTI AB RAZAK**

**Ogos 2016**

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Air minuman yang digunakan oleh populasi umumnya adalah dari air paip rumah, paip awam, lubang digerudi, telaga tertutup, mata air yang dilindungi dan air hujan yang dikumpulkan. Populasi di Pasir Mas menggunakan air paip sebagai sumber utama air minuman. Walaupun sumber air telah dirawat sebelum diedarkan kepada pengguna, air minuman masih boleh tercemar oleh logam berat disebabkan oleh sistem rawatan yang kurang efisien atau kebocoran dari sistem paip. Objektif pertama kajian ini adalah untuk mengetahui tahap pengetahuan, sikap dan amalan (KAP) mengenai pencemaran logam berat di dalam air minuman di Pasir Mas. Objektif kedua adalah untuk menentukan kepekatan logam berat (Al, Cr, Cu, Fe, Ni, Pb, Zn dan Cd) di dalam air minuman dan kuku jari kaki dari Pasir Mas. Objektif ketiga ialah untuk mengenal pasti sumber logam berat dalam air minum dari Pasir Mas. Objektif seterusnya adalah untuk menyiasat hubungan antara kepekatan logam berat (Fe, Cu dan Pb) dalam air minuman dan kuku jari kaki. Kajian ini juga mengenal pasti hubung kait antara faktor perancu dengan logam berat dalam kuku jari kaki. Akhir sekali, kajian ini menilai risiko kesihatan oleh pendedahan logam berat melalui air minuman di kalangan responden dari Daerah Pasir Mas. Seramai 214 responden dipilih secara rawak berstrata. Satu set soal selidik yang telah diubahsuai dilengkapkan oleh responden untuk mencapai objektif pertama. Kemudian, analisis logam berat dalam air minum telah dijalankan menggunakan teknik Spektrometri Penyerapan Atom Relau Grafit (GF-AAS) dan logam berat di kuku jari kaki telah dianalisis menggunakan Spektrometer Jisim Plasma Gandingan Teraruh (ICP-MS). Analisis statistik yang terlibat dalam kajian ini ialah analisis kelompok dan ujian Mann-Whitney U untuk objektif ketiga. Analisis korelasi telah dijalankan untuk menyiasat hubungan antara logam berat (Fe, Cu dan Pb) kepekatan dalam air dan kuku jari kaki minum. Akhir sekali, Regresi Linear Berganda (MLR) telah dijalankan untuk mengenal pasti hubungan di antara faktor perancu dengan logam berat dalam kuku jari kaki. Populasi dari Pasir Mas mempunyai pengetahuan yang baik (80%), sikap kurang positif (93%), dan amalan yang baik (81%) terhadap pencemaran logam berat dalam air minuman. Logam berat (Al, Cr, Cu, Fe, Ni, Pb, Zn dan Cd) yang dianalisis dalam kajian ini berada dalam julat piawaian kualiti air minuman yang telah ditetapkan oleh Kementerian Kesihatan Malaysia. Kepekatan logam berat dalam kuku jari kaki daripada penduduk Pasir Mas adalah 35.29 ± 39.22 μg/g (Fe), 3.89 ± 2.08 μg/g (Cu) dan 0.30 ± 0.34 μg/g (Pb). Analisis Kelompok (CA) digunakan untuk mengelaskan logam berat berdasarkan persamaan ciri-ciri fizikal dan kimia ke dalam kelompok untuk tujuan pembahagian sumber. Analisis Kelompok (CA) terhadap logam berat dalam air minuman menunjukkan tiga kelompok iaitu Ni, Pb dan Cr (K1), Cu, Zn dan Fe (K2) dan Al (K3). Oleh itu, Analisis Kelompok menunjukkan tiga sumber utama logam berat dalam air minuman. Kelompok 1 (Ni, Pb dan Cr) mungkin berasal dari larut lesap logam berat daripada saluran paip disadur besi dan keluli lembut. Kelompok 2 (Cu, Zn dan Fe) mungkin berasal dari hakisan saluran paip dan kehadiran logam berat dalam air Sungai Kelantan yang terjejas akibat dari aktiviti pertanian. Kelompok 3 (Al) mungkin berasal dari penggunaan alum dalam sistem rawatan air konvensional. Kajian ini juga menunjukkan tiada hubungan yang signifikan antara logam berat (Fe, Cu dan Pb) di dalam air minuman dan kuku jari kaki. Selain itu, kajian ini mendapati faktor perancu yang berkaitan dengan logam berat di dalam kuku jari kaki adalah pengambilan pemakanan (kerang, roti sandwic dan ikan laut). Sebanyak 17.5% variasi dalam kepekatan Fe dalam kuku jari kaki adalah di sumbang oleh Cu dalam kuku jari kaki dan pengambilan pemakanan (kerang dan roti sandwic). Model Cu menunjukkan kepekatan Fe dalam kuku jari kaki dan pengambilan kerang telah menyumbang kepada 13.5% variasi dalam kepekatan Cu dalam kuku jari kaki. Pengambilan pemakanan roti sandwic dan ikan laut menyumbang kepada 14.2% variasi dalam kepekatan Pb dalam kuku jari kaki. Penilaian Risiko Kesihatan (HRA) yang dijalankan dalam kajian ini pula menunjukkan tiada potensi risiko bukan kanser dan kanser melalui pengambilan logam berat dalam air minuman. Kesimpulannya, kajian ini menyediakan maklumat tentang pengetahuan, sikap dan amalan (KAP) penduduk Pasir Mas terhadap pencemaran logam berat dalam air minuman. Output kajian ini memberi informasi berkenaan sistem rawatan konvensional mampu untuk merawat air Sungai Kelantan dengan optimum sebelum digunakan sebagai air minuman. Kajian ini juga menunjukkan bahawa bahan paip seperti besi, keluli lembut dan plastik (PVC) boleh menjadi sumber kepada pencemaran logam berat dalam air minuman dirawat yang dibekalkan kepada penduduk di Pasir Mas. Output kajian ini membuktikan bahawa kuku jari kaki adalah penunjuk biologi yang kurang efisien untuk mencerminkan pendedahan terhadap logam berat (Fe, Cu dan Pb) melalui pengambilan air minuman. Akhir sekali, kajian ini menyediakan maklumat untuk mengisi jurang pengetahuan mengenai penilaian risiko kesihatan (HRA) berkaitan dengan pendedahan terhadap logam berat melalui pengambilan air minuman. Maklumat ini boleh memberi manfaat kepada pembuat keputusan dan pihak berkuasa untuk meningkatkan kualiti air minuman dan pengurusan pada masa hadapan.

**Kata kunci**: Air minuman, logam berat, kuku kaki, Penilaian Risiko Kesihatan, faktor perancu

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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**Declaration by Members of Supervisory Committee**

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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| | |
|---|---|
| < | Less than |
| > | More than |
| AKSB | Air Kelantan Sdn. Bhd. |
| Al | Aluminium |
| APHA | American Public Health Association |
| As | Arsenic |
| AT | Averaging time |
| BMI | Body mass index |
| BW | Body weight |
| CA | Cluster analysis |
| Cd | Cadmium |
| CDC | Centre of Disease Control and Prevention |
| CDI | Chronic Daily Intake |
| $C_{dw}$ | Heavy metal concentration in drinking water |
| Cr | Chromium |
| Cu | Copper |
| $Cu_{drinking\ water}$ | Copper concentration in drinking water |
| $Cu_{toenail}$ | Copper concentration in toenail |
| DID | Department of Irrigation and Drainage |
| DW | Drinking water |
| ED | Exposure duration |
| EF | Exposure frequency |
| FAO | Food and Agricultural Organisation |
| Fe | Iron |
| $Fe_{drinking\ water}$ | Iron concentration in drinking water |
| $Fe_{toenail}$ | Iron concentration in toenail |
| GF-AAS | Graphite Furnace Atomic Absorption Spectrophotometry |
| H | Hair |
| HDPE | High density polyethylene |
| HQ | Hazard Quotient |
| HRA | Health Risk Assessment |
| ICP-MS | Inductively Coupled Plasma - Mass Spectrometry |
| IR | Drinking water ingestion rate |
| IRIS | Integrated Risk Information System |
| JECFA | The Joint FAO/WHO Expert Committee in Food Additives |
| KAP | Knowledge, Attitude and Practice |
| MLR | Multiple Linear Regression |
| MMOH | Malaysian Ministry of Health |
| Mn | Manganese |
| Ni | Nickel |
| NWSC | National Water Services Commission |
| Pb | Lead |
| $Pb_{drinking\ water}$ | Lead concentration in drinking water |
| $Pb_{toenail}$ | Lead concentration in toenail |
| PVC | Polyvinyl chloride |
| RfD | Oral reference dose of heavy metal |
| SAJH | Syarikat Air Johor Holdings |
| Sf | Slope Factor |

| | |
|---|---|
| SYABAS | Syarikat Bekalan Air Selangor |
| TN | Toenail |
| U | Urine |
| UNESCO | United Nations Educational, Scientific and Cultural Organisation |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organisation |
| Zn | Zinc |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Sources of drinking water supply for global population are surface water (rivers, springs or lakes in a watershed) and groundwater. Furthermore, availability of drinking water supply depends on geography, climate, engineering, regulation, and competition for resources. Approximately 11% of global population do not have access to water (UNESCO, 2014; WHO, 2013). Another 89% of global population have access to improved drinking water source (UNESCO, 2014; WHO, 2013). World Health Organisation (2013) has stated that improved drinking water sources included piped water to the house, public pipe or standpipes, boreholes, protected dug wells, protected springs and rainwater collection. According to Department of Statistics Malaysia (2010), 93% of housing units in Malaysia are equipped with treated drinking water supply. For instance, drinking water supply in Malaysia is from rivers and groundwater. Malaysia used 99% drinking water supply from rivers (Azrina et al., 2011). River water in Malaysia is readily available and treated for domestic uses (FAO 2010; Lim et al., 2012). However, rapid development and industrial activity in Malaysia have deteriorated river water quality (Fulazzaky et al., 2010). Major pollution water sources in Malaysia are manufacturing industries, livestock farming and agro-industries (Aini et al., 2007). Furthermore, most of the supplied drinking water in Malaysia is treated using conventional treatment system. Conventional treatment system using alum salt as coagulant is proven to produce aluminium residue in the treated drinking water (Muyibi et al., 2004).

Drinking water can be defined as the water supplied to the consumer that can be safely used for drinking, cooking, and washing (DeZuane, 1997). A survey by Azrina et al., (2012) in a Malaysian urban area (Klang Valley) showed that majority of the respondents consumed tap water. Although drinking water is treated before being supplied, consumers commonly complaint on drinking water quality related to air in water, high turbidity, high colour and presence of foreign particles, taste and odour (Chan 2007). Meanwhile, a study by Aini et al., (2007) showed that Malaysian consumers are dissatisfied with drinking water quality only based on the appearance without realising that drinking water could be contaminated by toxic contaminants, even when the appearance of drinking water seems to be in good quality. Besides, treated drinking water is supplied to consumers through the pipeline system. Pipeline system has been used in Malaysia which varies from mild steel, galvanised iron, stainless steel and plastic (HDPE and PVC) (NWSC, 2015). For instance, galvanised iron pipe has been replaced by HDPE, PVC and stainless steel pipe in West Coast Peninsular Malaysia and South Malaysia such as states of Selangor and Johor (SYABAS, 2013; SAJH, 2012). However, states in East Coast Peninsular Malaysia such as Kelantan has replaced galvanised pipe with mild steel and HDPE pipes (AKSB, 2013). Due to the fact that mild steel pipe is prone to corrosion which is susceptible to heavy metal (Fe, Cu, Cr, Ni and Pb) release (Jung et al., 2009; Pearce, 2013). Thus, corrosion of pipeline can be a problem to drinking water quality in Kelantan.

The United States Environmental Protection Agency (USEPA) (2016) have listed six group of drinking water contaminants including microorganisms, disinfectants, disinfection by-products, inorganic chemicals, organic chemicals and radionuclides (Table 1.1). Inorganic chemical through heavy metal contamination in drinking water is a major concern due to heavy metal exposure, which will lead to various health effect such as liver and kidney damage, neurology impairment and increased risk of cancer (USEPA, 2016; Järup, 2003). Even though the drinking water is treated before being supplied, heavy metal still can present in treated drinking water. Heavy metal contamination in drinking water may be due to contaminated water source, usage of chemical in water treatment, inefficiency of water treatment system, and leaching out from corroded pipeline system (Tamasi and Cini, 2004; Farizwana et al., 2010). Furthermore, due to the unpleasant appearance of supplied drinking water, most consumers have installed private water filter at their home (Aini et al., 2007). This practice is a good alternative to ensure the quality of drinking water, however poor maintenance of the filter unit could also contribute to heavy metal contamination in drinking water. On top of that, household storage practice also could contribute to heavy metal contamination in drinking water (Brick et al., 2004). Besides, heavy metal present in drinking water is in soluble form and readily absorbable (Chiron et al., 2003). Thus, heavy metal can be easily absorbed and accumulated in human body (Chiron et al., 2003).

**Table 1.1: Drinking water contaminants**

| Contaminants | Potential Health Effects from Long-Term Exposure | Sources of Contamination |
|---|---|---|
| **Microorganisms** | | |
| Total Coliforms (including fecal coliform and *E. Coli*) | Not a health threat in itself but it is used to indicate whether other potentially harmful bacteria may be present. | Human and animal fecal waste. |
| *Cryptosporidium* | Gastrointestinal illness (such as diarrhea, vomiting, and cramps) | |
| **Disinfection byproduct** | | |
| Chlorite | Anemia; infants and young children: nervous system effects | Byproduct of drinking water disinfection |
| **Disinfectants** | | |
| Chlorine | Eye/nose irritation; stomach discomfort | Water additive used to control microbes |
| **Inorganic pollutants** | | |
| Arsenic | Skin damage or problems with circulatory systems, and may have increased risk of getting cancer | Erosion of natural deposits, runoff from glass and electronics production wastes |
| Cadmium | Kidney damage | Corrosion of galvanised pipes, erosion of natural deposits, discharge from metal refineries |
| Copper | Liver or kidney damage | Corrosion of household plumbing systems, erosion of natural deposits |
| Lead | Kidney problems; high blood pressure | |
| Chromium | Allergic dermatitis | Discharge from steel and pulp mills, erosion of natural deposits |
| **Organic pollutants** | | |
| Dichloromethane | Liver problems; increased risk of cancer | Discharge from drug and chemical factories |
| **Radionuclides** | | |
| Uranium | Increased risk of cancer, kidney toxicity | Erosion of natural deposits |

(Source: USEPA, 2009)

## 1.2 Problem statement

Kelantan river water is treated at Kelar Water Treatment Plant before being used as drinking water in Pasir Mas District (AKSB, 2014). However, Kelantan river water quality was deteriorating due to anthropogenic activities such as logging activity near the upper stream of the river, sand mining and agricultural activity along the riverside (Rahim and Shahwahid, 2011; Yen and Rohasliney, 2013; Yusoff et al., 2015; Asyirah et al., 2016). The increase of land use at the upper river for logging and agricultural activity has resulted changes of river water quality from class I-II at the upper stream to class III at the downstream of Kelantan River (Rahim and Shahwahid, 2011; Yusoff et al., 2015; Asyirah et al., 2016). Furthermore, uncontrolled sand mining activity along the Kelantan River was also contributed to the river water quality deterioration (Yen and Rohasliney, 2013). Besides, land clearing due to logging and agricultural activities and fertilizers run-off from agricultural area were the main source of heavy metal (Fe, Pb, Zn, Cu and Cd) pollution to Kelantan River (Ahmad, 2009; Yen and Rohasliney, 2013; Yusoff et al., 2015). A study done by Yusoff et al., (2015) have found that concentration of Fe in Kelantan river water was exceeded the permissible limit of 0.3 mg/L set by MMOH for raw water guideline with the highest concentration up to 4.37 mg/L.

Water supply categorised as class III such as Kelantan river water requires advanced water purification treatment to remove organic matter and other pollutants before being used as water supply (Kubota and Tsuchiya, 2010; Halim and Khan, 2012). Yet, water treatment plant at Kelar Water Treatment Plant is still practicing conventional treatment system (AKSB, 2014). Usage of alum in conventional water treatment system at coagulation stage will contribute to heavy metal residue in drinking water (Wang and Peng, 2010; Fu and Wang 2011). Moreover, previous studies in Malaysia have found that high concentration of Al in drinking water is due to the excess use of alum in water treatment process (Qaiyum et al. 2011; Farizwana et al., 2010; Dzulfakar et al, 2010). According to Wang and Peng (2010), pH adjustment during coagulation process is crucial to avoid heavy metal residues in treated drinking water. Additionally, higher alum dosage is needed to remove higher turbidity and dissolved organic carbon in raw water such as water from Kelantan river (Srinivasan and Viraraghavan, 2002; Halim and Khan, 2012).

On the other hand, there are possibilities of heavy metal being leached out into drinking water supply during transfer and storage stage (Lim et al., 2013). Treated water is distributed to the Pasir Mas population through pipeline system which consists of mild steel and HDPE pipes (AKSB, 2014). Moreover, majority of pipeline systems in Kelantan are using mild-steel pipe which has low resistance towards corrosion (AKSB, 2013; Pearce, 2013). In this regard, Mustapha and Anuar (2011) has reported that concentration of Fe in drinking water supplied to Pasir Mas District was higher than the drinking water guideline (0.3 mg/L) with concentration of 4.89 mg/L. Besides pipeline, fittings material such as brass and copper also contributed to leach out of heavy metal such as Pb and Cu into drinking water (Tam and Elefsiniotis, 2009). Apart from that, corrosion of pipeline and fittings depends on drinking water characteristic such as pH, high dissolved oxygen and temperature (Turek et al., 2011; Tam and

Elefsiniotis, 2009). Meanwhile, Lim et al. (2013) has found that Pb in first flush was higher than in the fully flush drinking water. The finding shows that pipeline corrosion and stagnation of water in the pipeline will allow contacts between water with leached Pb (Kim et al. 2011; Lim et al. 2013).

Drinking water studies in Malaysia have focused on concentration of heavy metal without further study on the risk and accumulation of heavy metal in population. Most of the heavy metal concentration in the Malaysian drinking water complied with standard from Malaysian Drinking Water Quality Standard, except for Pb, Al and Fe (Ong et al., 2007; Nalatambi, 2009; Azrina et al., 2011; Lim et al., 2013; Qaiyum et al. 2011; Farizwana et al., 2010; Dzulfakar et al, 2010). Although the heavy metal concentration were under permissible limit, there is lack of investigation on the possible sources of heavy metal in the treated drinking water at the point of use by population (Lim et al., 2013). Furthermore, literatures on knowledge, attitude and practice (KAP) related to heavy metal contamination in drinking water is limited. A study by Aini et al., (2007) found that the urban population (Seremban, Negeri Sembilan) practices good drinking water quality improving techniques, by using a filtration unit at home and boiling drinking water. Another study by Azlina et al., (2013) has found lack of knowledge regarding safe drinking water among the rural population (Kelantan). Apart from KAP, there have been few studies investigating health risk of heavy metal to the Malaysia population via drinking water ingestion using Health Risk Assessment (HRA) (Qaiyum et al., 2011; Dzulfakar et al., 2011). However, there is still limited quantitative data on KAP, HRA and biomarker pertaining to the exposure of heavy metal to the Malaysia population through drinking water ingestion pathway, particularly in areas which use treated Kelantan River water.

## 1.3 Study justification

Drinking water from Pasir Mas District has been reported to contain high Fe concentration (489µg/L) by Mustapha and Anuar (2011). Although heavy metal removal using an efficient conventional water treatment system could achieve heavy metal removal of 99% as reported by Chang and Wang (2007), other factors such as heavy metal leaching out during transfer and storage stage could also contaminate drinking water supply (Buchet and Lison, 2000; Lim et al., 2013). Thus, usage of mild steel pipe and fittings of brass and copper might be the factor of heavy metal contamination in drinking water in Pasir Mas District. Previous studies have found that corrosion of pipeline system is a factor of heavy metal contamination in drinking water (Buchet and Lison, 2000; Lim et al., 2013). Therefore, further research is needed to provide a better understanding that conventional treatment system is suitable to treat Kelantan river water which is classified as class III to be used as drinking water. This study is also required to provide knowledge of pipeline system material, which could be the source of heavy metal in treated drinking water supplied in Pasir Mas population.

Heavy metal contamination in Malaysian drinking water has been well studied (Ong et al., 2007; Nalatambi, 2009; Azrina et al., 2011; Lim et al., 2013; Qaiyum et al. 2011; Farizwana et al., 2010; Dzulfakar et al, 2010). However, there is lack of evidence that heavy metal in the drinking water could give adverse health effects to the Malaysia population. Furthermore, there is also very limited information regarding heavy metal accumulation in the Malaysia population due to intake of heavy metal through drinking water. Hence, this study will provide information to fulfil the knowledge gap on health risk assessment (HRA) and biomarker related to the exposure of heavy metal to population through drinking water ingestion pathway.

## 1.4 Significant of study

Outputs from this study included the level of knowledge, attitude and practice (KAP) of the Pasir Mas population regarding heavy metal contamination in drinking water. Detailed discussion of KAP level regarding heavy metal contamination in drinking water was provided to open wide field of information that will be very useful for authorities to improve drinking water management in the future.

This has study also shown variation, level and distribution of heavy metal concentration in drinking water and toenail from Pasir Mas District. The discussion presented were including the possible sources of heavy metal in drinking water. This study also provided evidence of incorporation of heavy metal investigation in drinking water and biomarker to give a clear picture on heavy metal accumulation in the Pasir Mas population via drinking water. This information provide a clear picture of biomarker as a useful tool to understand the end point of heavy metal once the elements ingested into the human body. This study also contributed an understanding of significant influential parameters or confounders to heavy metal accumulation in toenail, which may be applied for other exposure pathways in future studies.

Lastly, this study also provided evidence of application from Health Risk Assessment (HRA) are a useful tool to estimate more accurate health risk of a population. This will help decision makers to maintain or improve drinking water quality for the sake of public health.

## 1.5 Study limitation

Design of this study is cross-sectional study, which only provides a snapshot of the outcome and the characteristics associated with it, at a specific point in time (Levin, 2006). The heavy metal in drinking water data presented in this study represent at the point of sampling without consideration of seasonal factor which could affect the heavy metal distribution (Yusoff et al., 2015). However, Mann (2003) has stated that cross-sectional study is quick, less expensive and produces multiple outcomes. Thus, a broad base of knowledge from this study will be obtained to provide more information for future research (Levin, 2006).

Confounder is a contributing factor that may be resulting in underestimation and overestimation of the exposure (McNamee, 2003). The most important step to control confounders is to identify the possible confounders. In this study, possible confounders are demographic, dietary pattern and smoking habit which were obtained using questionnaire as these factors can increase or decrease heavy metal concentration in toenail. Confounders such as demographic, dietary pattern and smoking can be controlled by statistical method. Thus, statistical method using multiple linear regression can be applied to determine the role of confounders are associated with heavy metal concentration in toenail (Slotnick et al., 2008). Sample population chosen in this study is based on available respondents during the day of sampling. Sampling was conducted on weekdays and weekend, which resulting higher response rate among housewives compared to working men. However, previous studies had shown that gender were not a contributing factor to heavy metal concentration in toenail (Coelho et al., 2012; Barbosa et al., 2005).

Furthermore, this study only focused on heavy metal exposure through drinking water intake. This is due to the fact that heavy metal present in drinking water is in soluble form which is can be easily absorbed and accumulated in human body (Chiron et al., 2003). Other routes of exposure such as dietary intake, dermal exposure, inhalation exposure and occupational exposure were not considered in quantitative manner. This study is intended to study the heavy metal exposure on general adult population which does not requires population classified according to their occupational exposure. Similar studies has been conducted by Kavcar et al., (2009) which has reported exposure of heavy metal on general population in Italy. Furthermore, a study done by Saat et al., (2013) have found that toenail heavy metal concentration in farmers from Kelantan is not associated with their occupational exposure.

The order of important heavy metal exposure routes can be rank as absorption < inhalation < ingestion (Beckett et al., 2007; Cornelis and Nordberg, 2007). Ingestion route is indirect exposure by intake of food and drinking water into gastrointestinal tract (Beckett et al., 2007; Cornelis and Nordberg, 2007). Beckett et al., (2007) stated that exposure through the skin is usually less important than inhalation and ingestion pathways. Cornelis and Nordberg (2007) stated that intake of heavy metal through inhalation pathway is usually small compared to ingestion pathway. Thus, ingestion of food and drinking water is the major source of general population exposure (Beckett et al., 2007; Xu et al., 2006). Analysis of heavy metal in food is a complex process due to various pattern of a population diet and the analysis should represent cooking method (Cornelis and Nordberg, 2007; Żukowska and Biziuk, 2008). Food analysis for heavy metal also could contribute to more confounder factors such as source of the food item, cooking method and utensils used (Żukowska and Biziuk, 2008). Furthermore, heavy metal in drinking water also could be a factor of heavy metal in food item during washing and cooking process such as reported by Ndilila et al., (2013). Thus, this study only focused on drinking water to reduce the possible confounders and the confounders considered can be controlled using qualitative method and statistical analysis.

## 1.6 Conceptual framework

Figure 1.2 shows the conceptual framework of this study by estimating carcinogenic and non-carcinogenic health risk of heavy metal exposure through drinking water ingestion pathway, along with examining biomarker of exposure (toenail) with consideration of confounders.

Heavy metal occur in environmental media (water, soil and air) due to both natural occurrences and anthropogenic sources (Kavcar et al., 2009; Ahmad et al., 2009). Pollution of heavy metal in water bodies such as groundwater and surface water will affect the quality of drinking water sources, even the drinking water was treated before distributed to the population (Bobaker et al., 2014).

Heavy metal can be absorbed into human through three main routes which are ingestion, inhalation, and dermal contact (USEPA, 2011). However, ingestion of drinking water has been considered as the main pathway of heavy metal exposure due to the solubility of heavy metal in water, which are readily absorbable into the human body (Chiron et al., 2003). Carcinogenic and non-carcinogenic health risks through ingestion pathway were estimated using Health Risk Assessment (HRA).

There are a few biomarkers has been used to monitor exposure of heavy metal through drinking water, such as blood, urine, toenail, fingernail and hair (Esteban and Castaño, 2009). However, previous studies had suggested that toenail is the most suitable biomarker compared to blood, urine, fingernail and hair due to its rapid growth, less external contamination, adequate sample availability and incorporation of elements in the tissue (Adair et al., 2006; Sukumar, 2006; Slotnick et al., 2007).

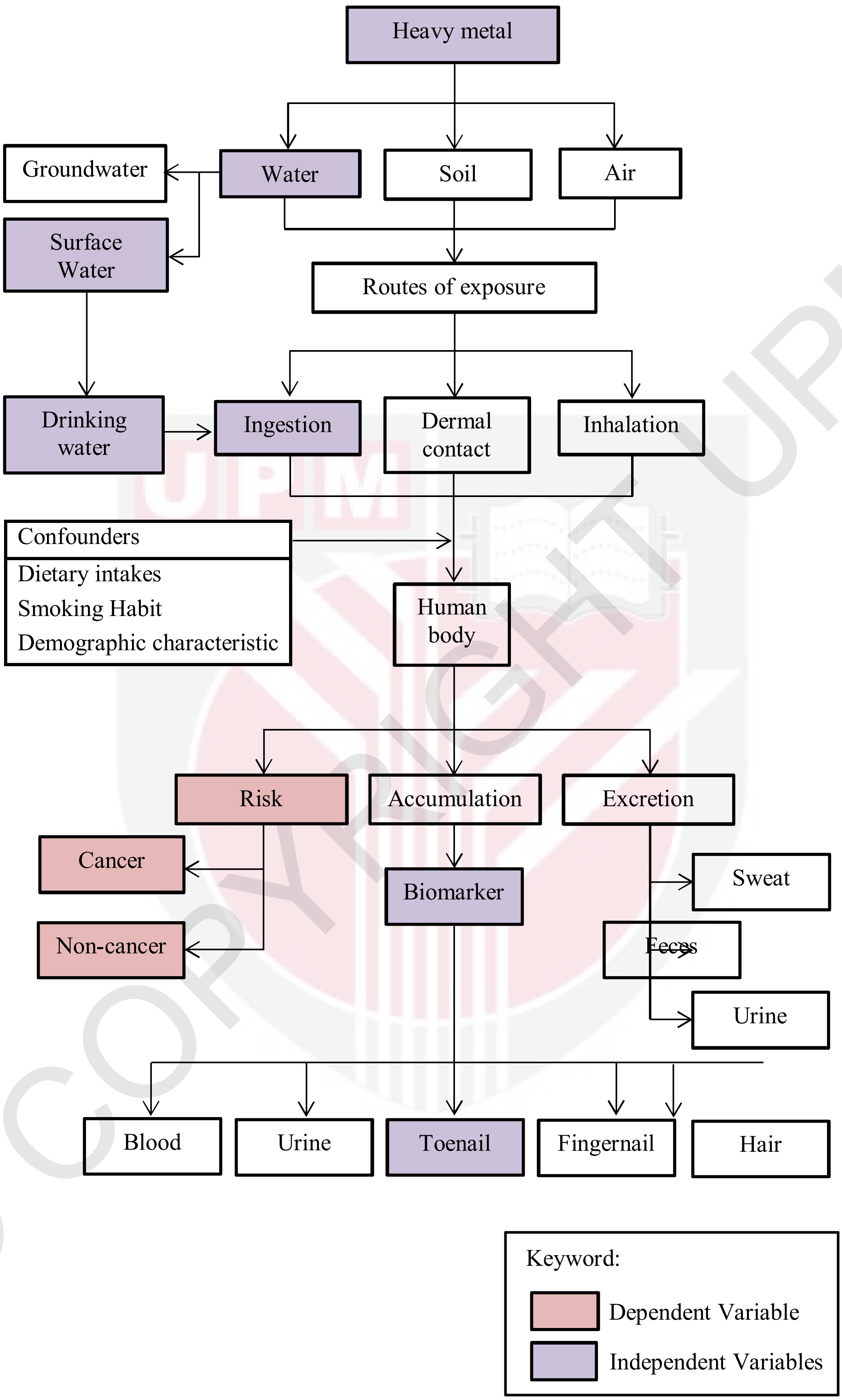


**Figure 1.1: Conceptual framework of this study**

## 1.7 Research objective and hypotheses

### 1.7.1 General objective

To estimate exposure and health risks of heavy metal (Al, Pb, Fe, Cu, Cd, Cr, Ni & Zn) through drinking water in the population of Pasir Mas District.

### 1.7.2 Specific objectives

1. To ascertain the level of knowledge, attitude and practice (KAP) on heavy metal contamination in drinking water in Pasir Mas population.
2. To determine heavy metal concentration in drinking water and toenail from Pasir Mas District.
3. To identify possible sources of heavy metal in drinking water from Pasir Mas.
4. To investigate correlations between heavy metal (Fe, Cu and Pb) concentration in drinking water and toenail.
5. To identify the associations of confounders with heavy metal in toenail.
6. To evaluate health risks for heavy metal exposure via drinking water among the population of Pasir Mas District.

### 1.7.3 Research hypotheses

1. There is significant sources of heavy metal in drinking water from Pasir Mas.
2. There is significant correlation between heavy metal concentration in drinking water and toenail.
3. There is significant associations between confounders and heavy metal in toenail.
4. There are health risks presents for heavy metal exposure via drinking water among the population of Pasir Mas District.

## 1.8 Conceptual Definition

### 1.8.1 Heavy metal

Heavy metal is defined as an element with densities greater than $5g/cm^3$ (Jaishankar et al., 2014; Tchounwou et al., 2014). Heavy metal is also considered as trace elements or trace metal because of their presence in trace concentrations (ppb range to less than 10ppm) in environmental matrices (Tchounwou et al., 2014). United State Environmental Protection Agency (USEPA, 2014) has listed Al, As, Pb, Fe, Cu, Cd, Cr, Ni & Zn as the target heavy metal analyte to be analysed in the environmental assessment programme under USEPA. Physical properties of heavy metal are listed as below (Cornelis and Nordberg, 2007):

1. High reflectivity that is responsible for the characteristic metallic cluster

2. High electrical conductivity, decreasing with increasing temperature

3. High thermal conductivity

4. Mechanical properties such as strength and ductility

### 1.8.2 Health Risk Assessment (HRA)

HRA is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future (USEPA, 2015).

### 1.8.3 Operational Definition

#### 1.8.3.1 Health Risk Assessment

Health risk assessment was directly calculated. Equation (1) shows calculation of chronic daily intake (CDI) (mg/kg/day) through ingestion route (USEPA, 2011; Lim et al., 2013). This equation was used to estimate exposure to each heavy metal.

$$CDI\ (mg/kg/day) = \frac{C_{dw} \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

Where;

CDI = Chronic Daily Intake (mg/kg/day)
$C_{dw}$ = Heavy metal concentration in drinking water (mg/L)
IR = Drinking water ingestion rate (L/day)
ED = Exposure duration (years)
EF = Exposure frequency (day/year)
BW = Body weight (kg)
AT = Averaging time (ED x 365 days)

Hazard quotient (HQ) was calculated to estimate non-carcinogenic risk using equation (2) (USEPA, 2011; Kavcar et al., 2009; Lim et al., 2013). RfD were obtained from the USEPA (IRIS, 2012).

$$HQ = \frac{CDI}{RfD} \quad (2)$$

Where;

HQ= hazard quotient,
CDI= chronic daily intake (mg/kg/day)
RfD= oral reference dose of heavy metal (mg/kg/day).

HQ was compared with the values of risk acceptability for non-carcinogenic health risks. It is assumed that no chronic non-carcinogenic health risks faced by population when HQ < 1.

Cancer risk associated with ingestion exposure was calculated using both equation (3) and equation (4) as shown below (USEPA, 2011).

$$\text{CDI } (mg/kg/\text{day}) = \frac{C_{dw} \times IR \times ED \times EF}{BW \times AT} \quad (3)$$

Where;

CDI = Chronic daily intake (mg/kg/day)
$C_{dw}$ = Heavy metal concentration in drinking water (mg/L)
IR = Drinking water ingestion rate (L/day)
ED = Exposure duration (years)
EF = Exposure frequency (day/year)
BW = Body weight (kg)
AT = Averaging time (25550 days)

$$\text{Lifetime cancer risk (LCR)} = \text{CDI} \times \text{SF} \quad (4)$$

Where;

CDI = Chronic daily intake (mg/kg/day)
SF = slope factor (mg/kg/day)

Slope factor was stated on USEPA's (2012) Integrated Risk Information System (IRIS). The acceptable LCR ranges from one in ten thousand to one in a million ($10^{-6} < LCR < 10^{-4}$).

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**APPENDIX G: Descriptive statistics of dietary intakes (g)**

| Food | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|
| rice (g) | 68.00 | 612.00 | 342.0400 | 135.67044 |
| sandwich bread (g) | .00 | 136.00 | 32.5421 | 19.93445 |
| eggs (g) | .00 | 64.00 | 21.2345 | 13.61215 |
| fish (g) | .00 | 128.00 | 49.2485 | 26.41287 |
| freshwater fish (g) | .00 | 84.00 | 7.2080 | 10.66382 |
| cockles (g) | .00 | 28.57 | 10.5003 | 10.29016 |
| squid (g) | .00 | 59.43 | 18.7089 | 15.50744 |
| prawn (g) | .00 | 171.43 | 45.1552 | 38.16068 |
| green leafy vegetables (g) | .00 | 240.00 | 169.4476 | 64.40182 |
| tuber vegetables (g) | .00 | 273.00 | 19.8233 | 38.52695 |
| manggo (g) | .00 | 140.00 | 24.4370 | 18.71771 |
| guava (g) | .00 | 320.00 | 49.8936 | 44.00883 |
| plain water (ml) | 750.000 | 4500.000 | 1988.33333 | 591.704870 |
| tea (ml) | .000 | 1000.000 | 167.14193 | 218.972757 |
| coffee (ml) | .000 | 400.000 | 89.28600 | 85.248785 |
| chocolate drink (ml) | .000 | 200.000 | 64.76093 | 70.315665 |
| fruit juice (ml) | .000 | 71.430 | 9.03773 | 17.658475 |

# LIST OF PUBLICATIONS

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Ab Razak, N.H., Praveena, S. M., & Hashim, Z. (2014). Toenail as a biomarker of heavy metal exposure via drinking water: a systematic review. *Reviews on environmental health. 30*(1): 1-7. (Scopus)

Ab Razak, N. H., Praveena, S. M., Aris, A. Z., & Hashim, Z. (2015). Drinking water studies: A review on heavy metal, application of biomarker and health risk assessment (a special focus in Malaysia). *Journal of epidemiology and global health*. 5(4): 297-310. (Scopus)

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## Under review

Investigation of the Possible Sources of Heavy Metal in Drinking Water and Biomonitoring of Heavy Metal Exposure through Drinking Water among Pasir Mas District Population. Submitted to *Chemosphere*.

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International Conference on Environmental Forensics (iENFORCE 2013). 12-13 Nov 2013. Putrajaya Mariott Hotel, Malaysia. Praveena, S. M., Mutalib, S. A., **Razak, N. H.**, Abidin, E. Z., & Aris, A. Z. (2014). Health Risk Assessment of Heavy Metal Exposure to Classroom Dust in Primary School, Serdang (Malaysia). In From Sources to Solution (pp. 83-87). Springer Singapore. **(Oral presentation)**.

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International Conference on Environmental and Occupational Health (ICEOH 2016). 11-13 Apr 2016. Putrajaya Mariott Hotel, Malaysia. **Ab Razak, N. H.**, Praveena, S. M., Aris, A. Z., & Hashim, Z. (2015). Possible Sources of Heavy Metal in Drinking Water and Exposure of Heavy Metal through Drinking Water Intake Among Pasir Mas Population. **(Oral presentation).**

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