UNIVERSITI PUTRA MALAYSIA

HEALTH RISK ASSOCIATED WITH NITRATE EXPOSURE IN GROUNDWATER AT MUKIM SALOR IN KOTA BHARU, KELANTAN

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BY

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Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

HEALTH RISK ASSOCIATED WITH NITRATE EXPOSURE IN GROUNDWATER AT MUKIM SALOR IN KOTA BHARU, KELANTAN

CHE MOHAMAD FARIZUAN

Introduction: Mukim Salor is one of the agriculture areas in Kelantan. Farmers usually use fertilizers for paddy and vegetables. The use of chemical fertilizers can lead to contamination of nitrate in groundwater by the leaching process. Kota Bharu is one of the cities in Malaysia that use groundwater as the main source of water supply. Daily use of groundwater that is contaminated with nitrate, can lead to health problems such as methemoglobinemia or "blue-baby syndrome" and shortness of breath. There is no data that show the nitrate level and hazard index from the nitrate exposure in groundwater at Mukim Salor. **Objectives**: To determine levels of nitrate in groundwater and health risk among respondents in Mukim Salor in Kota Bharu, Kelantan. **Methodology**: This study was conducted among residents at Mukim Salor in the district of Kota Bharu, Kelantan. A total of 50 respondents were chosen based on the inclusive and exclusive criteria. Groundwater samples were taken from each of the respondent's house. It was then analyzed by using a direct reading spectrophotometer HACH brand DR1900, while a set questionnaire was used for health risk assessment of the exposure. **Results and Discussion**: The mean ± SD of nitrate level was 1.08 ± 0.64 while the range was from 0.00 to 2.8 mg/L. According to p-value (0.38), it indicated that there was no significant difference of nitrate levels between these 3 villages. Nitrate level in groundwater at Mukim Salor has not exceeded the standard and there was no significant risk of nitrate contamination in ground water whereas the HI is less than 1. **Conclusion**: The groundwater at Mukim Salor was considered safe for drinking and cooking purpose. However, everyone should be concerned about the nitrate level in drinking water because it will give a bad health impact to the consumers.

Keywords: Nitrate, groundwater, contamination, health risk assessment, Mukim Salor

ABSTRAK

RISIKO KESIHATAN YANG BERHUBUNGKAIT DENGAN PENDEDAHAN NITRAT DI DALAM AIR BAWAH TANAH DI MUKIM SALOR, KOTA BAHARU, KELANTAN

CHE MOHAMAD FARIZUAN BIN CHE ISMAIL

Pengenalan: Mukim Salor adalah salah satu kawasan pertanian di Kelantan. Petani biasanya menggunakan baja untuk padi dan sayur-sayuran. Penggunaan baja kimia boleh membawa kepada pencemaran nitrat dalam air bawah tanah melalui proses larut lesap. Kota Bharu adalah salah satu bandar di Malaysia yang menggunakan air bawah tanah sebagai sumber utama bekalan air. kegunaan harian air bawah tanah yang tercemar dengan nitrat, boleh membawa kepada masalah kesihatan seperti methemoglobinemia atau "sindrom biru-bayi" dan sesak nafas. Tiada data yang menunjukkan tahap nitrat dan indeks bahaya daripada pendedahan nitrat dalam air bawah tanah di Mukim Salor. **Objektif:** Untuk menentukan tahap nitrat dalam air bawah tanah dan risiko kesihatan di kalangan responden di Mukim Salor, Kota Bharu, Kelantan. **Kaedah:** Kajian ini dijalankan di kalangan penduduk di Mukim Salor di daerah Kota Bharu, Kelantan. Seramai 50 orang responden telah dipilih berdasarkan kriteria yang inklusif dan eksklusif. sampel air bawah tanah telah diambil dari setiap rumah responden. Ia kemudiannya dianalisis dengan menggunakan bacaan terus spektrofotometer HACH jenama DR1900, manakala borang soal selidik digunakan untuk penilaian risiko kesihatan pendedahan. **Keputusan dan Perbincangan**: Min ± SD tahap nitrat adalah 1.08 ± 0.64 manakala julat adalah 0.00-2.8 mg / L. Mengikut nilai-p (0.38), ia menunjukkan bahawa tidak terdapat perbezaan yang signifikan tahap nitrat antara 3 buah kampung. Tahap nitrat dalam air bawah tanah di Mukim Salor tidak melebihi standard dan tidak ada risiko besar pencemaran nitrat dalam air bawah tanah manakala HI adalah kurang daripada 1. **Kesimpulan**: Air bawah tanah di Mukim Salor dianggap selamat untuk diminum dan tujuan memasak. Walau bagaimanapun, semua orang harus mengambil berat tentang tahap nitrat dalam air minuman kerana ia akan memberi kesan kesihatan yang tidak baik kepada pengguna.

Kata kunci: Nitrat, air bawah tanah, pencemaran, penilaian risiko kesihatan, Mukim Salor.

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LIST OF ABBREVIATIONS

kg	Kilogram
%	Percentage
CDI	Chronic Daily Intake
C	Nitrate Concentration in Water
DI	Average Daily Intake rate of Water
BW	Body weight
HI	Hazard Index
RfD	Reference Dose
N	Sample Size
P	Prevalence
e	Probability error

CHAPTER 1

INTRODUCTION

1.1 Background

Nitrate (NO_3^{-}) is one of the compounds of nitrogen (Crohn, 2004). It exists together with different types of nitrogen in a complex cycle (Fields, 2004). Nitrogen in soil and water usually come from atmospheric deposition, uses of compost, fertilizer, waste material, dead plant and animal tissue (Burow, Nolan, Rupert, & Dubrovsky, 2010). Most of the nitrogen on earth is in the atmospheres, which consist of 78% N_2 gas and there are originating mainly from power plant emission, internal combustion engines, fertilizer and manure (Chern, George & Jeff, 1999).

Nowadays, agriculture is the one of the industry grow well because of the advanced technologies and can give a good profit for the business. This industry has pros and cons. For example, the increase the productivity, the more profit they get while in order to increase the production farmer need to use the fertilizer in high amount for their crop. The production of artificial fertilizers and intensification of agriculture globally has increased the amount of reactive nitrogen in terrestrial and aquatic ecosystems (Galloway et al., 2008). The increasing use of fertilizer has created environmental problems such as accumulation of nitrate in freshwater systems (Vitousek et al., 1997), including groundwater (Taylor and Townsend, 2010). Excessive use of fertilizer and improper management of other nitrogen sources can increase the rate of movement and the magnitude of groundwater contamination (Mahler, Colter & Hirynck, 2007). Nitrate can enter to the groundwater by the leaching process. Leaching is the downward movement of water and nitrate through the soil (Song et al., 2009).

Daily use of groundwater that is contaminated with nitrate, can lead to health problems such as methemoglobinemia or "blue-baby syndrome" and shortness of breath (US EPA, 1991). Others studies stated that implicated of nitrate exposure as a possible risk factor associated with lymphoma, hypertension, thyroid disorder and birth defects (Chern, George & Jeff, 1999). In addition, some research has suggested that nitrate may play a role in spontaneous miscarriages, thyroid disorders, birth defects, and in the development of some cancers in adults (Woolverton, 2015).

Health risk assessment is the process of quantifying the probability of a harmful effect to individuals or populations from certain human activities (California Environmental Protection Agency's OEHHA, 2001). Health risk assessment also can be used identify serious health hazards and determine practical recommendations for reducing exposure to toxins (US EPA, 2000).

1.2 Problem Statement

Mukim Salor is one of the agriculture areas in Kelantan. Farmers usually use fertilizers for paddy and vegetables. The use of chemical fertilizers can lead to contamination of nitrate in groundwater by the leaching process (Jalali, 2005). Kota Bharu is one of the cities in Malaysia that use groundwater as the main source of water supply. Groundwater is an important water source in Kota Bharu, Kelantan and 70 percent of residents in Kota Bharu rely on groundwater as their primary source (Husin, 2012). Groundwater provides about 50 percent of drinking water to all around the world and the groundwater should be protected from contamination by any substances such as nitrate that might cause health problem (Burow et al., 2010).

There is no data that show the nitrate level and hazard index from the nitrate exposure in groundwater at Mukim Salor. Water containing less than 10 mg/L of nitrate is considered safe for all humans and livestock (Mahler, Colter & Hirynck, 2007). The problem to be highlighted is whether the level of nitrate in groundwater at Mukim Salor is safe or not for drinking and cooking purposes. From this study, the data obtain were compared with National Drinking Water Quality Standard and were used to determine the Hazard Index from exposure to nitrate.

1.3 Study Justification

This study is to determine nitrate level in groundwater at Mukim Salor, Kota Bharu, Kelantan. The main economic activity in Mukim Salor is agriculture. The most common fertilizer used to support the growth of the plant are urea and NPK fertilizer (Xu et al., 2013).

In Kelantan and Perlis, groundwater is being significant utilized for public water supply while in Terengganu, Pahang, Sarawak and Sabah groundwater use for a secondary source of water supply (Halwani, 2012). Drinking water used by people in Kota Bharu are mostly from groundwater (Hao, 2010). Groundwater plays an important role not only drinking water but also use for cooking.

1.4 Conceptual Framework

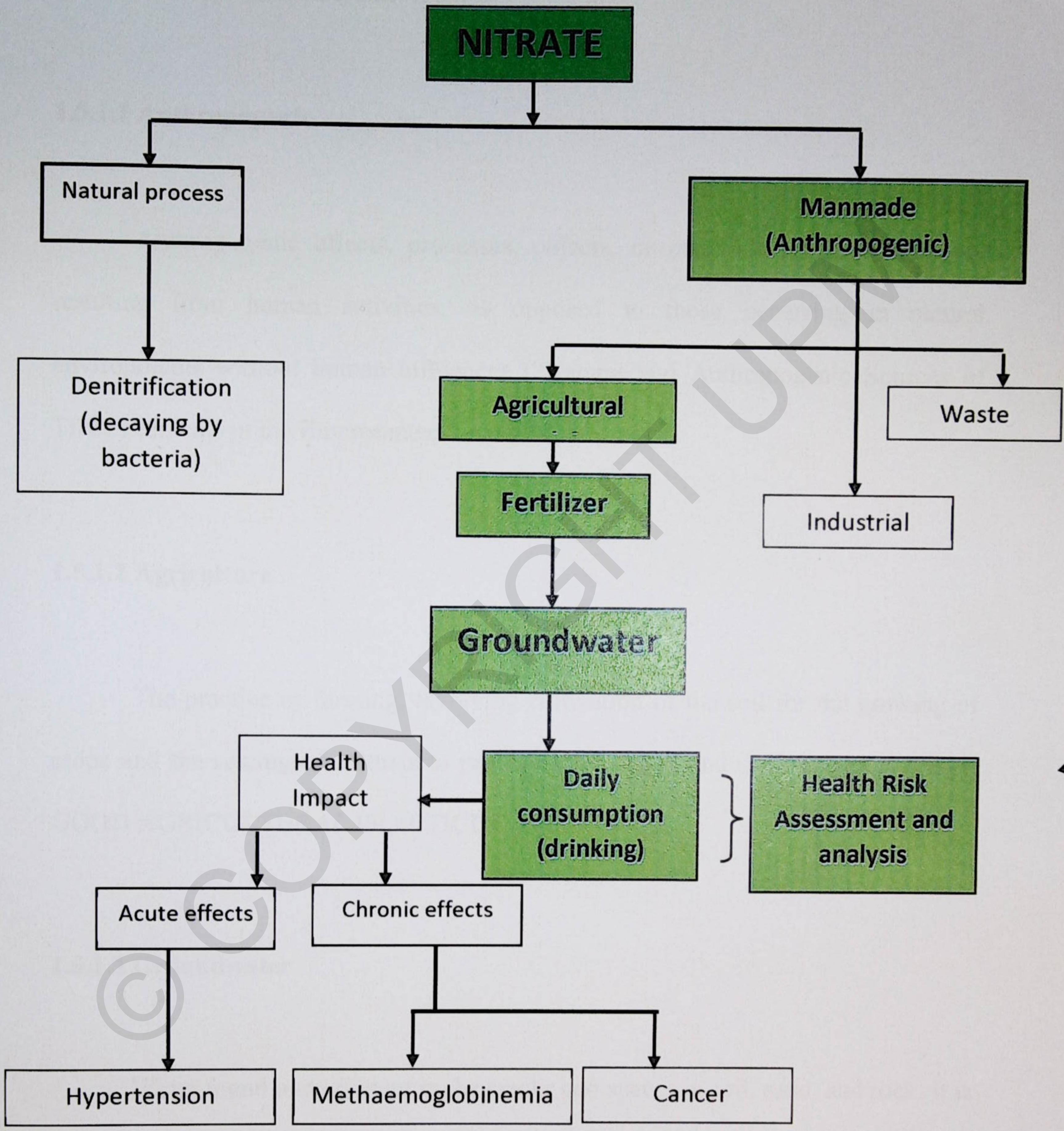

Figure 1.1: Conceptual framework (Moore et al., 2011)

1.5 Definition

1.5.1 Conceptual Definition

1.5.1.1 Anthropogenic

Anthropogenic effects, processes, objects, or materials are those that are resulting from human activities, as opposed to those occurring in natural environments without human influences ("Natural and Anthropogenic Sources of Trace Elements in the Environment," n.d.).

1.5.1.2 Agriculture

The practice of farming, involving cultivation of the soil for the growing of crops and the rearing of animals to provide food, wool, and other products ("FOR GOOD AGRICULTURAL PRACTICE Contents," n.d.).

1.5.1.3 Groundwater

Water found underground in the cracks and spaces in soil, sand, and rock. It is stored in and transports slowly through geologic formations of soil, sand, and rocks called aquifers (Burow et al.,2010).

1.5.1.4 Health risk assessment

Health risk assessments are used to determine a particular chemical poses a significant risk to human health. The risk assessment process is typically described as consisting of five basic steps (California Environmental Protection Agency, 2000):

I. Hazard identification

II. Exposure assessment

III. Dose-response assessment

IV. Risk characterization

V. Analysis

1.5.2 Operational Definition

1.5.2.1 Drinking Water

Drinking water samples were collected directly from each respondent's groundwater source.

1.5.2.2 Nitrate Level

Nitrate in groundwater samples was analyzed by direct reading spectrophotometer HACH brand DR1900.

1.6 Objective

1.6.1 General Objective

To determine levels of nitrate in groundwater and health risk among respondents in Mukim Salor in Kota Bharu, Kelantan.

1.6.2 Specific Objective

1. To determine the socio-demography of residence at Mukim Salor.
2. To determine nitrates level in groundwater at Mukim Salor.
3. To compare nitrates level of groundwater with National Drinking Water Quality Standards.
4. To compare nitrates level between National Drinking Water Quality Standards among the three villages
5. To determine the Hazard Index from exposure to nitrate.

1.7 Research Hypothesis

1. There is a significant difference of nitrate level in groundwater between the three villages.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrate

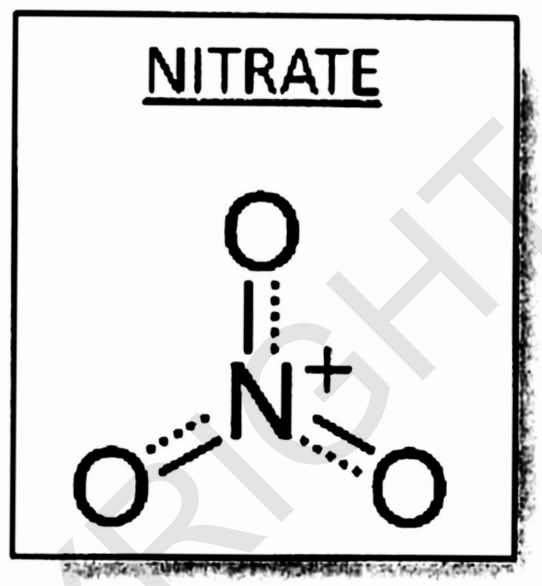

Figure 2.1 : Nitrate ion (Harrison, 2003)

Nitrates and nitrites are naturally occurring inorganic nitrogen ions found in soil, water, and some foods (Fields, 2004). They are also a natural part of the human diet. However, excessive consumption of drinking water or eating food from areas where ground water has become contaminated by excessive nitrate from fertilizers or improper manure management can cause serious adverse health effects (Fan & Steinberg, 1996).

Nitrogen in soil and water invents from atmospheric deposition, applications of fertilizer, manure, waste material and dead plant and animal tissue (Chern, George & Jeff, 1999). The decomposition of organic materials in soils releases ammonia. This ammonia oxidizes to form nitrate and nitrite. Though both compounds can be found in groundwater and soils, nitrate is more common (Number, 2014). Nitrate is also an inorganic ion and common nitrogenous compound that occur from nitrogen cycle and anthropogenic (man-made) sources (ATSDR, 2011).

2.2 Source Of Nitrate

Nitrate come in two different sources which natural and anthropogenic or known as man-made (Mahler, Colter & Hirynck, 2007). For the natural sources, it comes from the denitrification process where in the absence of oxygen, the bacteria process nitrate to gain the oxygen they need to break down the food source (Nitrogen et al., n.d.). Agriculture and industrial activities are the examples of the anthropogenic source of the nitrate and most of this originates from agricultural sources divided almost equally among legumes, manure, and commercial fertilizer (Sadler et al., 2016). Nitrate also comes from atmospheric sources including combustion of gasoline in automobiles, the breakdown of nitrogen fertilizers and manure, and lightning (Chern, George & Jeff, 1999). According to ARGOSS (2001), nitrate also comes from septic and sludge disposal.

2.2.1 Nitrogen Cycle

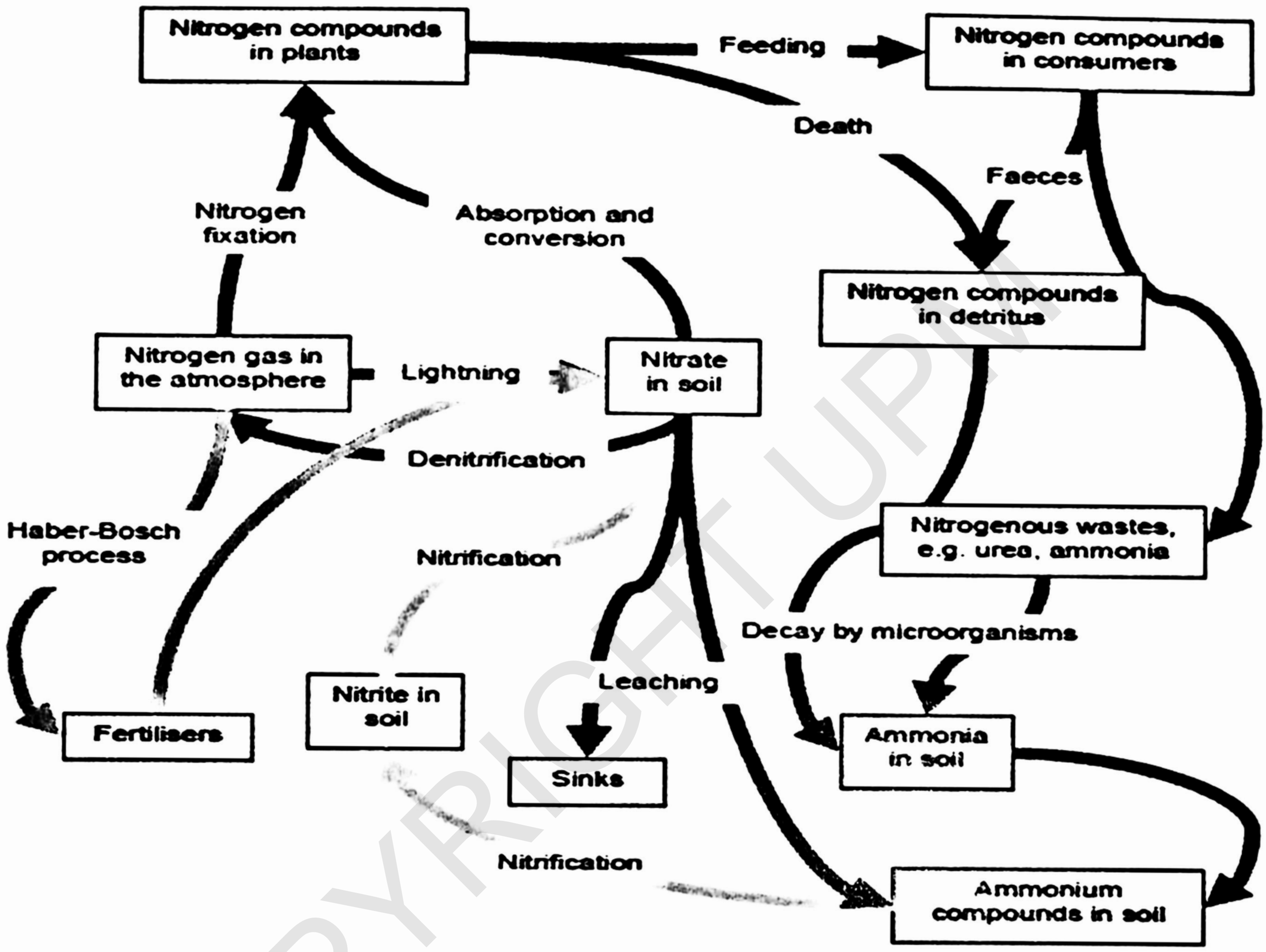

Figure 2.2: Nitrogen Cycle (RSC, n.d).

Nitrogen Cycle is a cycle within the biosphere which involves the atmosphere, hydrosphere, and lithosphere (RSC, n.d.). The conversion of nitrogen can be carried out through both biological and physical processes (Vitousek et al., 1997). There are six major transformations processes of nitrogen in this cycle through the biosphere, atmosphere, and geosphere which are nitrogen fixation, nitrogen uptake, nitrogen mineralization, nitrification, ammonification, and denitrification (Fields, 2004).

In nitrogen fixation, the process involved is the conversion of nitrogen gas (N_2) to ammonium (NH_{4+}) and nitrogen uptake is the process where the ammonium (NH_{4+}) that are produced by nitrogen-fixing bacteria is quickly taken up by the plant (Harrison, 2003). Based on the present study there are some factors that can affect biological nitrogen fixation which are temperature, moisture, use of nitrogen fertilizer and acid solid (Montanez, 2000).

Nitrogen mineralization is the process by which organic nitrogen (N) is converted to plant-available inorganic forms (Crohn, 2004) or it is a process where after nitrogen is incorporated into organic matter, it is converted back into inorganic nitrogen and also known as decay (Harrison, 2003).

Nitrification is a process where ammonium that is produced during decomposition is converted to nitrate (NO_{3}-) (EPA, 2002). It is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, for example, ammonia or nitrite (Fields, 2004).

Ammonification is the process of when an organism excretes waste or die, the nitrogen in its tissues is in the form of organic nitrogen such as amino acids while, denification is a process of oxidized forms of nitrogen such as nitrate (NO_{3}-) and nitrite (NO_{2-}) are converted to dinitrogen (N_2) and to nitrous oxide gas (NO_2) (The Nitrogen Bomb, 2014).

Human activities are greatly increasing the amount of nitrogen cycling between the living world and the soil, water, and atmosphere (RSC, n.d.). Excessive nitrogen additions can pollute ecosystems and alter both their ecological functioning and the living communities they support (Vitousek et al., 1997).

2.2.2 Nitrate In Groundwater

Groundwater is the water under the earth's surface that flows freely through small pores and cracks in rock and soil and can be pumped from wells. Groundwater supplies approximately about 50 percent of drinking water to all around the world (Mahler, Colter & Hirynck, 2007). Groundwater is important not only because it supplies drinking water but also because it provides water to cooking and for daily use.

Nitrate contamination in groundwater is derived typically from sources such as agricultural land runoff, leaching of nitrogen fertilizers, intensive animal feeding operations, food processing, and industrial waste discharge (Jalali, 2005). The increasing use of artificial fertilizers, the disposal of wastes particularly from animal farming and changes in land use are the main causes responsible for the progressive increase in nitrate levels in groundwater supplies over the last 20 years (WHO, 2011). Nitrate that is not used by plants can build up in and move through the soil (Vitousek et al., 1997). Precipitation, irrigation, and sandy soils allow nitrate to move around and find its way into surface water and ground water (Burow et al., 2010).

2.3 Drinking Water Quality Standard

Drinking Water Quality Surveillance is a unit of Ministry of Health Malaysia which responsible to set the guidelines regarding safe and potable water supply throughout Malaysia. The guideline and standard were set based on the guidance from the World Health Organization, Western Pacific Regional Centre for the Promotion of Environmental Planning and Applied Studies (WHO)/PEPAS. According National Drinking Water Quality Standards, the acceptable value and maximum acceptable value of nitrate level in drinking water are 10 mg/L.

2.4 Health Risk Assessment

High levels of nitrates in groundwater may pose a risk to human health. Health risk assessment is a process to determine a harmful effect from human activities (US EPA, 2000). The likelihood of adverse non-carcinogenic health impact can be expressed by using Hazard Index (Sadler et al., 2016). Hazard Index is a dimensionless quantity, and the RfD values that go in its denominator are such that the critical value for HI is unity where if the HI value more than 1 (HI>1) will show a significant risk level, the higher the value, the greater the likelihood of adverse non-carcinogenic health impact (Gerba, 2000). The rfd value that used in this study is 1.6 mg/kg/day (US EPA, 2000).

2.5 Health Effect

Humans can be exposed to nitrate through food and water. In most populations, short-term exposure to even fairly large amounts of nitrate produces no immediate health effects (Ward et al., 2005). However, sensitive populations like babies, people in poor health, and the elderly can be susceptible to problems from short-term nitrate exposure(Fan & Steinberg, 1996). Water containing less than 10mg/L of nitrate is considered safe for all humans and livestock. Water containing between 11 and 20 mg/L of nitrate is generally safe for human adults and all livestock. However, it should not be given to infants. Nitrate levels between 21 and 40 mg/L considered safe for short-term use by adults but for long-term it can affect human health. Water containing more than 40 mg/L of nitrate should not be used for drinking purposes (Mahler, Colter & Hirynck, 2007).

2.5.1 Blue-baby syndrome

Infants younger than six months of age are especially sensitive to nitrate poisoning, which may result in serious illness or death (Sadler et al., 2016). The illness happens when nitrate is converted to nitrite in the baby's body (Mahler, Colter, & Hirnyck, 2007). Nitrite reduces the amount of oxygen in the baby's blood, causing shortness of breath and blueness of the skin or known as a blue baby syndrome (Jalali, 2005). The technical term for this condition is methemoglobinemia. This illness can cause the baby's health to deteriorate rapidly over a period of days (Fan & Steinberg, 1996).

2.5.2 Others Health Effects

The consumption of high level of nitrate not only causes methemoglobinemia in the newborn infants, but also can cause thyroid disorders and birth defects (Moore et al., 2011). Others studies, stated that the nitrate exposure in drinking water increases risks of hypertension (Woolverton, 2015). There is no evidence that nitrate or nitrite causes cancer in laboratory animals or humans. Studies have shown that diets lacking dietary fiber and including foods with high levels of nitrate and nitrite such as smoked meats, may promote stomach cancers. However, studies have not indicated that drinking water high in nitrate is associated with stomach cancer (California Department of Health Services, 2000).

CHAPTER 3

METHODOLOGY

3.1 Introduction

In methodology, the list of the subsections was study design, study location, study population, sampling, data collection method and instrumentation, risk assessment, data analysis and ethical consideration.

3.2 Study Design

The study design for this project was a cross-sectional study in order to determine the nitrate level in the groundwater at the Mukim Salor in Kota Bharu, Kelantan.

3.3 Study Location

The study locations were Mukim Salor which consists of Kampung Salor, Kampung Teluk Chengal and Kampung Jerulong in Kota Bharu, Kelantan. Mostly the houses in these areas were near to agriculture area which is paddy field. It was believed to consist of nitrate in groundwater with regards to usage of inorganic fertilizer for their crops. Thus, these areas were at high risk for nitrate residue to leach down into the groundwater.

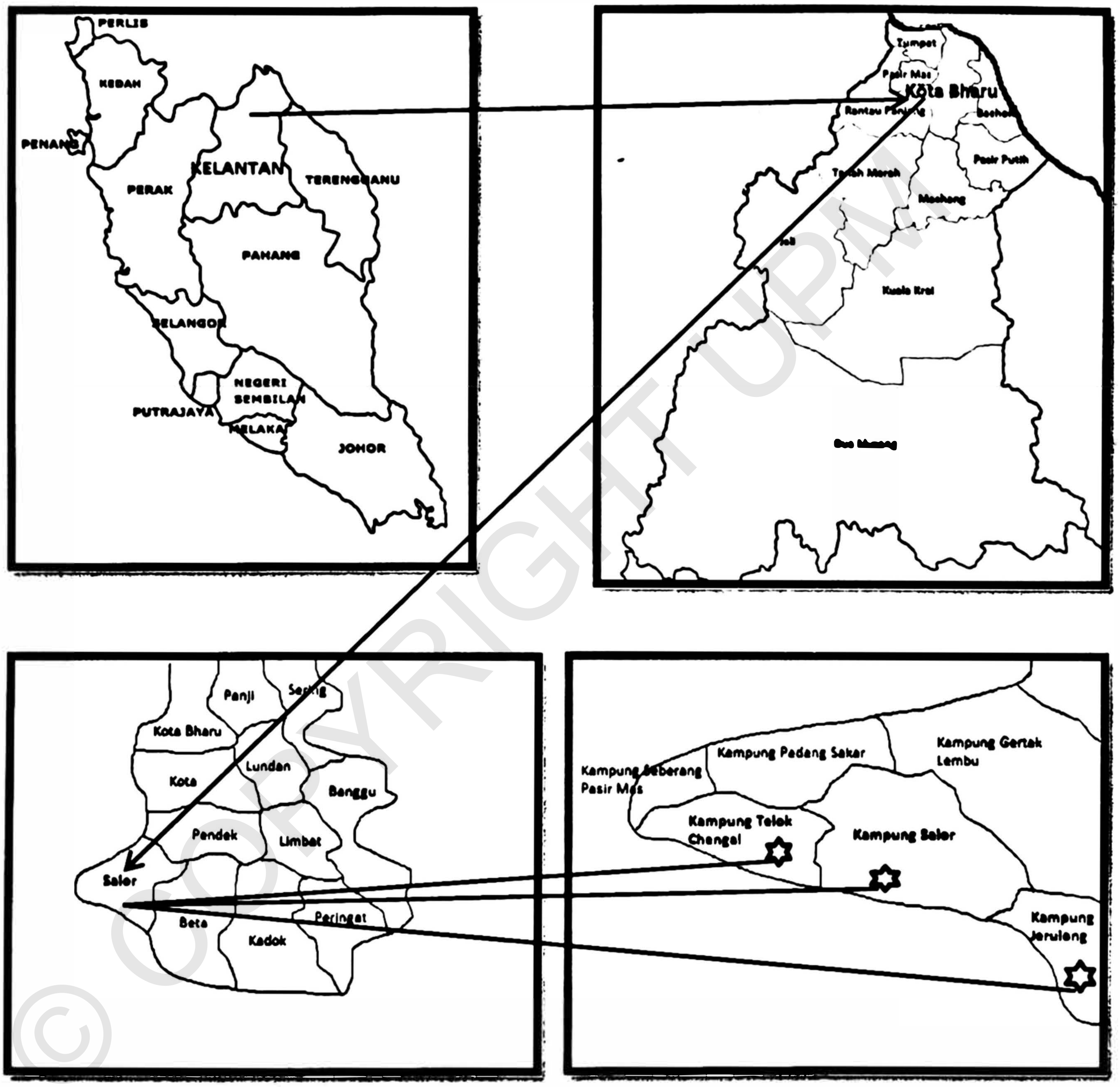

Figure 3.1: Study Location

Research Location: Mukim Salor in Kota Bharu, Kelantan.

3.4 Study Population

The study populations were residents of Mukim Salor who used groundwater as the main source for drinking and another daily usage.

3.5 Sampling

3.5.1 Study sample

The study was conducted on a respondent who meets the inclusion criteria only.

3.5.2 Sample Size

The sample size was calculated using formula (Equation 1) by Kirkwood and Sterne (2003).

$$N = \frac{P(1-P)}{e^2}$$

(Equation 1)

Where,

N= sample size

P=Prevalence

e=Probability error

The previous study by Alif and Shaharuddin (2014), mentioned that the prevalence of nitrate level in groundwater which above than 10 mg/L is 8.7% (0.57). So the sample size calculated as below.

$$N = \frac{0.0875(1 - 0.0875)}{0.05^2} = 32$$

As showed by the count over, the estimation of 32 considered as least sample size required. To ensure the study is statistically significant and taken into consideration damaged data, the sample size was taken is 50 samples.

3.5.3 Sampling method

The sampling method used in this study was purposive sampling. The respondents were selected based on inclusive criteria.

3.5.4 Sampling unit

The sampling unit for this study was residents living in Mukim Salor, who fulfilled the inclusive criteria.

Inclusion criteria:

I. Age 18 years old and above.

II. The respondent lifelong resident Mukim Salor in Kota Bharu, Kelantan.

III. The respondent uses groundwater as his/her main source of water supply for his/her daily usage.

Exclusion criteria:

I. Respondents who use water filtered systems that can treat nitrate in water like ion exchange and reverse osmosis.

3.6 Study Instrumentation And Data Collection

3.6.1 Questionnaire

A set of questionnaire comprised of three sections were administered to each respondent. Part A of the questionnaire contained questions regarding the socio-demographic information, Part B contained questions about the information of daily intake of drinking water and Part C contained questions related to the duration of resident. All the information obtained from the questionnaire was used in health risk assessment which to determine the hazard index of the exposure. This questionnaire was referred from the previuos study conducted by Alif and Shaharuddin (2014).

3.6.2 Body weight

The body weights of the respondents were measured using a Seca Weight scale. The readings were taken three times and then averaged. The unit was in kilogram (kg).

Figure 3.2: Seca weight scale

3.6.3 Drinking water daily intake

The daily intake rate of water among respondents was measured by using a standard cup of 250 ml, and every respondent needs to recall back their water consumption amount in a day based on that standard cup in order to calculate the Chronic Daily Intake (CDI) and Hazard Index (HI).

3.6.4 Water Sampling

3.6.4.1 Water analysis

The water was analyzed by using direct reading spectrophotometer HACH brand DR1900. This meter was used to determine the level of nitrate in groundwater.

Figure 3.3: Spectrophotometer HACH brand DR1900

3.6.4.1.1 Analyse of Nitrate

Nitrate in groundwater was analyzed by using USEPA method 8039 (Hach, 2005). The NitraVer 5 nitrate reagent powder pillow was being used. The step as below:

Prepare blank: Pipet was used to add 10.0 mL of sample to the sample cell.

↓

Prepare sample: Pipet was used to add 10.0 mL of sample to the sample cell.

↓

Stopper was used to shake the cell for one minute.

↓

The instrument timer was started for five minute.

↓

When the timer expired, the blank sample cell was cleaned.

↓

The blank was inserted into the cell holder.

↓

Push ZERO. The displayed showed 0.00 mg/L $NO_3^{-}N$.

↓

The prepared sample cell was cleaned.

↓

The sample prepare was inserted into the cell holder.

↓

Push READ. The result was shown in mg/L $NO_3^{-}N$.

3.7 Risk Assessment (Calculation)

In order to determine the exposure of nitrate in drinking water, chronic daily intake (CDI) was first calculated using the following equation.

$$CDI = \frac{C \times DI}{BW} \quad \text{(Gerba, 2000)}$$

(Equation 1)

Where,

CDI = Chronic Daily Intake (mg/kg/day),

C = Nitrate concentration in water (mg/L),

DI = Average daily intake rate of water (L/day),

BW = Body weight (kg).

Then to conclude the significant different exposure and overall potential for non-carcinogenic health effects posed by nitrate in drinking water, the Hazard Index (HI) were calculated using the following equation:

$$HI = \frac{CDI}{RfD} \quad \text{(Gerba, 2000)}$$

(Equation 2)

Where,

HI = Hazard Index,

CDI = Chronic Daily Intake (mg/kg/day),

RfD = Reference dose (mg/kg/day

HI value more than 1 (HI>1) will show a significant risk level, the higher the value, the greater the likelihood of adverse non-carcinogenic health impact. The rfd value that was used in this study was 1.6 mg/kg/day (US EPA, 2000).

3.8 Data Analysis

Data analysis was performed using the Statistical Program for Social Science software (SPSS for Windows) version 22.

For descriptive data, univariate analysis was used to determine nitrate level, socio-demographic information of respondents, water consumption and results of water analysis in percentage, mean, standard deviation, range, percentiles, maximum and minimum values.

Then, the normality test in this study was performed by using Kolmogorov-Smirnov Test where $p>0.05$ indicated a normally distributed data. The parametric test was used if data is parametric or normal distribution and the non-parametric test was used if the data is not normally distributed.

3.9 Ethical consideration

Ethical issues pertaining to this study was presented and approved by Ethics Committee, Faculty of Medicine and Health Science, Universiti Putra Malaysia.

I. The respondents were given some explanation about the whole of the study activities involved.

II. The respondents were given some explanation about the health risk assessment that was held in term of the purpose of the assessment, the procedure was taken, and also respondents' right in this study.

III. Approval letter was given to the village representative to obtain the consent of the resident involved in this study.

IV. Approval letter was given to the respondents to get their consent to be a participant in this study.

CHAPTER 4

RESULTS AND DATA ANALYSIS

4.1 Background of Respondent

4.1.1 Socio-demographic of Respondent

In this study, the total numbers of respondents who participated were 50 (Table 4.1). Race of all respondents was Malay. Figure 4.1 showed the percentage of participation between genders. It showed that there was a higher percentage of females than males' participation by 54% and 46 %, respectively.

The mean and standard deviation for the age of respondents was 50.28 ± 15.547. The median was 52 years old. Age ranged from 18 to 76 years old.

This study was conducted in three villages which are Kampung Salor, Kampung Telok Chengal, and Kampung Jerulong. Table 4.3 showed the frequency of respondents between villages. It was shown that the participation of respondents from Kampung Salor was 32%, Kampung Telok Chengal was 26% and Kampung Cengal Pulas was 42%(Figure 4.2).

Table 4.1 Number of respondents

Statistics of Respondents		
Valid	50	50
Missing	0	0

Table 4.2 Comparison between male and female respondents

	Frequency	Percent	Cumulative Percent
Male	23	46	46
Female	27	54	100
Total	**50**	**100**	

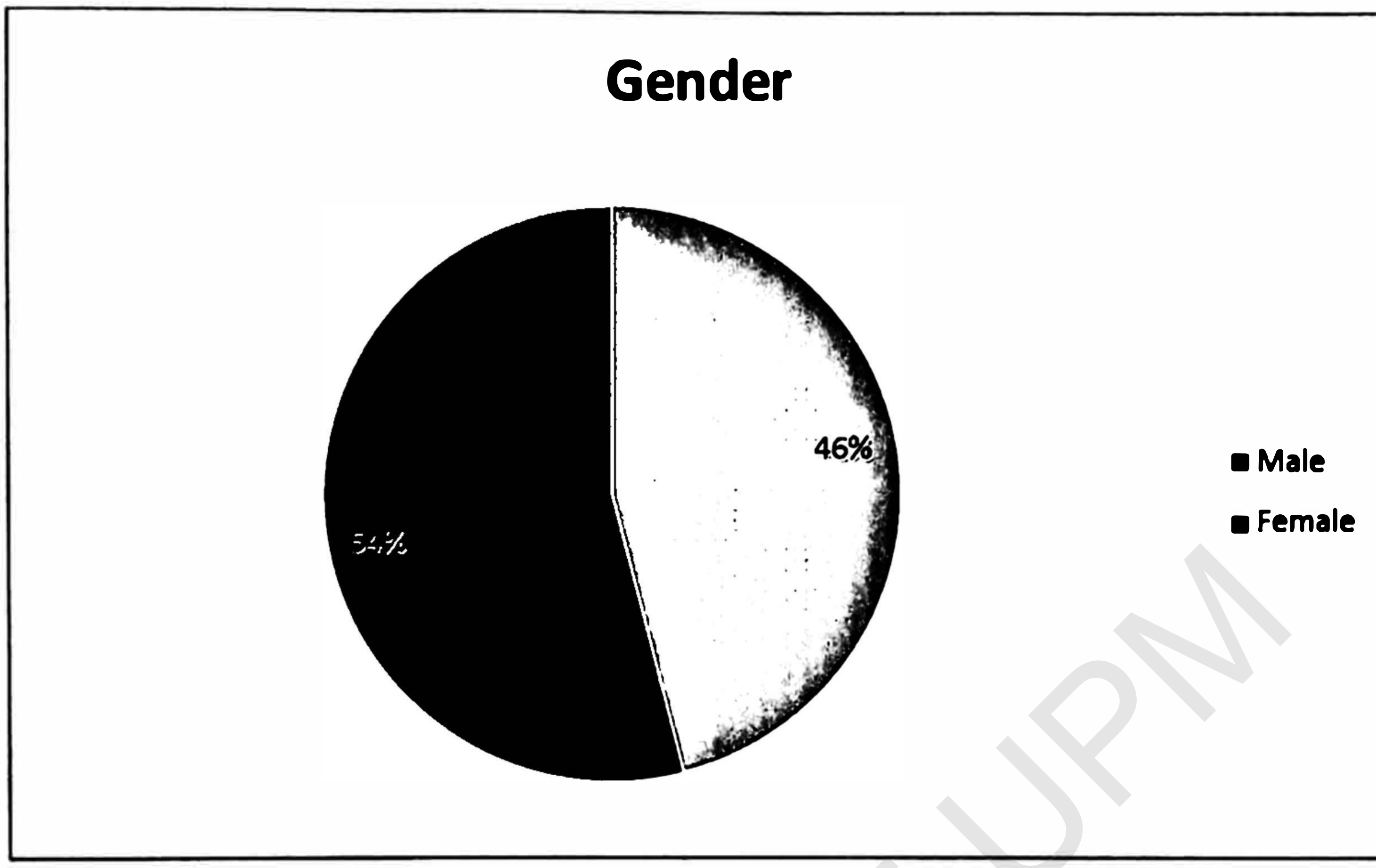

Figure 4.1 Comparison between male and female respondent

Table 4.3 Frequency of respondents between villages

	Frequency	Percent	Cumulative Percent
Kg Salor	16	32	32
Kg Telok Chengal	13	26	58
Kg Jerulong	21	42	100
TOTAL	**50**	**100**	

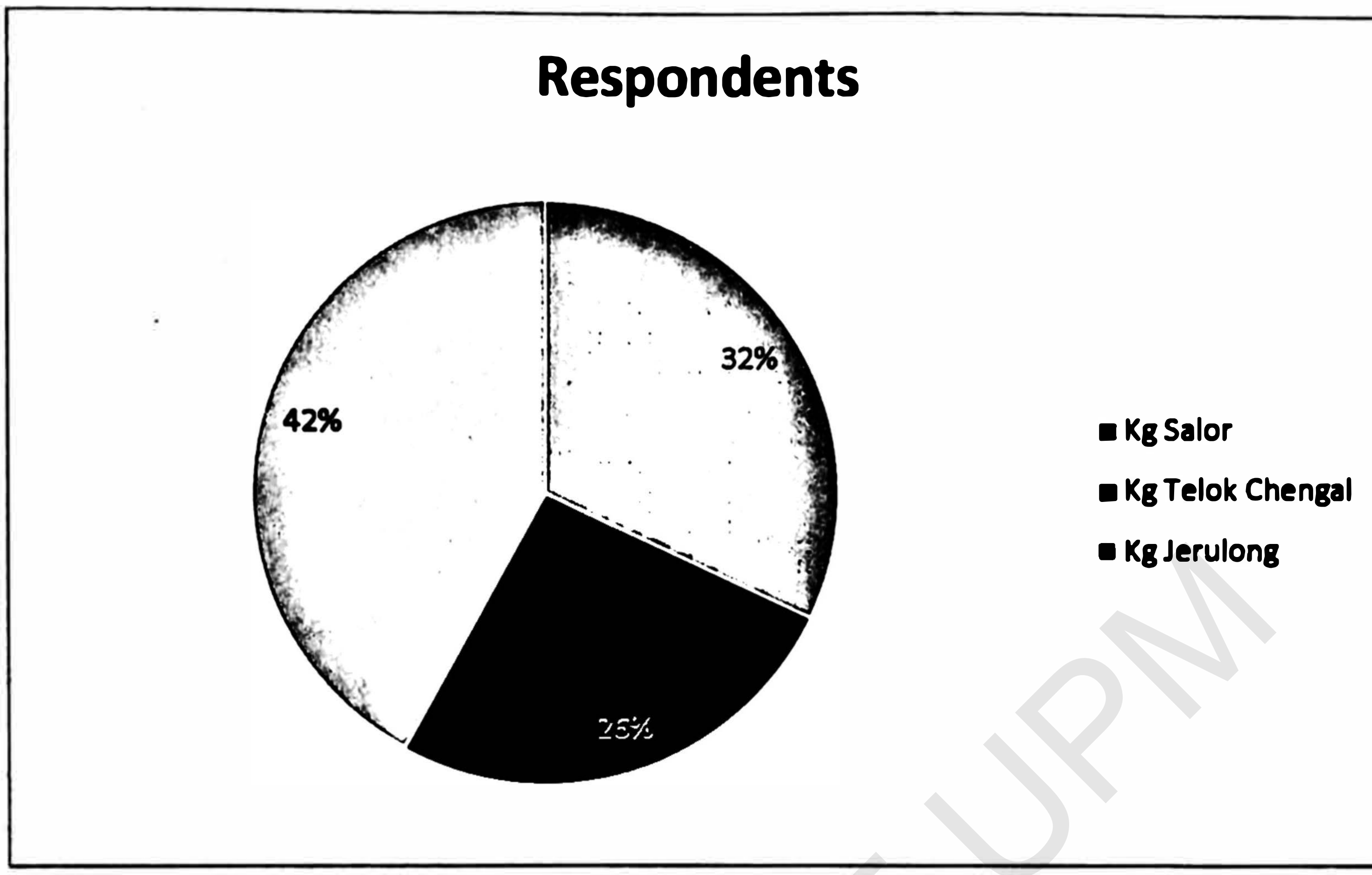

Figure 4.2 Frequency of respondents between villages

Table 4.4 showed the frequency of education level of respondents. Based on the information in Figure 4.3, it was found that most of the respondents only attended secondary school with the percentage of 42% and 8% of respondents were attended university. While, there was only 2% of respondents choose others as their education level, respectively.

Table 4.4 Frequency of education level

	Frequency	Percent	Cumulative Percent
Never Attended School	15	30	30
Primary School	9	18	48
Secondary School	21	42	90
University	4	8	98
Others	1	2	100
TOTAL	**50**	**100**	

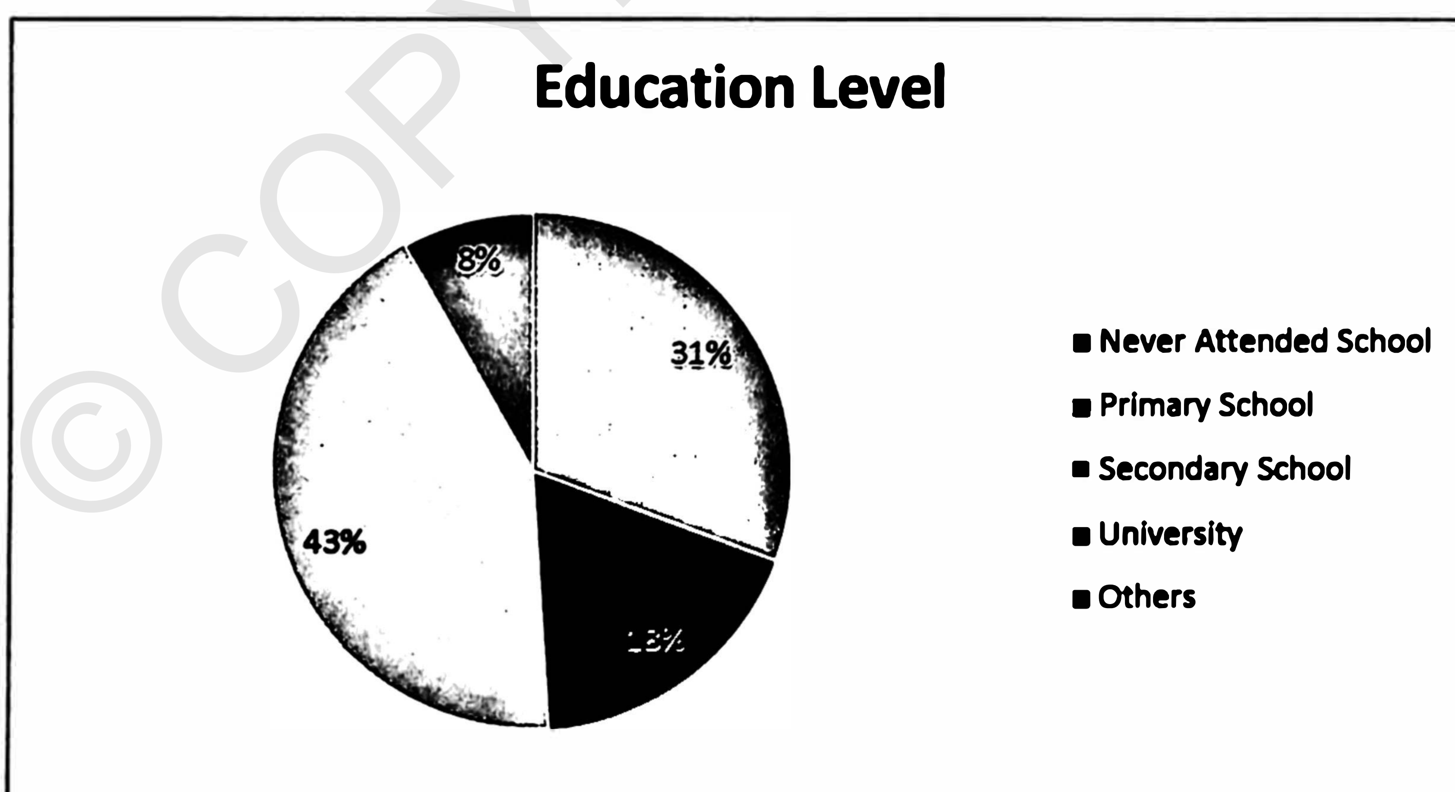

Figure 4.3 Frequency of education level

In this study, the information on the occupation of the respondents was collected and recorded (Table 4.5). Figure 4.4 was indicated that most of the respondents were unemployed with 54%, compared to self-employed and government workers with 42% and 4%, respectively.

Table 4.5 Frequency occupation of respondents

	Frequency	**Percent**	**Cumulative Percent**
Self-employed	21	42	42
Unemployed	27	54	96
Government Workers	2	4	100
TOTAL	**50**	**100**	

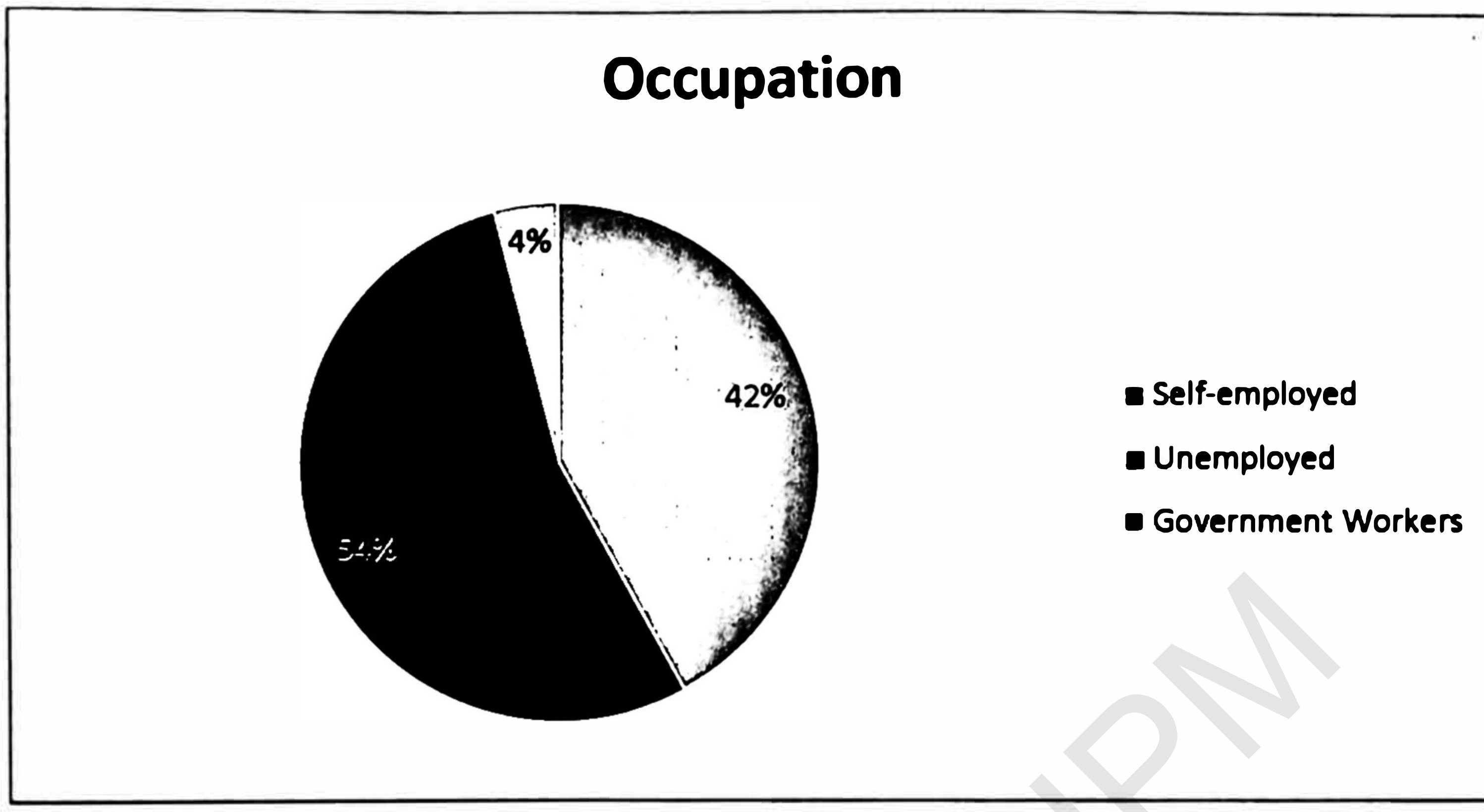

Figure 4.4 Frequency Occupation of respondents

Table 4.6 showed the frequency income of the respondents. Based on the information regarding income of respondents in Figure 4.5, it was found that most of the respondents had a salary between RM500-RM1000 with the percentage of 50%. While there was 34% of respondents had income below than RM300. Whereas 6% for the respondents had income RM300-RM500 and RM1000-RM2000, followed by only 4% of respondents had to salary more than RM2000.

Table 4.6 Frequency incomes of respondents.

	Frequency	Percent	Cumulative Percent
< RM300	17	34	34
RM300-RM500	3	6	40
RM500-RM1000	25	50	90
RM1000-RM2000	3	6	96
> RM2000	2	4	100
TOTAL	**50**	**100**	

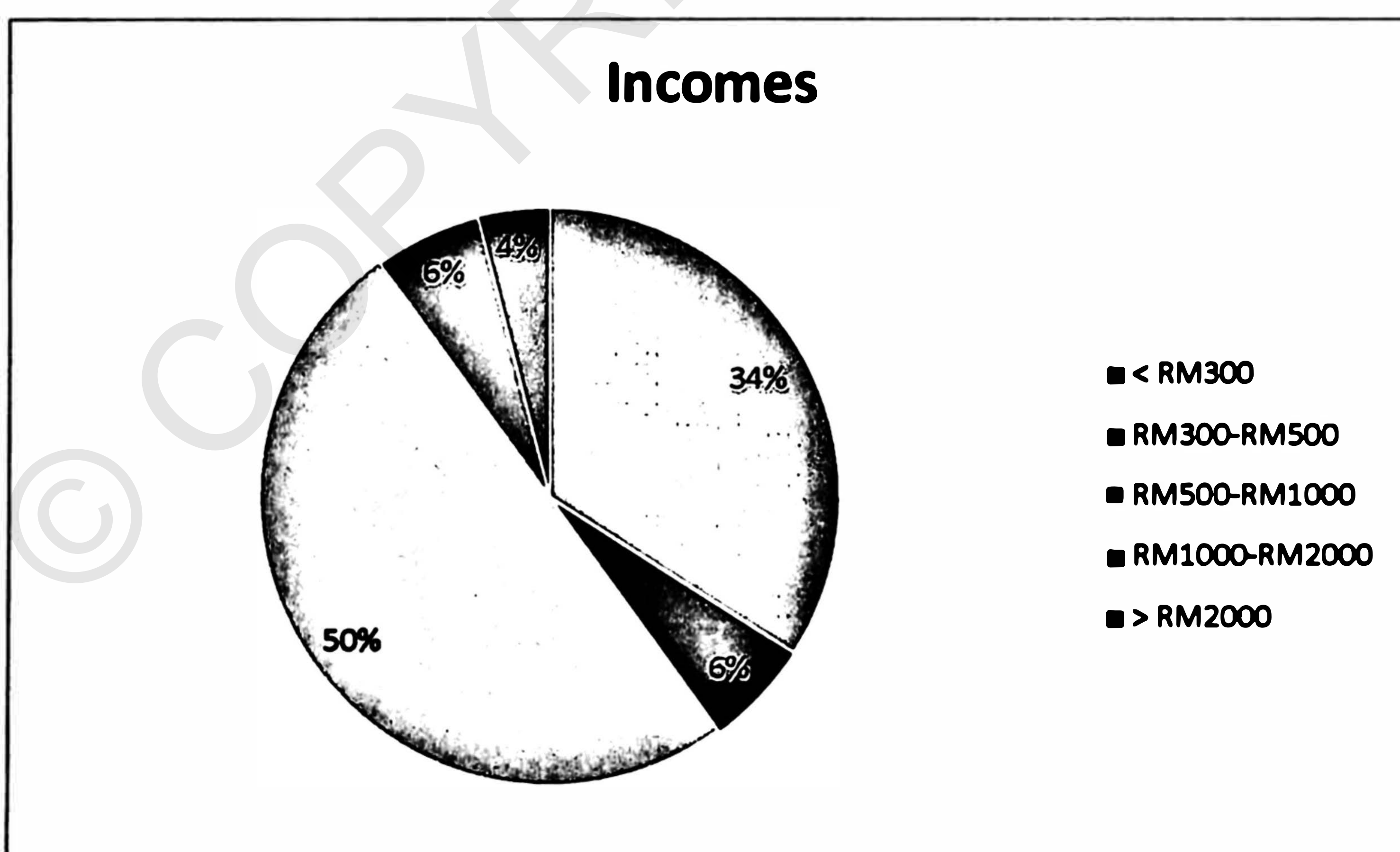

Figure 4.5 Frequency Incomes of Respondents.

4.1.2 Drinking Water Information

Table 4.8 showed information about daily water intake (L/day) and sources of water quality. Based on the questionnaires that were distributed, it was found that about all of the respondents which is 100% used groundwater for drinking and 98% of the respondents used for cooking purposes. While 96% of the respondents said they used groundwater for domestic usage.

For daily water intake, the mean ± S.D (L/day) was 2.00 ± 0.80 and had ranged between 0.75 – 3.75 L/day. From the survey, 68% of the respondents were satisfied with water quality from their wells while only 32% were no satisfied with water quality from their wells.

Table 4.7 Drinking Water Information

Variables	%	mean ± S.D (L/day)	Range (L/day)
Water for drinking			
Yes	98		
No	2		
Water for cooking			
Yes	100		
No	0		

Water for domestic usage			
Yes	96		
No	4		
Daily Water Intake (L)		2.00 ± 0.80	0.75 – 3.75
Water Quality			
Satisfied	68		
Not satisfied	32		

4.2 Nitrate Level in Groundwater

Table 4.8 Nitrate level in groundwater samples

Variables	mean ± S.D (mg/L)	Range (mg/L)
Nitrate	1.08 ± 0.64	0.00 to 2.8 mg/L

N=50

In this study, the mean ± SD of nitrate level was 1.08 ± 0.64 while the range was from 0.00 to 2.8 mg/L. This amount was considered as the low level of nitrate in groundwater.

4.3 Comparison of Nitrate Levels Between Villages (Kruskal-Wallis test)

Table 4.9 Comparison of nitrate level between 3 villages

Variables	No.of samples	Median	X^2	p-value
Kg Salor	16	21.38	1.93	0.38
Kg Telok Chengal	13	26.88		
Kg Jerulong	21	27.79		

In this study, nitrate levels between the three villages were compared using Kruskal-Wallis test. According to p-value (0.38), it indicated that there was no significant difference of nitrate levels between these 3 villages (Table 4.9).

4.4 Comparison of Nitrate Level of Each House With National Drinking Water Quality Standards (NDWQS)

With regards to National Drinking Water Quality Standards, the maximum concentration limit (MCL) for nitrate is (MOH, 2011). Figure 4.6 indicated that nitrate levels from all sampling sites were below 10 mg/L, which is below the maximum limit of National Drinking Water Quality Standards.

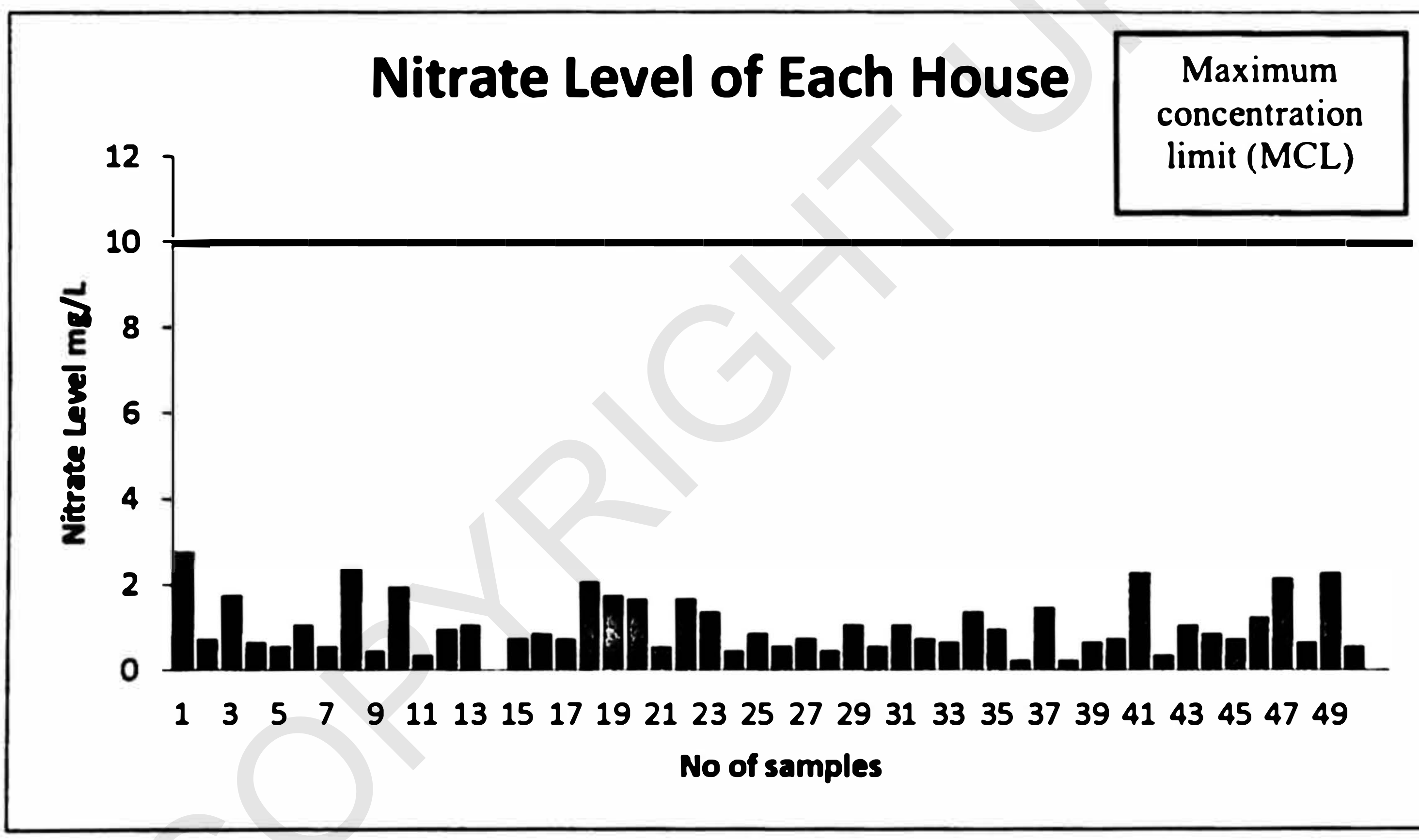

Figure 4.6: Comparison of nitrate level among study locations with national Standard

In this study, the highest reading of nitrate level was 2.8 mg/L. Hence, the results obtained was indicated that the nitrate level did not exceed the maximum concentration limit (MCL) for nitrate which is at 10 mg/L as referred to the to National Drinking Water Quality Standards.

4.5 Health risk assessment information (Chronic Daily Intake (CDI) estimation & Hazard Index)

Based on the table below, the exposure of nitrate on respondent was estimated as Chronic Daily Intake (CDI). From the table, it is shown that the mean for respondent's body weight was 64.02 kg. While the mean for respondent's daily water intake was 2.0 L/day. The CDI value was calculated using an equation as stated in Chapter 3. Table 4.10 shows the estimation for respondent's chronic daily intake was 0.03 mg/kg/day.

Table 4.10 Chronic Daily Intake (CDI) estimation

	Nitrate level (mg/L)	*DI (L/day)	*W (kg)	*CDI (mg/kg/day/
Mean	**1.08**	**2.0**	**64.02**	**0.03**

Hazard Index (HI) was calculated by dividing the CDI value with reference dose (RfD) as shown in Chapter 3. Referring to United State Environmental Protection Agency (USEPA), reference dose (RfD) for nitrate is 1.6 mg/kg. A Hazard Index reading greater than 1 indicates the potential for an adverse effect to occur.

Table 4.11 showed the HI estimated. HI value for all respondents were less than 1 (50 respondents-100%).

Table 4.11 Hazard Index (HI) Estimating Among Respondents

Hazard Index (HI)	No. of Respondent	Percentage (%)
HI<1	50	100
HI>1	0	0

The results showed that HI values for all respondents were less than 1 and this indicated the risk of the adverse effect of nitrate pollution in groundwater in the selective areas was negligible.

CHAPTER 5

DISCUSSIONS, CONCLUSION, STUDY LIMITATIONS AND RECOMMENDATIONS

5.1 Discussions

5.1.1 Socio-Demography Background

This study involved a total of 50 respondents from three villages which are Kampung Salor, Kampung Telok Chengal and Kampung Jerulong. All the respondents were selected based on the inclusive criteria which were 18 years old and above, the respondent lifelong resident Mukim Salor in Kota Bharu and the respondent use groundwater as his/her main source of water supply for his/her daily usage. Respondents who used water filtered system were excluded in this study.

The selection of respondents was done as to control the representativeness of nitrate levels in drinking water but the confounding factors including exposure to nitrate from other sources were not controlled.

5.1.2 Nitrate Level in Groundwater

From the data analysis, the mean ± SD of nitrate level was 1.08 ± 0.64 while the range was from 0.00 to 2.8 mg/L. This amount was considered as the low level of nitrate in groundwater. The study related to nitrate in groundwater was done at Pasir

Puteh by Aliff and Shahruddin (2014), show that the nitrate level in Pasir Puteh also was at a low level which the ranged was between 0.20 to 5.20 mg/L. There are some potential sources of nitrate which include fertilizer, animal waste, and mineralization of soil organic nitrogen like in plant residues, bacterial biomass, and soil constituents (Ghafari, Hasan, & Aroua, 2008). But, in this study it more focusing on the use of fertilizer. It was found in the present study that the leaching of NO_3 from agriculture areas has become an important environmental issue because high NO_3 in water supplies can cause ecological damage and health hazards (Jalali, 2005).

Whereas, the low level of nitrate obtained in this study could possibly cause by the rainy season. The nitrate sampling was done after the rainy season. The was some factors that can influence the level of nitrate in groundwater for example rainfall, temperature and wind stress (Xu et al., 2013). According to CCME (2007), the level of nitrate in the groundwater at a high rate during the beginning of rainy season and it will decrease throughout the rainy season and remain constant at the low level during the dry season. In others studies conducted in Kansas U.S by Perry, Robbins, & Barnes (1988), the increase of rainfall intensity has a clear positive correlation with leaching loss, such that an increase in rainfall intensity results in a parallel increasing rate of leaching loss thus, nitrate level in groundwater increased. According to the nitrogen migration characteristics and cumulative leaching loss amount, it can be concluded that the nitrogen leaching mainly takes place at the first stage of rainfall (Xu et al., 2013).

Another factor that may lead to low level of nitrate in this study was sampling time. The water sampling was done after the paddy rice had been harvested and there is no fertilizer had been applied.

5.1.3 Comparison of Nitrate Levels Between Villages (Kruskal-Wallis test)

From the data analysis performed by using Kruskal-Wallis test, the results obtained showed that there was no significant difference of nitrate levels between these three villages. (p-value 0.64).

One of the factors that lead to this situation may possibly cause the quantity or rate of fertilizer application during the previous season to crop. Excessive use of fertilizer will cause accumulation of nitrate in groundwater (Son et al., 2009). Nitrate pollution of groundwater became the potentially prominent threat to the health of the local population (Zhu, 2002). Other factors may cause by the same type and quantity of fertilizer was used in agriculture area in Mukim Salor. The use of urea fertilizer as the main source of nitrogen (N) in the initial stage is aimed to cultivating lush vegetative growth and to produce 1000 kg result, as much as 15-20 kg of N required by rice plants (AgroStreet, 2015). Fertilizer management practices like rate, source, and timing have highly influenced the level of nitrate in groundwater (Rajmohan & Elango, 2005).

5.1.4 Comparison of Nitrate Level of Each House With National Standard

Based on figure 4.6, the highest reading of nitrate level was 2.8 mg/L. Hence, the results obtained was indicated that the nitrate level did not exceed the maximum concentration limit (MCL) for nitrate which is at 10mg/L as referred to the Malaysian National Standard of Drinking Water Standard (NSDWQ,2000). The present study by Alif and Shaharuddin (2014) also reported similar trend which showed that all nitrate level of the study areas did not exceed 10mg/L or the permissible World Health Organisation (WHO, 2008) value. For the study was conducted in Semarang, Indonesia the nitrate levels in drinking water wells ranged from 0.01 to 84 mg/L with only 2 values in excess of the WHO guideline value of 50 mg/L (Sadler et al., 2016). Other studies by Jalali (2005) found that only 16% of water samples in Hamadan, Iran were classified as having a low risk to human health or environment. In general, groundwater beneath areas of intensive agriculture in the Hamadan area can be classified mostly as a medium to high in NO^3 content (Jalali, 2005).

It was similar to a study by Rajmohan and Elango (2005) in Tamil Nadu, India indicated that nitrate levels of groundwater were different among sampling locations and types of soil. In the previous study conducted by Xu et al., (2013) in China stated that nitrogen losses through leaching vary across a field due to differences in soil physical properties and nitrogen status of the soil.

Others factors that affect the nitrate level is large amounts of N-fertilizer and poorly managed irrigated systems may lead to nitrogen oxides (NO) leaching and pollution of groundwater (Sadler et al., 2016). Other study states that the nearer the wells to septic tank the high level of nitrate in the well (Azwan et al., 2010).

As stated at the above, the maximum level of nitrate in this study was 2.8 mg/L and considered safe for drinking and cooking purpose.

5.1.5 Health risk assessment information (Hazard Index)

The results showed that HI values for all respondents were less than 1 and this indicated the risk of an adverse effect of nitrate pollution in groundwater in the selective areas was negligible. A Hazard Index reading greater than 1 indicates the potential for an adverse effect to occur (NJDEP, 2007).

A previous study conducted in Hambota area, shown that there was a potential health risk due to exposure to drinking water contaminants of fluoride and nitrate (Sciences, 2014). Studies of the related contribution of foods and drinking water to the daily intake of nitrate have shown that the food contribution can be greater than that of drinking water (Himmi et al., 2009) and high intake of Asian vegetables alone can exceed the recommended daily intake for nitrate (Ziarati, 2012). Others study also shown health risk indicators such as chronic daily intake (CDI) and health risk index (HRI) has been recorded as less than 1, indicating no health risk to the local people in Pakhtunkhwa, Pakistan due to water consumption (Khan et al., 2013). While, the highest HQ or HI values which greter than 1 were observed at Angunukolapellassa area due to the consumption of contaminated groundwater at Angunukolapellassa area (Sciences, 2014).

5.2 Conclusion

This study was conducted to determine the effect of nitrate exposure in groundwater that was associated with health risk. Water is of a great significance for people, since it is used for drinking and in food. As a human, we must receive a certain amount of water every day. Otherwise, if does not obtain the required amount of water for several days, we will die. It is obvious the water that we consumed should be clean and pure. Water that contains any harmful bacteria or chemicals may cause various diseases. So, the contamination of water from nitrate or any other contaminants should be avoided as it.

In this study, the nitrate levels ranged was from 0.00 to 2.80 mg/L. The highest reading of nitrate level was 2.80 mg/L and its safe because not exceed the level of nitrate which can cause adverse health effect is 10 mg/L. Hence, groundwater in these three villages is considered safe for drinking and cooking purposes. In addition, Hazard Index (HI) in this study was below 1 which indicates no adverse effect due to nitrate exposure.

However, everyone should be concerned about the nitrate level because from day to day the use of fertilizer has increased in order to maximize the production of crops.

3.4 Study Limitations

i. The information given from respondents could not be determined to be 100 % valid in term of this limitation we assumed the answer and the information given met validity purposes. Recall bias may contribute to this study.

ii. This study was a cross-sectional study that measures nitrate for a specific period only. The data which is collected in this study might be insufficient to support the argument on ground water contamination in Kota Bharu.

5.3 Recommendation

This study was a cross-sectional study. The data was collected at one point in time only and thus it cannot conclude the causal relationship between the variables. Further studies are recommended where the data can be collected at the different point in time.

The results obtained may not represent the whole district or state since this study only focuses on three villages. The data also cannot be generalized to the entire Mukim Salor. Thus, an additional investigation should be conducted within a wider population and larger sample size only that the results of the study can be derived to the population.

As a recommendation, people who live near to the agriculture area should use a water filtered system that can trap and remove nitrate in water. Other than that, the local authorities should perform their role in monitoring the level of nitrate and others. The level of nitrate must be monitored from time to time especially at the risk areas such as location nearby the agriculture areas. Besides, local authorities also should undertake proactive and precaution steps so that the contamination of nitrate in groundwater can be prevented.

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APPENDIX 1: ETHICAL COMMITTEE CONSENT LETTER

ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS
(JKEUPM)
UNIVERSITI PUTRA MALAYSIA

Research title	:	**Health Risk Associated With Nitrate Exposure In Groundwater At Mukim Salor In Kota Bharu, Kelantan**
Study Site	:	**Kota Bharu, Kelantan**
JKEUPM Ref No.	:	**FPSK(EXP16-OSH)U025**
Researcher	:	**Che Mohamad Farizuan Bin Che Ismail**
Supervisor	:	**Dr. Shaharuddin Bin Mohd Sham**

Documents received and reviewed with reference to the above study:

1. Ethics Application Form. Version 1 dated 18/10/2016
2. Respondent Information Sheet & Consent (English) Version 1 dated 18 10/2016
3. Proposal (English). Version 1 dated 18/11/2016
4. Questionnaire (Malay), Version 1 dated 18/10/2016
5. Curriculum Vitae of:
   a. Dr. Shaharuddin Bin Mohd Sham

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.

Decision by JKEUPM:

[X] Approved

[X] **Permission MUST BE OBTAINED from the respective hospitals/ institutions before conducting the research**

[ ] Disapproved

Please note that the approval is valid until 29 November 2017

Researchers should comply with the following:

I. Complete a Study Final Report upon study completion (Form D).

II. Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

APPENDIX 2: INFORMATION SHEET

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA,' 43400 UPM SERDANG, SELANGOR, MALAYSIA.

FORM B1: RESPONDENT'S INFORMATION SHEET AND CONSENT

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. **STUDY TITLE**

   Health risk associated with nitrate exposure in groundwater at Mukim Salor in Kota Bharu, Kelantan.

2. **INTRODUCTION**

   Nitrate (NO^{3-}) is one of the compounds of nitrogen. It exists together with different types of nitrogen in a complex cycle. Nitrogen in soil and water usually come from atmospheric deposition, uses of compost, fertilizer, waste material, dead plant and animal tissue. Excessive use of fertilizer and improper management of other nitrogen sources can increase the rate of movement and the magnitude of groundwater contamination. This study is to determine levels of nitrate in groundwater and health risk among respondents.

3. **WHAT WILL YOU HAVE TO DO?**

   The respondents will be asked to complete the questionnaire. The questionnaire need to be filled with information regarding the socio-demographic information, information of daily intake of drinking water, duration of residence and health information. Then, researcher will measure the level of nitrate in your groundwater by using the YSI multimeter. Researcher also will take a weight measurement of the respondents to calculate the risk assessment.

4. **WHO SHOULD NOT PARTICIPATE IN THE STUDY?**

   Respondents are not selected based on these criteria:

   i. Respondents who use water filtered systems.
   ii. Respondents who use other than groundwater as their main water supply for their daily needs

5. **WHAT WILL BE THE BENEFITS OF THE STUDY:**

   (a) TO YOU AS THE SUBJECT?

   Respondents will determine the level of nitrate in their groundwater.

   (b) TO THE INVESTIGATOR?

   The researcher hope that the finding of this study will provide evidence based data on the association of health risk with nitrate exposure in groundwater.

6. **WHAT ARE THE POSSIBLE RISKS?**

   There is no possible risk if take part in this study.

9. CONSENT

I .... Identity Card No. .... address...
....hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ....
(Respondent)

Date :..

Signature ....
(Witness)

Name :..

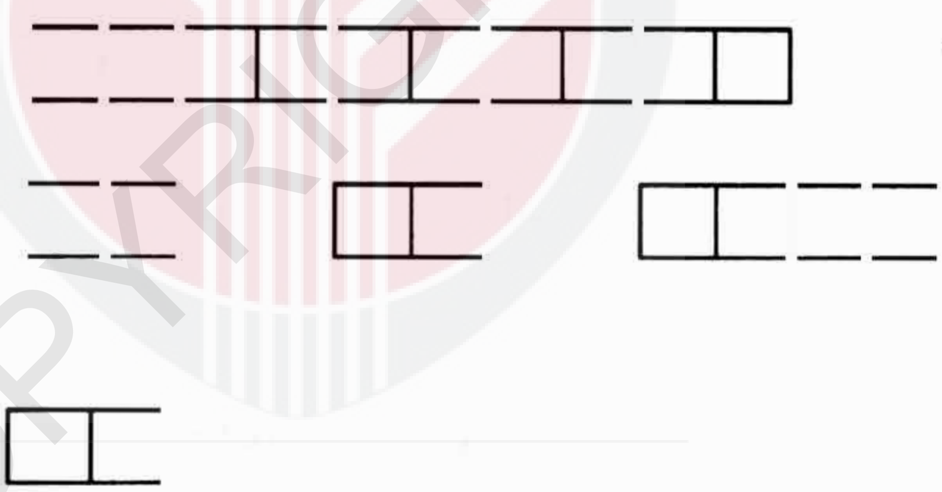
I/C No. :..

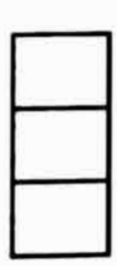
I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ....

Signature ....
(Researcher)

APPENDIX 3: QUESTIONNAIRE (BAHASA MELAYU VERSION)





☐ Universiti

☐ Lain-lain

10. Berat : ....kg

11. Tinggi : ....cm

Bahagian B: Maklumat penggunaan air paip

1. Apakah punca air paip di rumah?

   ☐ Jabatan Air Negeri Kelantan

   ☐ Telaga

   ☐ Lain-lain, sila nyatakan ....

2. Berapa gelas air yang anda minum setiap hari?

   .... gelas (200 ml)

3. Penggunaan air dari dapur:

   ☐ Memasak

   ☐ Minum

   ☐ Kegunaan domestik

   ☐ Lain-lain, sila nyatakan ....

4. Adakah anda berpuas hati dengan kualiti air paip yang digunakan?

   ☐ Ya ☐ Tidak ☐ Tidak pasti

5. Adakah anda menggunakan sistem penapisan air persendirian di rumah?

   ☐ Ya ☐ Tidak

   Jika ya, sila nyatakan jenama yang digunakan: ....

Bahagian C: Maklumat persekitaran tempat tinggal

6. Adakah terdapat kawasan perindustrian/ pertanian berdekatan dengan tempat tinggal anda?

☐ Ya ☐ Tidak ☐ Tidak pasti

7. Apakah jenis pempaipan air di rumah?

☐ Logam

☐ PVC

☐ Tidak pasti

8. Bilakah rumah ini dibina?

☐ 10-15 Tahun

☐ 16-20 Tahun

☐ > 20 Tahun

TERIMA KASIH ATAS KERJASAMA ANDA

-TAMAT-