UNIVERSITI PUTRA MALAYSIA

HEALTH RISK ASSOCIATED WITH NITRATE EXPOSURE IN GROUNDWATER AT MUKIM TELIPOT IN PASIR PUTEH, KELANTAN

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BY

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ABSTRACT

HEALTH RISK ASSOCIATED WITH NITRATE EXPOSURE IN GROUNDWATER AT MUKIM TELIPOT IN PASIR PUTEH, KELANTAN

RAJA MUHAMAD NASHRIQ

Introduction: Agriculture activities in Malaysia have increased from time to time. Modern agriculture has been practiced by farmers to increase their productivity. Nitrogen fertilizer is used mostly by the farmers in rural areas. Nitrates are known to be essential components for making protein in plants. High nitrate levels in water can cause methemoglobinemia or blue baby syndrome, a condition found especially in infants under six months and in the long-term, could be potentially carcinogenic to humans. **Objectives**: The objective is to determine levels of nitrate in groundwater and to perform health risk assessment from nitrate exposure among respondents in Mukim Telipot, Pasir Puteh, Kelantan. **Methodology**: This study has be conducted among residence at Mukim Telipot. Fifty respondents have been choosen based on the inclusive criteria and they were interviewed. Underground water sample was taken in each of the respondent's house. The water sample was analyzed by using YSI multimeter and a set questionnaire was answered by each respondent for health risk assessment of the exposure. **Results and Discussion**: For the outcome, the nitrate concentration in groundwater at Mukim Telipot 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 Telipot was considered safe for drinking and cooking purpose.

Keywords: Nitrate level, Underground water, Water contamination, Potentially carcinogenic, Health risk assessment.

ABSTRAK

HEALTH RISK ASSOCIATED WITH NITRATE EXPOSURE IN GROUNDWATER AT MUKIM TELIPOT IN PASIR PUTEH, KELANTAN

RAJA MUHAMAD NASHRIQ

Pendahuluan: Aktiviti pertanian di Malaysia telah meningkat dari semasa ke semasa. Pertanian moden telah diamalkan oleh para petani untuk meningkatkan produktiviti mereka. Pertanian moden bermakna perladangan intensif atau pertanian intensif di dalam sektor pertanian untuk meningkatkan pengeluaran. Baja nitrogen digunakan kebanyakannya oleh petani di kawasan luar bandar. Nitrat dikenali sebagai komponen penting untuk membuat protein dalam tumbuhan. Tahap nitrat yang tinggi dalam air boleh menyebabkan methemoglobinemia atau 'blue baby syndrome', satu penyakit terutama pada bayi di bawah enam bulan dan dalam jangka masa panjang boleh berpotensi menjadi karsinogen kepada manusia. **Objektif**: Objektif kajian adalah untuk menentukan tahap nitrat dalam air bawah tanah dan melaksanakan penilaian risiko kesihatan daripada pendedahan nitrat di kalangan responden di Mukim Telipot, Pasir Puteh, Kelantan. **Kaedah**: Kaedah kajian ini telah dijalankan di kalangan penduduk di Mukim Telipot. 50 responden telah dipilih berdasarkan kriteria yang inklusif dan mereka telah ditemuramah. Sampel air bawah tanah telah diambil di setiap rumah responden. Sampel air dianalisis dengan menggunakan YSI multimeter dan soal selidik telah dijawab oleh setiap responden untuk penilaian risiko kesihatan pendedahan. **Keputusan dan Perbincangan**: Keputusuan kajian adalah keseluruhan kepekatan nitrat dalam air bawah tanah di Mukim Telipot tidak melebihi standard dan tidak ada risiko daripada pencemaran nitrat dalam air bawah tanah manakala HI adalah kurang daripada 1. **Kesimpulan**: Air bawah tanah di Mukim Telipot adalah dianggap selamat untuk tujuan minuman dan masakan.

Kata kunci: Tahap nitrat, Air bawah tanah, Pencemaran air, Yang berpotensi karsinogen, Penilaian risiko kesihatan.

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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

Nitrates are inorganic compounds made up of nitrogen and oxygen, NO_3 (one nitrogen and three oxygen molecules). These compounds combine with other elements like sodium and potassium to make sodium nitrate or potassium nitrate, which are used in fertilizer industries.

Although Malaysia is a newly industrialised country, agriculture is still an important aspect of its economy. In order to increase productivity, farmers use fertilizers for their crops . This kind of activity involves high use of inputs such as capital, labour and heavy use of pesticides, herbicides, insecticides and chemical fertilizers relative to land area.

In a provincial zone, the majority of the farmers utilized nitrogen compost as a part of their farming. These composts contain nitrates. Nitrates really are key segments for making protein in plants..

As a result, plants grown on nitrogen fertilizer are healthier and yields are better and bigger. However, exposure to nitrate results in multiple health effects, and it particularly affects human health. Groundwater contamination is frequently associated with agricultural activities such paddy plant.

Nowadays, the well is still mostly used for the drinking water by the villagers in Kelantan. The groundwater may be contaminated when the fertilizer spread in the soil and enter the well. The drinking water is no longer safe to consume because the water that contains nitrate can cause adverse effects. Nitrate could affect human health when its level exceeds 45 mg /L of NO_3 (US EPA, 1991).

One of the Nitrate's health effects is shortness of breath and blue baby syndrome (US EPA, 1991). This happens when the high level of nitrate are found in drinking water.

1.2 Problem Statement

Sufficient water supply of appropriate quality is a key ingredient in the health and well-being of humans and ecosystems and for social and economic development (Water, U. N. 2014). It is well known that agriculture is the single largest user of freshwater resources, using a global average of 70% of all surface water supplies. Except for water lost through evapotranspiration, agricultural water is recycled back to surface water and/or groundwater. Currently, the degradation of the quality of water supply has risen due to anthropogenic factors including agricultural practices and industrial activities (Mohamed Azwan et al., 2010).

Although Malaysia is a developed country but agriculture is an important sector of the Malaysian economy in which there has been a substantial development in the cultivation of rubber, oil palm, cocoa, fruits, and vegetables. The developments of agriculture activities have also resulted in increased use of commercial fertilizers (Phosphate fertilizer, urea, and NPK fertilizer).

Moreover, nitrate is one of the most common contaminants in groundwater that originate from either fertilizer or sewage. Problems arise when the level of nitrate in well water is not safe for drinking and cooking purposes. Some parts of rural Kelantan, especially in Pasir Puteh, rely on the rural water supply system, in which some residents are still using groundwater as the main water source.

1.3 Study Justification

This study was carried out to determine nitrate concentration level in groundwater at Mukim Telipot which are Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas in Pasir Puteh, Kelantan. The main economic activity in Pasir Puteh is agriculture for example paddy, tobacco plant and vegetables. The main crop is paddy. Usually, they use urea and NPK fertilizer to support plant growth. NPK stands for nitrogen(N), phosphorous(P) and potassium(K).

The drinking water of people in Kelantan was mostly from groundwater. Groundwater also plays a very important role in public water supply system. Approximately 70% of total water supply in the state including Pasir Puteh was derived from groundwater (Mohamed Azwan et al., 2010).

In Pasir Puteh, most of the paddy fields were near villager's home. Therefore, nitrate in well water may exist due to the location of the well which is near the paddy fields thus obtaining data on the nitrate concentration is essential for the further plan to overcome this problem because it is can give great effect to human health.

1.4 Conceptual Framework

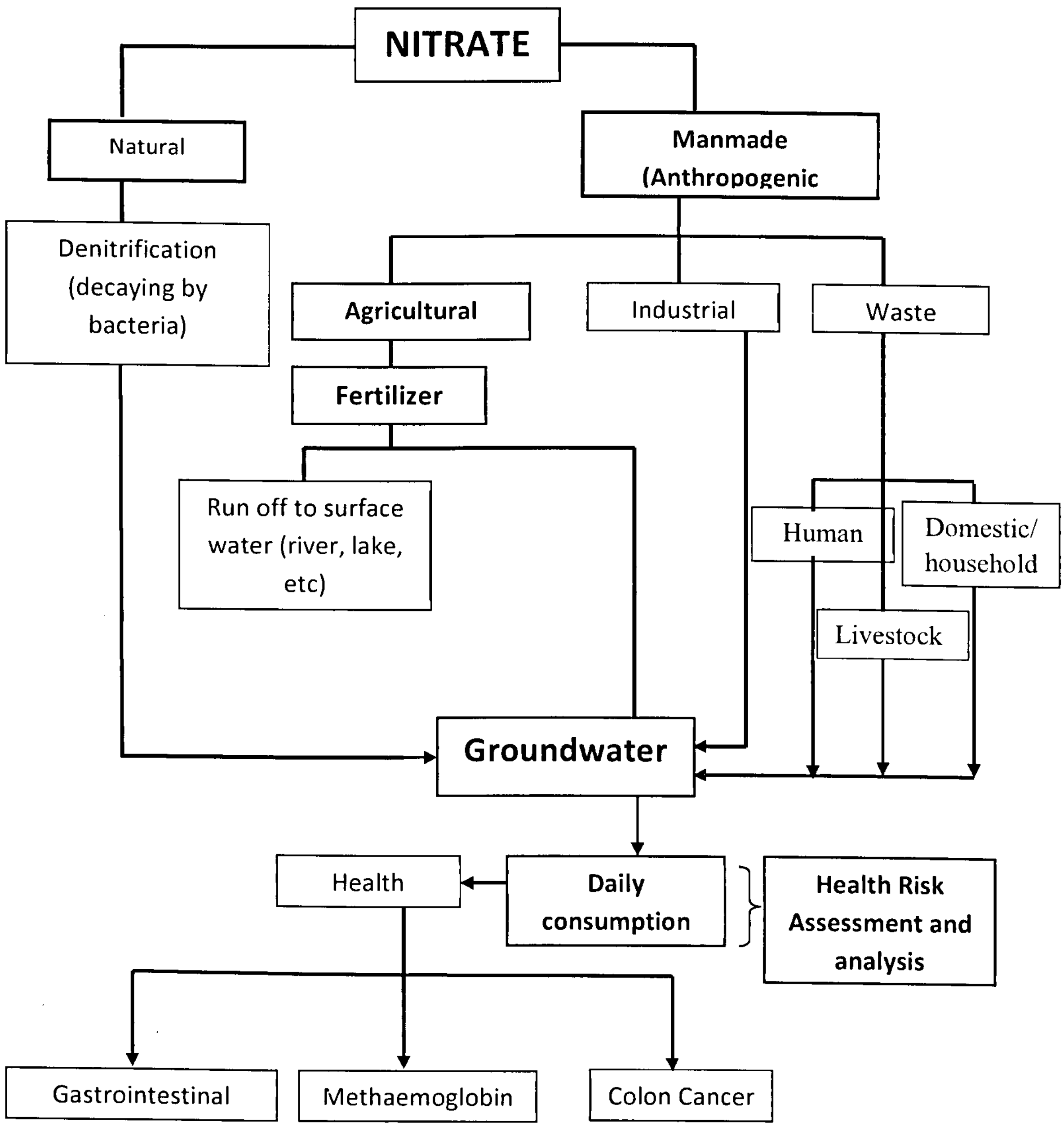


Figure 1.1: Conceptual framework (Moore et al., 2011; Rosen et al., 2006)

1.5 Definition

1.5.1 Conceptual Definition

1.5.1.1 Drinking Water

Drinking water or potable water is water pure enough to be consumed or used with low risk of immediate or long-term harm. The drinking water quality is a powerful environmental determinant of health (WHO, 2010).

1.5.1.2 Nitrate Level

According Interim National Standard for Drinking Water Quality for Malaysia (NSDWQ), the maximum allowable nitrate concentration in drinking water is 45 mg/L.

1.5.1.3 Health Risk Assessment

Health risk assessment is the process of quantifying the probability of a harmful effect to individuals or populations from certain human activities. The risk assessment process is typically described as consisting of five basic steps (California Environmental Protection Agency's OEHHA, 2001):

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 are collected direct from each respondent's groundwater source. Then, the water is poured into pre-clean 250ml high density polyethylene (HDPE) bottles.

1.5.2.2 Nitrate level

Nitrate in groundwater samples were analysed by YSI Professional Plus multimeter.

1.5.2.3 Health Risk Assessment

According to US EPA (1992), estimation of daily exposure of individuals for nitrate through ingestion is by calculating the chronic daily intake (CDI) as the exposure metric. Then, to estimate the non-carcinogenic risk of nitrate, the hazard index (HI) is calculated using the equation in Chapter 3.

1.6 Objective

1.6.1 General Objective

To determine the levels of nitrate in groundwater and relate to health risk assessment among respondents in Mukim Telipot in Pasir Puteh, Kelantan

1.6.2 Specific Objective

1. To determine the socio-demography of residence at Mukim Telipot.

2. To determine nitrates level in groundwater

3. To compare nitrates level of groundwater with National Standard for Drinking Water Quality among each house.

4. To compare nitrates level between National Standard for Drinking Water Quality among the three villages.

5. To determine the Hazard Index from exposure to nitrate.

1.7 Research Hypothesis

1. Nitrate concentration in groundwater in Pasir Puteh is higher than the National Standard for Drinking Water Quality, Malaysia.

2. There is a significant difference of nitrate level in groundwater among each study location.

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

4. HI of the respondents >1.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrates in Environment

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO_3) is the stable form of combined nitrogen for oxygenated systems. Although chemically unreactive, it can be reduced by microbial action. The nitrite ion (NO_2) contains nitrogen in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR Life Systems, Inc., 1987).

Nitrite oxidizes easily into nitrate. Thus, Nitrate is more frequently found in groundwater and surface water. Nitrate-containing compounds in the soil are generally soluble, which means they dissolve easily in water. Nitrates thus flow easily into groundwater. Nitrates and nitrites both occur in soil and water. Microbes breakdown animal and human organic wastes in soil and water. This breakdown process converts wastes into ammonia, which then oxidizes into nitrite and nitrate. 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 collects from both common and anthropogenic sources. Nitrates happen in soil and water (ATSDR, 2011).

2.2.1 Nitrogen Cycle

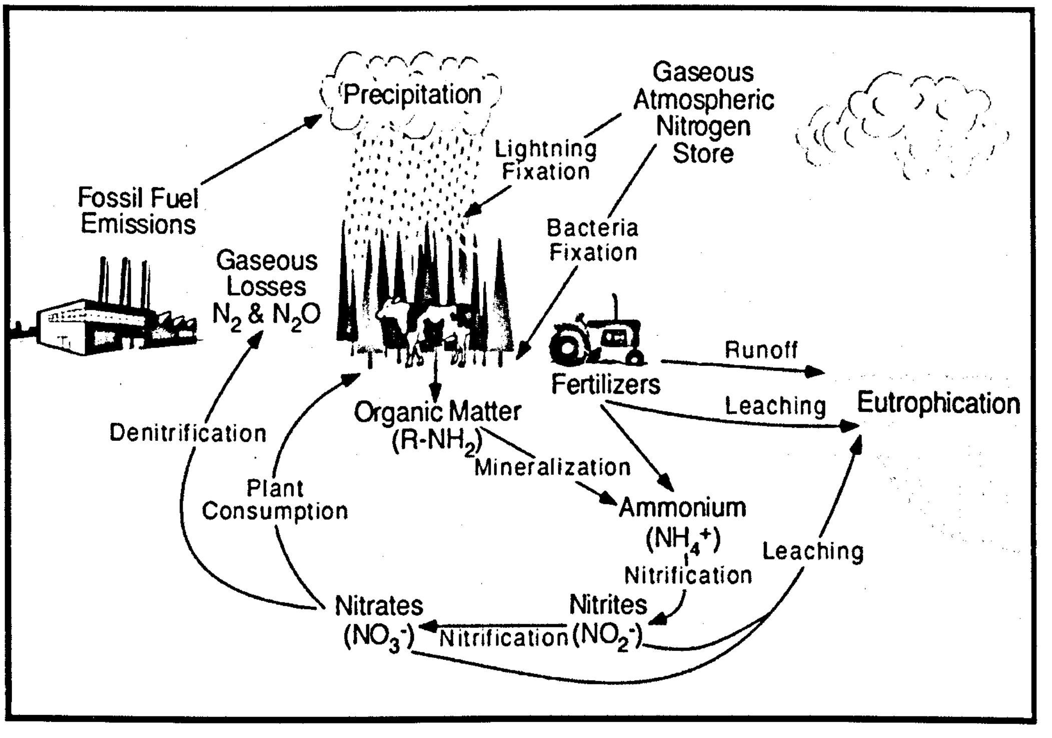


Figure 2.1 : The process of nitrogen cycle (Pidwirny,2006)

Nitrogen is an important component of DNA, RNA and protein; as the building blocks of life (Harrison, 2003). All organisms that needs nitrogen (Fields, 2004). Nitrogen represents about 78% of the atmosphere which is usually unavailable for organisms using because of the strong triple bond between the N atoms that are bound tightly in pairs as N_2. This N_2 molecule is relatively inert or unreactive (Harrison, 2003).

The movement of nitrogen in atmosphere, biosphere and geosphere is known as nitrogen cycle process (Harrison, 2003). 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.

2.2.2 Nitrogen cycle processes

2.2.2.1 Nitrogen fixation

In nitrogen fixation, the process involved is the conversion of N_2 to ammonium (NH_4^+). N_2 is a very stable compound due to the triple bond and it needs a large amount of energy to break the bond; at least eight electrons and sixteen ATP molecules (Bernhard, 2012). The only way of organisms can catch nitrogen directly from the atmosphere is known as nitrogen-fixing organisms (Harrison, 2003). Bacteria that is able to fix nitrogen through metabolic processes including from the genus Rhizobium. This nitrogen-fixing bacteria usually form symbiotic relationships with host plant such as that is occur in the legume family. However, some nitrogen can also be fixed abiotically by lightning or through industrial processes such as fossil fuels combustion (Bernhard, 2012).

2.2.2.2 Nitrogen uptake

This is the process where the ammonium (NH_4^+) that are produced by nitrogen-fixing bacteria is quickly taken up by plant (Harrison, 2003).

$$NH_4^+ \longrightarrow \text{organic N}$$

2.2.2.3 Nitrogen Mineralization

Nitrogen mineralization is a process where after nitrogen is incorporated into organic matter, it is converted back into inorganic nitrogen; also known as decay (Harrison, 2003). This occurs when organism die and decomposers such as bacteria and fungi consume the organic matter and undergo the process of decomposition. During the process, a significant amount of nitrogen contained inside the dead organism is then converted into ammonium.

2.2.2.4 Nitrification

Nitrification is a process where ammonium that is produced during decomposition is converted to nitrate (NO_3^-), (Harrison, 2003). According to Bernhard (2012), nitrification is a process of ammonia converted to nitrite and then to nitrate. Oxygen is needed for this process. Ammonium ions (NH_4^+) are positively charged particles and only stick to negatively charged particles such as clay and soil organic matter. Therefore, the positive charge will prevent the ammonium from being washed out of the soil or leached by rainfall. In contrast, the negatively charged nitrate ion (NO_3^-) is not held by soil particles and it can be washed out of the soil that leading to decrease in soil fertility and nitrate enrichment of downstream surface and groundwater.

2.2.2.5 Ammonification

This 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. Microorganisms such as fungi are decomposing the tissue and releasing inorganic nitrogen such as ammonium back into the ecosystem (Harrison, 2003).

2.2.2.6 Denitrification

The 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). Denitrification is an anaerobic process which mostly occurs in soils, sediments and anoxic zones in lakes or oceans. This process is important in removing fixed nitrogen such as nitrate from the ecosystem by returns it to the atmosphere in inert form (N_2) (Bernhard, 2012).

2.2.3 Groundwater

Groundwater is known as a major supply for many activities such as in industrial, domestic, agricultural and most importance as drinking sources (Gao et al., 2012). According to West (2001), groundwater is the water that flow freely under the earth's surface that passes through pores of soils and cracks on rock. The groundwater is not important only for the above activities, but also to recharge rivers, lakes, streams and wetlands. Groundwater comes from varies sources, but primarily from rainfall. The flowing motion of groundwater is from upland areas to lower land areas.

As reported by West (2001), contamination mostly occurs due to human activities. The contamination of groundwater can affects human health. Generally, the contaminant is undetected until it perceived in water supply well. Once contamination of groundwater occurs, it is difficult to remove it since it will disperse and remains for a long period of times.

2.2.4 Natural source

The nitrogen cycle represents one of the most important nutrient cycles found in terrestrial ecosystems (Pidwirny, 2006). The nitrogen cycle is the natural process by which nitrogen is converted between its various chemical forms and this transformation can be carried out via both biological and non-biological processesThe nitrogen cycle is of particular interest to ecologists because nitrogen availability can affect the rate of key ecosystem processes, including primary production and decomposition (Absolute Astronomy, 2011).

2.2.5 Anthropogenic source

Anthropogenic activities or man-made source such as use of fertilizers, fossil fuel combustion and release of nitrogen in wastewater have also dramatically altered the global nitrogen cycle (Absolute Astronomy, 2011).

2.3 Exposure to Nitrate and Health Effects

Problems caused by the nitrate contamination in drinking water have been studied since 80's and 90's (Forman et al., 1985). Payne (1993) also reported that people who consumed drinking water that contaminates with nitrate will suffer from enlargement of thyroid gland, cancers, hypertension and stomach cancer.

ATSDR (2011) reported that nitrate contamination causes health risks to humans. It may cause 'blue baby syndrome' or methemoglobinemia among infant. Methemoglobinemia is actually an excess of methemoglobin in the blood. Methemoglobinemia causes cyanosis (blue skin) of limbs/trunk, weakness, and rapid heart rate. If methemoglobinemia progresses in severity, central nervous system depression can occur, as a can headache, dizziness, fatigue, difficulty in breathing, and nausea. Finally, severe methemoglobinemia can cause lethargy, brief loss of consciousness, irregular heartbeat, shock, convulsions, coma, and even death. Methemoglobin levels greater than 50% is potentially fatal (Nathan et al., 1977).

Rademacher et al. (1992) reported, that nitrate exposure in drinking water increases risks such as gastric cancer, thyroid disorder, birth defect and hypertension.

Schubert et al. (1997) also reported, several cases of miscarriages on peoples who consumed nitrate-contaminated drinking water.

As well, Weyer et al. (2001) and Moore et al. (2011) claimed, that exposure of nitrate at low levels for many years could cause certain types of cancer such as stomach, esophagus, lungs, colon, bladder, testicles, ovaries, urogenital tract and non-Hodgkins lymphoma.

Many gastrointestinal illnesses are related to nitrates. The consumption of nitrate contaminated water causes multiple digestive tract impairment (Rao, 2006). There is some evidence that long-term effects, such as gastric cancer, might result from exposure to even smaller amounts of nitrate if they expose to the N-nitroso compounds; the form when nitrate reacts with certain conditions and in which it is a potent carcinogen in many species (Walter et al., 2009).

CHAPTER 3

METHODOLOGY

3.1 Introduction

For the methodology part, it was divided into few subsections which are study design, study location, sampling, data collection method and instrumentation, risk assessment, quality control, ethics committee approval and data analysis.

3.2 Study Design

The study design for this project was a cross-sectional study. The equipment used in this study includes plastic 250ml HDPE bottles. The bottles were used to store samples to analyze for nitrate content in the water. Sample analysis was analyzed with YSI Professional Plus multimeter.

3.3 Study Location

The study locations is Mukim Telipot which consists of Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas in Pasir Puteh, Kelantan. Mostly the houses in these areas were near to agriculture area which is paddy field. This is 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.

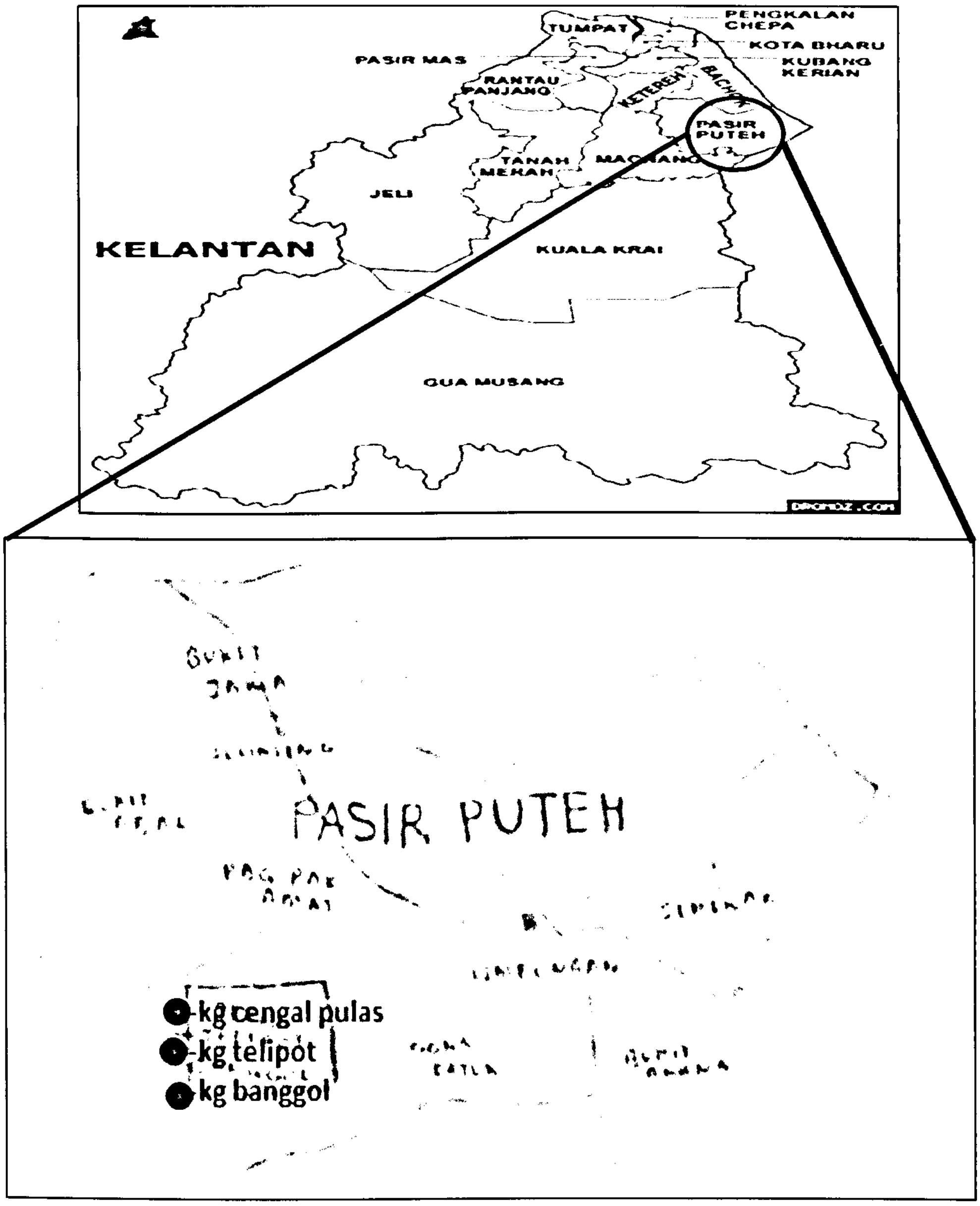


Figure 3.1: Study Location

3.4 Study Population

The study populations were residents of Mukim Telipot consist of Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas in Pasir Puteh, Kelantan, who used groundwater as the main source for drinking and other daily usage.

3.5 Sampling

3.5.1 Study Sample

The study was conducted on respondent who meet 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

Based on Alif (2014), the prevalence of nitrate level of groundwater from wells sampled which was above of 45mg/L is 8.7% (0.57). So the sample size was calculated as below.

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

As indicated by the count over, the estimation of 32 was considered as least sample size required. To ensure that the study was statistical significant and taken into consideration damaged data and non-response survey, the sample size was increased from actual sample size. Thus, the final sample size was 50.

3.5.3 Sampling Method

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

3.5.4 Sampling Unit

The sampling unit for this study was a resident living in Mukim Telipot, who fulfilled the inclusive criteria.

Inclusion criteria:

I. Aged 18 years old and above was considered as an adult.

II. The respondent was lived more than five years as resident of Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas in Pasir Puteh, Kelantan.

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

Exclusion criteria:

I. Respondents who used water filtered systems.

II. Respondents who used other than groundwater as their main water supply for their daily needs.

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, Part C contained questions related to duration of residence and Part D contained questions related to health information.

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 averaged. The unit was in kilogram (kg).

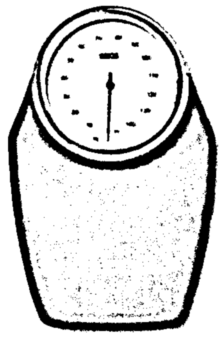


Figure 3.2 Seca weight scale

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 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

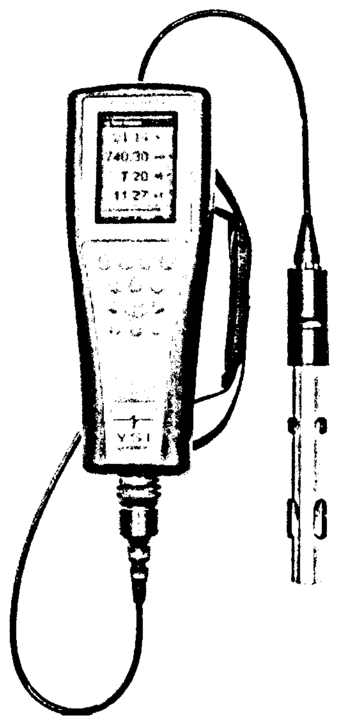


Figure 3.3 YSI Professional Plus Multimeter

The water was analyzed by using YSI Professional Plus multimeter. The readings were taken three times and the average of the readings was the actual reading. This multimeter provided extreme flexibility for the measurement of a variety of combinations for dissolved oxygen, conductivity, specific conductance, salinity, resistivity, total dissolved solids (TDS), pH, ORP, pH/ORP combination, ammonium (ammonia), nitrate, chloride and temperature.

3.7 Risk Assessment (Calculation)

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

$$CDI = \frac{C \times DI}{BW}$$

(Equation 2)

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).

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

$$HI = \frac{CDI}{RfD}$$

(Equation 3)

Where,

HI = Hazard Index,

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

RfD = Reference dose (mg/kg/day

HI value more than 1 (HI>1) is considered a significant risk level, the higher the value, the greater the likelihood of adverse non-carcinogenic health impact. RfD value 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 not normally distributed data. The parametric test was used if data was parametric or normal distribution. While, if it was not normally distributed, a non-parametric test was used to analyse the data.

3.9 Ethical consideration

Ethical issues pertaining to this study was presented and approved by the 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 were held in term of the purpose of the assessment, the procedure taken, and also respondents' right in this study.

III. Approval letter were 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 participant in this study.

3.10 Study Limitation

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

ii. The study was conducted during the rainy season, in which directly affect the outcome of study once fertiliser is applied.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Background of Respondent

4.1.1 Socio-demographic of Respondent

The data collection was carried out from December 2015 to February 2016 at the respective areas.

In this study, the total numbers of respondents who participated were 50 (Table 4.1). Race of all respondents were Malay. Figure 4.1 showed the percentage of participation between genders. It showed that there were a higher percentage of females than males' participation by 52% and 48%, respectively.

The mean and standard deviation for the age of respondents was 46.5 ± 12.797. The median was 48 years old. Age ranged from 26 to 78 years old.

This study was conducted in three villages which are Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas. Table 4.3 showed the frequency of respondents between villages. It was shown that the participation of respondents from Kampung Telipot was 32%, Kampung Banggol and Kampung Cengal Pulas were 34% for both (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	24	48	48
Female	26	52	100
Total	**50**	**100**	

Figure 4.1 Comparison between male and female respondents

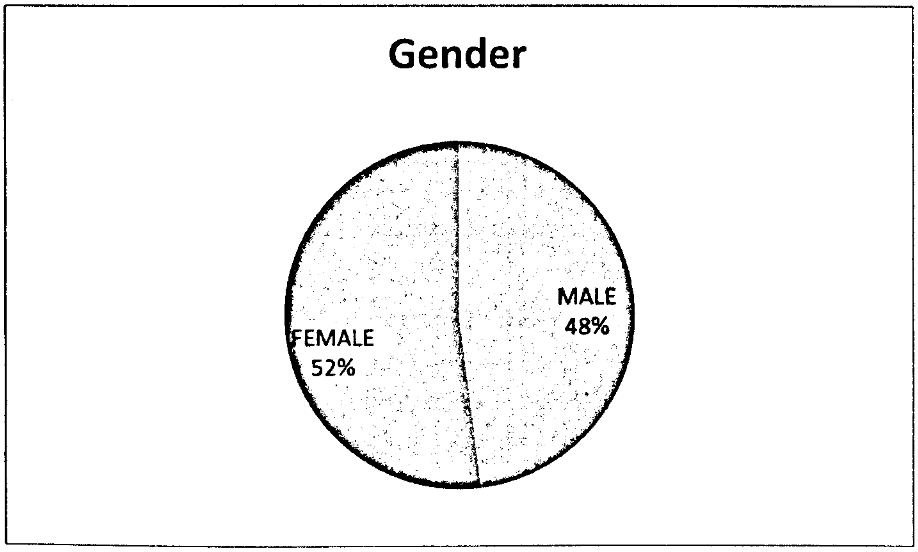


Table 4.3 Frequency of respondents between villages.

	Frequency	Percent	Cumulative Percent
KG TELIPOT	16	32	32
KG BANGGOL	17	34	66
KG CENGAL PULAS	17	34	100
TOTAL	**50**	**100**	

Figure 4.2 Frequency of respondents between villages

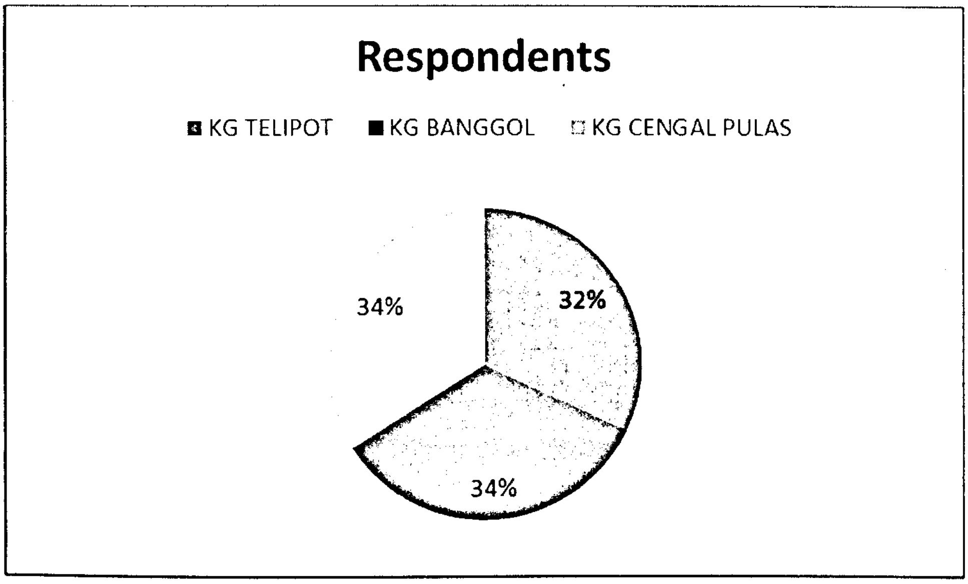
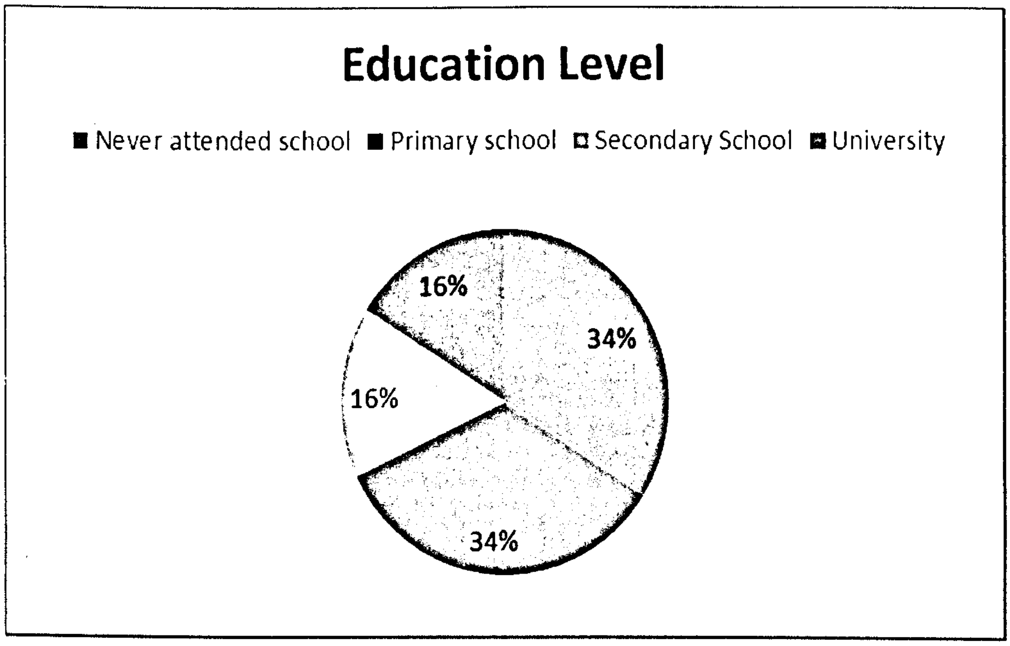


Table 4.4 showed the frequency of education level of respondents. Based on the information in Figure 4.3, it was found that most of the respondents had never attended school and only attended primary school with the percentage of 34% for both. While, there was only 16% of respondents were attended secondary school and university, respectively.

Table 4.4 Frequency of education level.

	Frequency	Percent	Cumulative Percent
Never Attended School	17	34	34
Primary School	17	34	68
Secondary School	8	16	84
University	8	16	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 self-employed gave the highest percentage of occupation of the respondents with 50%, compared to government workers and unemployed of 14% and 36%, respectively.

Table 4.5 Frequency occupation of respondents

	Frequency	Percent	Cumulative Percent
Self-employed	25	50	50
Unemployed	18	36	86
Government Workers	7	14	100
TOTAL	**50**	**100**	

Figure 4.4 Frequency Occupation of respondents

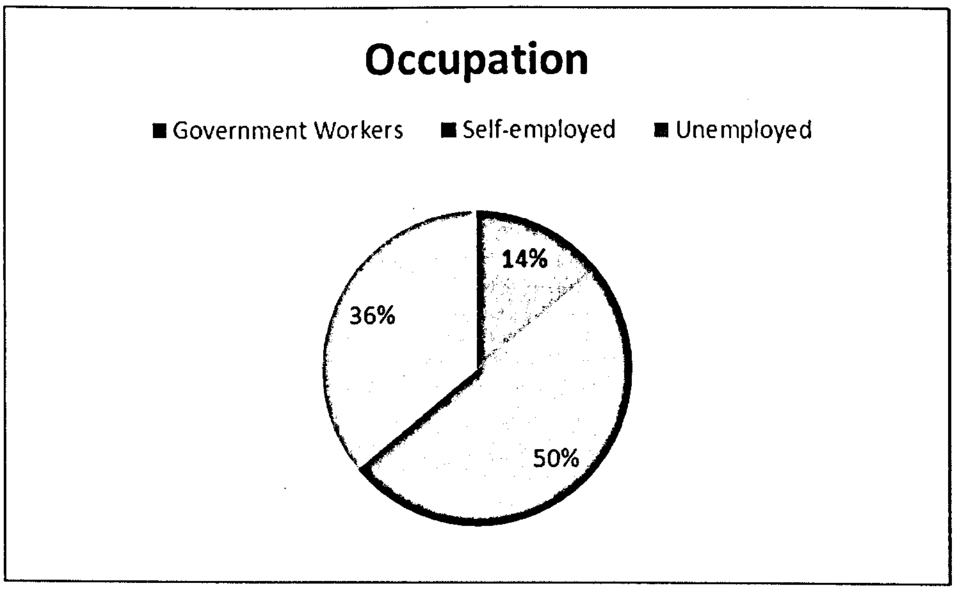
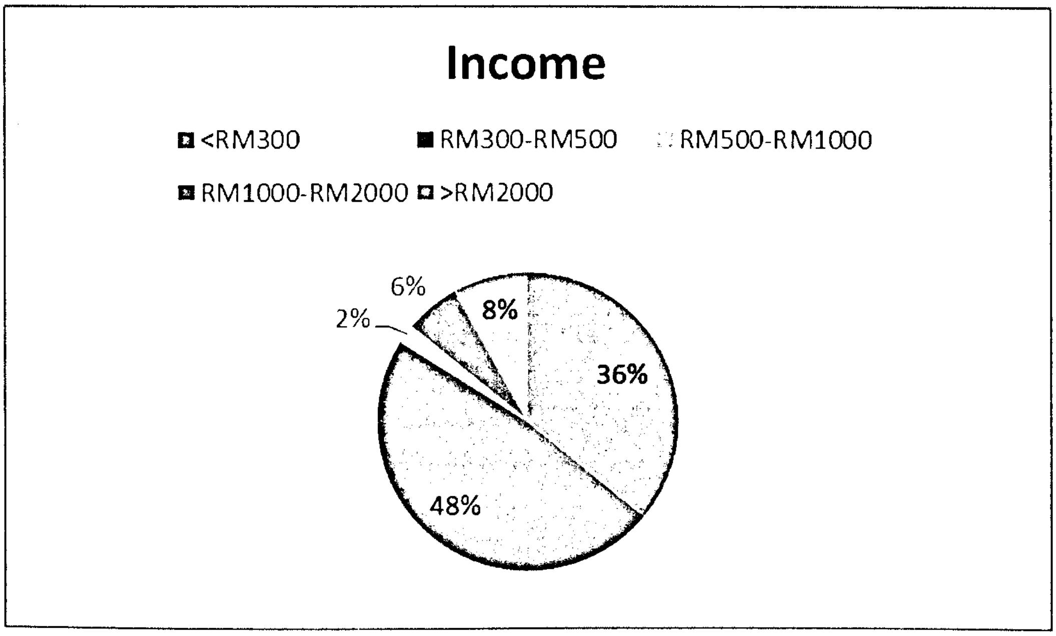


Table 4.6 showed the frequency income of the respondents. Based on the information regarding income of respondents in Figure 4.5, it was found that most of the respondents had a salary between RM300 to RM500 with the percentage of 48%. While, there was 36% of respondents had income below than RM300. Whereas, only 8% of the respondents had income more than RM2000, followed by 6% with an income of RM1000 to RM2000 and only 2% of respondents had salary within RM500 to RM1000.

Table 4.6 Frequency incomes of respondents.

	Frequency	**Percent**	**Cumulative Percent**
< RM300	18	36	36
RM300-RM500	24	48	84
RM500-RM1000	1	2	86
RM1000-RM2000	3	6	92
> RM2000	4	8	100
TOTAL	**50**	**100**	

Figure 4.5 Frequency Incomes of Respondents.



4.1.2 Well Information

In this study, the information on the age of the well was recorded. There were 50 wells were selected for samples. From the Table 4.7, it was shown that most of the samples were collected from the well with age more than 20 years which is 37 (74%) of wells out of 50. While, about 11(22%) of the wells were age within 16 to 20 years and only 2 (4%) wells were age between 10 to 15 years.

Table 4.7 Well information

Variables	No. of samples	%
Age of Well		
10-15 years	2	4
16-20 years	11	22
>20 years	37	74

4.1.3 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 42 (84%) of the respondents used groundwater for drinking and cooking purposes. While another 16% of the respondents used groundwater for domestic usage.

For daily water intake, the mean ± S.D (L/day) was 1.188 ± 0.24381and had ranged between 0.6 – 1.6 L/day. From the survey, all of the respondents were satisfied with water quality from their wells.

Table 4.8 Drinking Water Information

Variables	%	mean ± S.D (L/day)	Range (L/day)
Water purposes	84%(Drinking and Cooking)		
	16%(Domestic usage)		
Daily Water Intake		1.188 ± 0.24381	0.6 – 1.6
Water Quality	100%(satisfied)		

4.2 Nitrate Level in Groundwater

Table 4.9 Nitrate level in groundwater samples

Variables	mean ± S.D (mg/L)	Range (mg/L)
Nitrate	11.6062 ± 5.57611	2.14 to 22.10 mg/L

N=50

In this study, the mean ± SD of nitrate level was 11.6062 ± 5.57611 while the range was from 2.14 to 22.10 mg/L. This amount was considered as low level of nitrate in groundwater. However, the level of nitrate in groundwater can increase due to a few factors such as the use of fertilizers, waste disposal especially from animal farms, and septic tank (Haycock, 1990). But, in this study, the relationship of nitrate level in groundwater was related to the use of fertilizer. This happened because of irrigated agriculture practice utilizes a huge amount of fertilizers, which may lead to groundwater contamination (Ghaderi et al., 2012).

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. According to CCME (2007), rainfall would influence the concentration of nitrate, as it penetrates the groundwater at a high rate during the beginning of rainy season, decrease throughout the rainy season and remain constant at the low level during the dry season. It caused the water table going up and nitrate in the unsaturated zone (such as soil) become mobilized into groundwater. Therefore, the nitrate level was higher in this situation. Nitrate will accumulate in the soil during a dry season and decrease of mobilization into stream and groundwater, thus it will resulted in lowering nitrate concentration in the groundwater.

Another factor that may leads to low level of nitrate in this study was sampling time. The water sampling was done after the paddy rice had been harvested. This may happen because of fertilizer had not been applied extensively. Thus, the amount of fertilizer's leaching into groundwater were low.

4.3. Comparison of Nitrate Level Between Each Study Location (Independent t-test)

Table 4.10 showed the comparison of nitrate levels of groundwater between each of the study location. The mean ± S.D (mg/L) obtained was 11.6062 ± 5.57611. In this study, independent t-test was used to compare the difference of nitrate levels between each house.

The p-value obtained for this comparison was 0.00. Therefore, it can be indicated that there was a strong significant difference of nitrate levels between each house in this study area since p-value was less than 0.05 ($p<0.05$) (Table 4.7).

Table 4.10 Comparison of Nitrate Level of Groundwater Between Each Study Location

Variables	mean ± S.D (mg/L)	Range (mg/L)	p-value
Nitrate	11.6062 ± 5.57611	2.14 to 22.10 mg/L	0.00

From the results obtained, it indicated that there was a significant difference of nitrate levels between each study location. The p-value for this comparison was 0.00. It was similar to a study by Rajmohan and Elango (2005) who indicated that nitrate levels of groundwater were varied among sampling locations.

The distance of the well to agriculture land also a causal factor in determining the nitrate levels in groundwater. According to Weyer (2001), agriculture activities were contributed to high nitrate levels in groundwater.

Additionally, the distance of septic tanks to the sampling point or well could probably be the factors that affects the nitrate concentration. As reported by Mohamed Azwan et al. (2010), the nearer the wells to septic tanks would cause high level of nitrate.

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

In this study, nitrate levels between the three villages were compared using Kruskal-Wallis test. The results obtained showed that Kampung Cengal Pulas had highest mean ± S.D (mg/L) of nitrate levels which is 12.45 ± 5.35 than Kampung Banggol and Kampung Telipot with mean ± S.D (mg/L) of 11.42 ± 5.36 and 10.90 ± 6.25, respectively.

The factor that leads to this situation may possibly caused by the quantity or rate of fertilizer application during the previous season to crop. Another reason might be caused by the leaching process of excessive nitrate to the groundwater. As stated by Roy et al. (2000), excessive fertilizer application can cause a state of pollution in groundwater. However, from the results obtained, it showed that there was no significant difference of nitrate levels between these three villages. (p-value 0.68)

According to p-value (0.680), it indicated that there was no significant difference of nitrate levels between these 3 villages (Table 4.11).

Table 4.11 Comparison of nitrate level between 3 villages

Variables	No.of samples	mean ± S.D (mg/L)	Range (mg/L)	p-value
Kg Telipot	16	10.90 ± 6.25	2.14 to 22.10	
Kg Banggol	17	11.42 ± 5.36	4.42 to 19.44	0.68
Kg Cengal Pulas	17	12.45 ± 5.35	5.29 to 21.68	

4.5 Comparison of Nitrate Level of Each House With National Standard

With regards to Malaysian National Standard of Drinking Water Standard (NSDWQ), the maximum concentration limit (MCL) for nitrate is 45mg/L (MOH, 2011). Figure 4.6 indicated that nitrate levels from all sampling sites were below 45mg/L, which is below the maximum limit of NSDWQ.

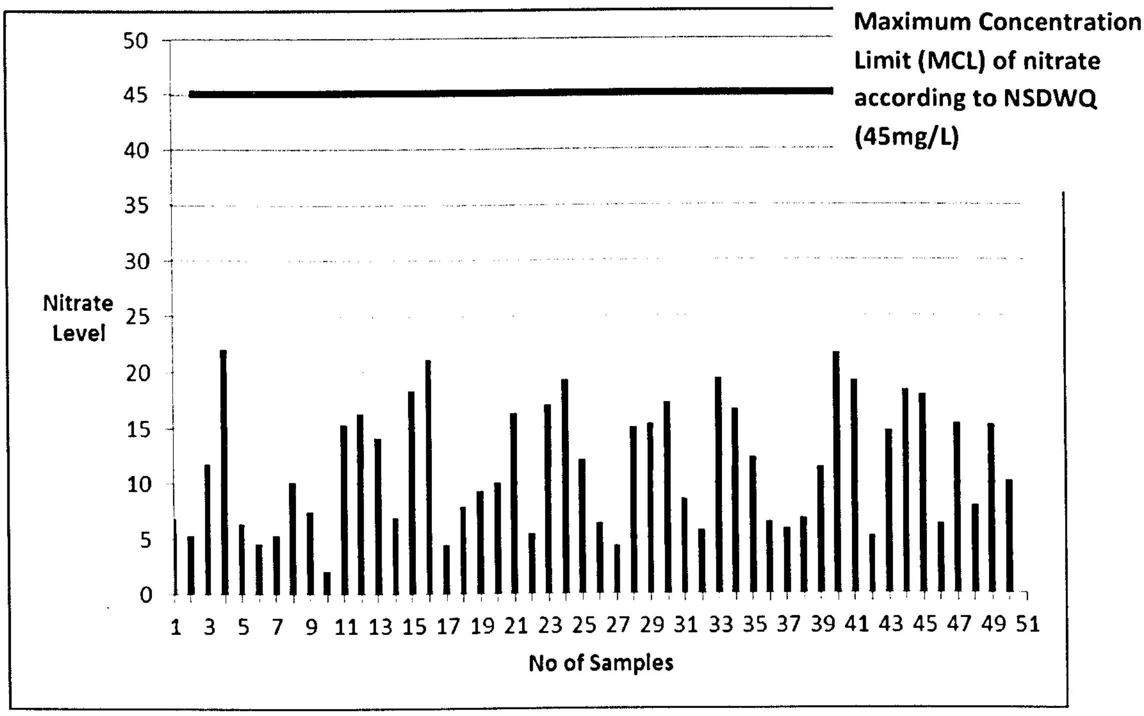


Figure 4.6: Comparison of nitrate level among study locations with national standard

In this study, the highest reading of nitrate level was 22.10 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 45mg/L as referred to the Malaysian National Standard of Drinking Water Standard (NSDWQ,2000).

A previous study by Alif and Shaharuddin (2014) also reported similar trend which showed that all nitrate level of the study areas did not exceed 45mg/L or the permissible World Health Organisation (WHO, 2008) value.

As stated at the above, the maximum level of nitrate in this study was 22.10 mg/L and considered safe for drinking and cooking purpose.

4.6 Exposure Information (Chronic Daily Intake)

Table 4.12 showed the results of Chronic Daily Intake (CDI) estimation of respondents. From the table, it is shown that the mean ± S.D for respondent's body weight was 61.57± 11.63212 kg. While the range for respondent's body weight was between 42-94 kg.

Based on the table below, the exposure of nitrate on respondent was estimated as Chronic Daily Intake (CDI). The CDI value was calculated using an equation as stated in Chapter 3. Table 4.12 shows the mean ± S.D for CDI (mg/kg/day) was 0.2395± 0.15127 and the range for CDI was between 0.01-0.78 mg/kg/day.

Table 4.12 Chronic Daily Intake (CDI) estimation

	Nitrate level (mg/L)	***DI (L/day)**	***W (kg)**	***CDI (mg/kg/day/**
Mean	11.6062	1.188	61.57	0.2395
Median	10.825	1.2	59.5	0.2219
SD	5.57611	0.24381	11.63212	0.15127
Range	2.14 to 22.10	0.6 – 1.6	42-94	0.01-0.78

4.7 Health risk assessment information (Hazard Index)

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 potential for an adverse effect to occur (NJDEP, 2007).

Table 4.13 showed the HI estimated. The range of HI obtained was between 0.01-0.49. While the mean ± S.D was 0.1497 ± 0.09454. As shown in Table 4.10, HI value for all respondents were less than 1 (50 respondents-100%).

Table 4.13 Hazard Index (HI) Estimating Among Respondents

Hazard Index (HI)	**No. of Respondent**	**Percentage (%)**	**Mean ± S.D**	**Range**
HI<1	50	100	0.1497 ± 0.09454	0.01-0.49
HI>1	0	0		

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

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study was conducted to determine the effect of nitrate exposure in groundwater that was associated with health risk. As we know, water is one of the most important elements in our daily life. The quality of water for daily uses must always be clean and safe for drinking and cooking purposes. Contamination of water from nitrate or any other contaminants should be avoided as it.

The results obtained in this study also showed a strong significant difference of nitrate levels between each location as nitrate levels may differ according to different location and geological areas. Therefore, the second hypothesis was failed to be rejected.

However, the results of nitrate level between the three villages, Kampung Telipot, Kampung Banggol and Kampung Cengal Pulas has no significant difference among each other. Thus, the third hypothesis was rejected.

In this study, the highest reading of nitrate level was 22.10mg/L and it is considered safe because not exceed level of nitrate which can cause adverse health effect is 45mg/L. Hence, groundwater in these three villages are 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. In short, the fourth hypothesis was rejected.

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.

5.2 Recommendations

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 different point in time.

The results obtained may not represent the whole district or state since this study only focuses on three villages. 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, if there are peoples want to build a new well, they should make it deep enough. This is in order to extend the past of water table into the groundwater aquifer as the concentration of nitrate are decreases as depth of well are increases.

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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APPENDICES

APPENDIX A: QUESTIONAIRE

Semua maklumat adalah untuk kegunaan kajian sahaja dan maklumat ini akan dianggap sulit. Maklumat anda hanya digunakan untuk membahagi responden mengikut kategori yang sama.

Arahan: Sila jawab semua soalan dan tandakan (/) pada ruang yang disediakan.

Bahagian A: Maklumat responden

1. Nama ....
2. Alamat ....

....

....

3. No tel ☐☐☐☐☐☐☐☐☐☐ :

4. Tarikh lahir ☐☐ hr ☐☐ bln ☐☐☐☐ thn

:

5. Umur ☐☐ tahun :

6. Jantina : ☐ Lelaki ☐ Perempuan

7. Pekerjaan : ....

8. Pendapatan sebulan : RM ....

9. Taraf pendidikan :

- ☐ Tidak bersekolah
- ☐ Sekolah rendah
- ☐ Sekolah menengah
- ☐ 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

Bahagian D: Maklumat kesihatan

9. Adakah anda merokok?

☐ Ya ☐ Tidak

10. Adakah anda mengambil minuman beralkohol?

☐ Ya ☐ Tidak

Jika ya, sila nyatakan berapa botol sehari? ....botol

11. Adakah anda mengalami kegetaran badan yang serius?

☐ Ya ☐ Tidak ☐ Tidak pasti

12. Adakah anda mempunyai masalah pergerakan yang lambat (mengambil masa untuk melakukan sesuatu tugasan mudah)?

☐ Ya ☐ Tidak ☐ Tidak pasti

13. Adakah anda sering kali bersifat pelupa?

☐ Ya ☐ Tidak ☐ Tidak pasti

14. Adakah anda mempunyai masalah baru dengan perkataan apabila bercakap atau menulis?

☐ Ya ☐ Tidak ☐ Tidak pasti

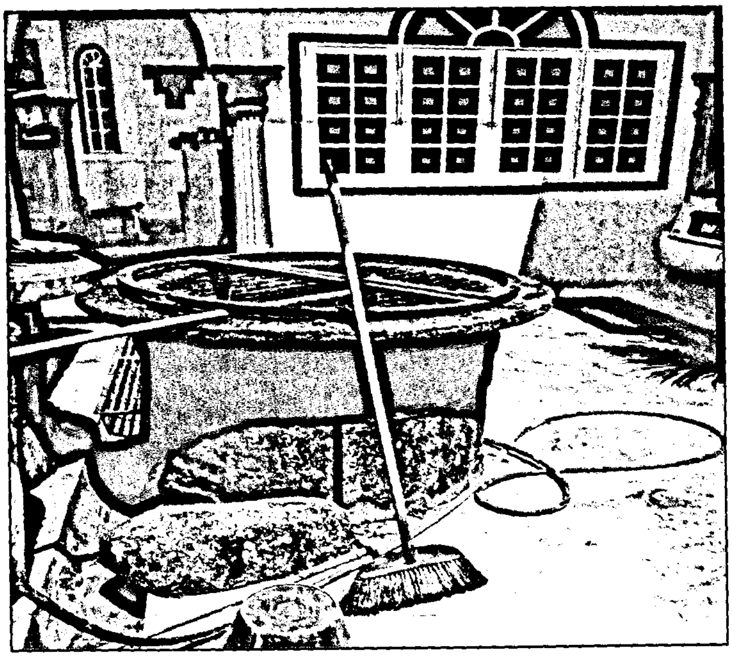
15. Adakah keluarga anda mempunyai sejarah penyakit Alzheimer?

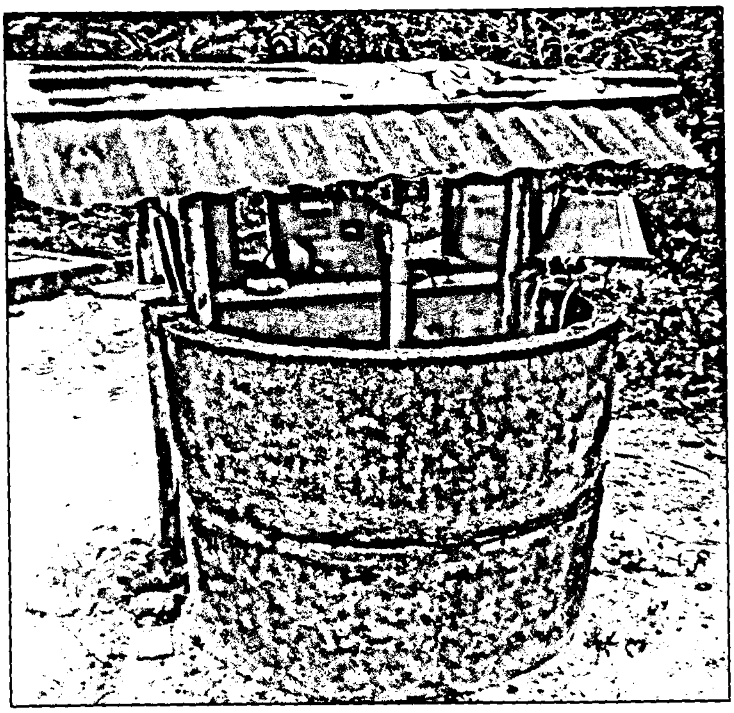
☐ Ya ☐ Tidak ☐ Tidak pasti

TERIMA KASIH ATAS KERJASAMA ANDA

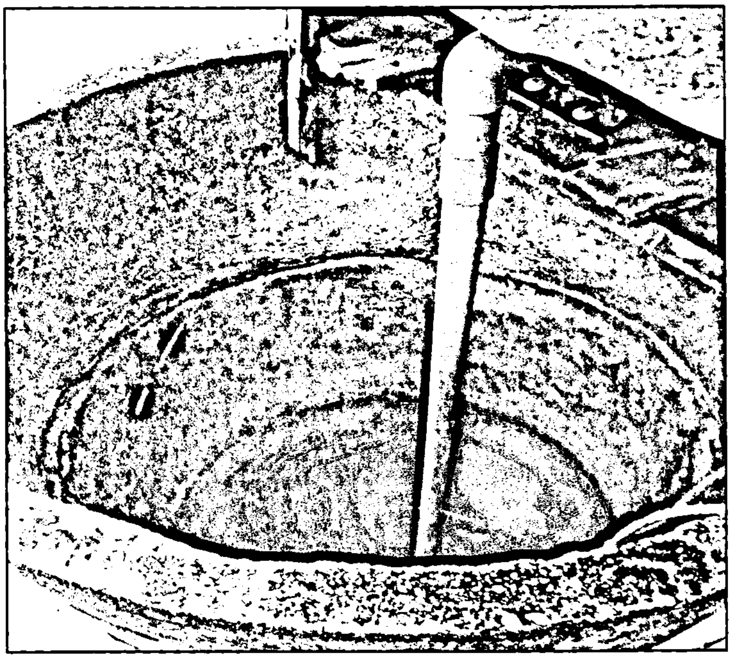
-TAMAT-

APPENDIX B: PICTURE OF WELLS IN KG TELIPOT





PICTURE OF WELLS IN KG BANGGOL



PICTURE OF WELLS IN KG CENGAL PULAS