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CONTAMINATION OF NITRATE IN GROUNDWATER AND EVALUATION OF HEALTH RISK IN KAMPUNG KETING, BACHOK, KELANTAN: A CROSS-SECTIONAL STUDY

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BY

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ABSTRACT

CONTAMINATION OF NITRATE IN GROUNDWATER AND EVALUATION OF HEALTH RISK IN KAMPUNG KETING, BACHOK, KELANTAN: A CROSS-SECTIONAL STUDY

Muhammad Azri Mat Yusof

Introduction: Nitrate is one of the compounds of nitrogen that is formed in the nitrogen cycle from both natural and anthropogenic sources. Most of rural areas in Kelantan still depend on well water as their primary water source. Their main economic activity is agriculture which uses high amounts of nitrate fertilizer to nurture their crops. The increased use of nitrate fertilizers is risk factor associated to health problems such as methemoglobinemia and cancers. Health risk assessment can be conducted to quantify the probability of harmful effects of nitrate to individuals or populations from certain human activities. **Objectives:** To determine levels of nitrate in groundwater and to perform health risk assessment among respondents in Kampung Keting, Bachok, Kelantan. **Methodology:** A total of 47 respondents were chosen based on inclusive and exclusive criteria and groundwater samples were collected from each of the respondent's houses. The samples were then analyzed by using a portable Hanna Instrument multimeter model HI98191 and probe model HI4113 (manufactured in USA), while a set of questionnaires were distributed among respondents to gather information for health risk assessment of the exposure. **Result and Discussion:** The result obtained shows that nitrate levels in groundwater did not exceed the maximum acceptable value of Drinking Water Quality Standard (44.3 ppm nitrate – NO_3) with a mean ± sd of 5.34 ± 4.94 (ppm). Spearman's rho correlation analysis shows that only depth of well (meter) is correlated (r = - 0.348) to nitrate levels ($p<0.05$). The Hazard Index (HI) for the study population was less than 1. **Conclusion:** This study conclude that the groundwater analyzed in the study area was considered safe for drinking and cooking purposes. The result for HI indicated that the non-carcinogenic risk related to nitrate was not significant to the study population. However, nitrate levels in drinking water should be concerned by the consumers as it will give bad health impact to them in long-term exposures.

Keywords: Nitrate, groundwater, maximum acceptable value, hazard index.

ABSTRAK

PENCEMARAN NITRAT DALAM AIR BAWAH TANAH DAN PENILAIAN RISIKO KESIHATAN DI KAMPUNG KETING, BACHOK, KELANTAN: KAJIAN KERATAN RENTAS

Muhammad Azri Mat Yusof

Pengenalan: Nitrat adalah salah satu daripada sebatian nitrogen yang dibentuk dalam kitaran nitrogen dari sumber semula jadi dan antropogenik. Kebanyakan kawasan luar bandar di Kelantan masih bergantung kepada air telaga sebagai sumber air utama mereka. Aktiviti ekonomi utama mereka ialah pertanian yang mana menggunakan baja nitrat yang tinggi untuk memelihara tanaman mereka. Peningkatan penggunaan baja nitrat mempunyai kemungkinan faktor risiko yang berkaitan dengan masalah kesihatan seperti methemoglobinemia dan kanser. Penilaian risiko kesihatan boleh dilakukan untuk mengukur kebarangkalian kesan berbahaya nitrat kepada individu atau populasi daripada aktiviti manusia yang tertentu. **Objektif:** Menentukan tahap nitrat di dalam air bawah tanah dan melaksanakan penilaian risiko kesihatan dalam kalangan responden di Kampung Keting, Bachok, Kelantan. **Metodologi:** Sejumlah 47 responden dipilih berdasarkan kriteria inklusif dan eksklusif dan sampel air tanah dikumpulkan dari setiap rumah responden. Sampel kemudian dianalisis dengan menggunakan model multimeter Hanna Instrumen mudah alih HI98191 dan kuar model HI4113 (dibuat di Amerika Syarikat), manakala satu set soal selidik diedarkan dalam kalangan responden bagi mengumpulkan maklumat untuk pendedahan penilaian risiko kesihatan. **Keputusan dan Perbincangan:** Hasil yang diperoleh menunjukkan bahawa kadar nitrat dalam air bawah tanah tidak melebihi nilai kepekatan maksimum Piawaian Kualiti Air Minum (44.3 ppm nitrat - NO3) dengan min ± sd adalah 5.34 ± 4.94 (ppm). Analisis hubung kait Spearman menunjukkan bahawa hanya kedalaman telaga (meter) berhubung kait ($r = -0.348$) dengan tahap nitrat ($p < 0.05$). Indeks Bahaya (HI) untuk populasi kajian kurang daripada 1. **Kesimpulan:** Sebagai kesimpulannya, air bawah tanah yang dianalisa di kawasan kajian ini dianggap selamat untuk tujuan minum dan memasak. Hasil untuk HI menunjukkan bahwa risiko bukan karsinogen yang berkaitan dengan nitrat tidak berbahaya bagi populasi kajian. Walau bagaimanapun, tahap nitrat dalam air minuman harus menjadi perhatian pengguna kerana ia akan memberikan impak kesihatan yang buruk kepada mereka untuk pendedahan jangka panjang.

Kata kunci: Nitrat, air bawah tanah, nilai kepekatan maksimum, indeks bahaya.

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CDI	Chronic Daily Intake
CRC	Colorectal Cancer
DWQS	Drinking Water Quality Standard
EIA	Environmental Impact Assessment
GPS	Global Positioning System
HI	Hazard Index
HIA	Health Impact Assessment
HQ	Hazard Quotient
INWQS	Interim Water Quality Standard of Malaysia
ISA	Ionic Strength Adjuster
MAV	Maximum Acceptable Value
NADH	Nicotinamide Adenine Dinucleotide
NOC	N-Nitroso Compouds
RfD	Reference Dose
US EPA	United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

1.1) Background

Nitrate is an inorganic compound that is made up from nitrogen and oxygen, NO_3^- (one nitrogen and three oxygen molecules) and can be found in nature and food that we eat (Nitrate, 2018). Nitrate is soluble in water, easily leaches through soil and then it accumulates in groundwater (Jaturong et al., 2015). Over the last century, nitrogen cycle dramatically altered by human activities and causes nitrate (NO_3^-) accumulating in water resources such as in groundwater and surface water (Aida Soraya Shamsuddin et al., 2014). Some of the human activities that contributed to the contamination of nitrate are agriculture activities, farming activities, industrial activities, disposal of solid waste and etc. Based on Aida Soraya Shamsuddin et al. (2016) stated that many studies showed there is correlation between contamination of nitrate with agriculture activities due to the extensive use of nitrate fertilizer. Furthermore, short distance of well water from agriculture activity potentially increase the contamination of nitrate in drinking water.

A significant concern about nitrate that present in drinking water is contamination of nitrate can lead to health problem such as methemoglobinemia among infants and cancers in digestive tract of adults. Age, gender, genetics or other health condition are the factors of severity of methemoglobinemia and infants under 6 months is susceptibility to this syndrome (Jaturong at al., 2015). Moreover, women during first trimester of pregnancy probability will having birth defects due to the exposure of high nitrate content in drinking water from groundwater (Brender et al., 2013). In addition, Mina Parvizishad et al. (2016) mentioned that if consuming drinking water and food that contain high nitrites and nitrates level can cause health effects such as methemoglobinemia, cancer, diabetes mellitus, enlargement of the thyroid gland and so on. Plus, the study also mentioned that nitrate and nitrite have positive effects at low concentration such as maintain blood pressure regulation, as a protective effect on cardiovascular system, and maintain homeostasis (the stability) of vessels in the body.

In order to determine whether the consumers were exposed to the diseases or not, health risk assessment can be conducted. Health risk assessment is the process to evaluate the probability of adverse health effect in human due to exposure to an environment stressor (US EPA, 2016). Health risk assessment also play an important role in health promotion and disease prevention both in individually and population level since 1950s (Orlando et al., 2018).

1.2) Problem Statement

The sole provider that provide water services in Kelantan is Air Kelantan Sdn. Bhd. (AKSB). The conflict begins when most of people complaint about the water services provided by AKSB was smelly and coloured water supply (Mahirah Kamaludin, Khalid Abdul Rahim & Alias Radam, 2013). Plus, Association of Water and Energy Research Malaysia (2011) also stated that water supply in Kelantan was dirty and smelly, frequent water disruptions and low coverage performance. One of the reason is because the water prices in Kelantan is cheap and the water company is unable to maintain its operation and thus, leads to water wastage. This situation leads the consumers to use groundwater and presently, Kelantan is the largest groundwater operator in Malaysia. About 35% of the population in Kelantan depends on groundwater as their main source of water supply (Idrus et al., 2014).

On previous study, well water sample in Bachok contain higher nitrate than other districts in Kelantan due to the location of well water within tobacco plant and paddy field. However, the concentration of nitrate was still lower than the limit of 10mg/L by the Interim Water Quality Standard of Malaysia (INWQS) (Noor Wahida Mahasim et al., 2014). Moreover, agriculture activity is the main occupation in Bachok which mainly paddy farming (Aida Soraya Shamsuddin et al., 2016) and majority of the houses are located near the paddy fields (Muhamad Nur Fakhri MR and Shaharuddin MS, 2017). Therefore, most of the resident exposed to the nitrate contamination due to the water supply from groundwater for cooking and drinking purposes. This is because, agriculture activity used high volume of nitrogenous fertilizers and then, it will leach into the soil and contaminate the near groundwater. This will pose a negative effect to human health (Noraziah Jamaludin, Shaharuddin Mohd Sham and Sharifah Norkhadijah Syed Ismail, 2013).

The problem to be highlighted is whether the level of nitrate in groundwater at Bachok is safe or not for drinking and cooking purposes. The data obtained were compared with Drinking Water Quality Standard and were used to determine the Hazard Index from exposure to nitrate.

1.3) Study Justification

Bachok is one of the district areas that still use well water as the main source of water to fulfill their daily needs. They depend on groundwater (well water) due to limited piped water supply in the area (Muhamad Nur Fakhri MR and Shaharuddin MS, 2017). Furthermore, the wells are located near the agriculture activity such as tobacco, paddy, rubber plantation and orchards. Each year they used high amount of fertilizers containing phosphates and nitrates in order to obtain high yields and faster growing crops (Noor Wahida Mahasim et al., 2014).

1.4) Research question

1) Is the age of well, depth of well and distance of well from paddy field area give effect to the nitrate level in groundwater?
2) Is there any significant health risk due to nitrate level in groundwater?

1.5) Research Objectives and Hypothesis

1.5.1) General Objective:

To determine levels of nitrate in groundwater and health risk among respondents in Kampung Keting, Bachok, Kelantan.

1.5.2) Specific Objectives:

a. To determine the weight of respondent in order to calculate health risk assessment
b. To identify the age, depth, and distance of the well
c. To identify the nitrate level in groundwater
d. To determine the association between age of well, depth of well, and distance of well from paddy field area with nitrate level
e. To determine the health risk associated with nitrate level in groundwater

1.5.3) Research Hypothesis:

There is an association between age of well, depth of well and distance of well from paddy field area with level of nitrate.

1.6) Conceptual Framework

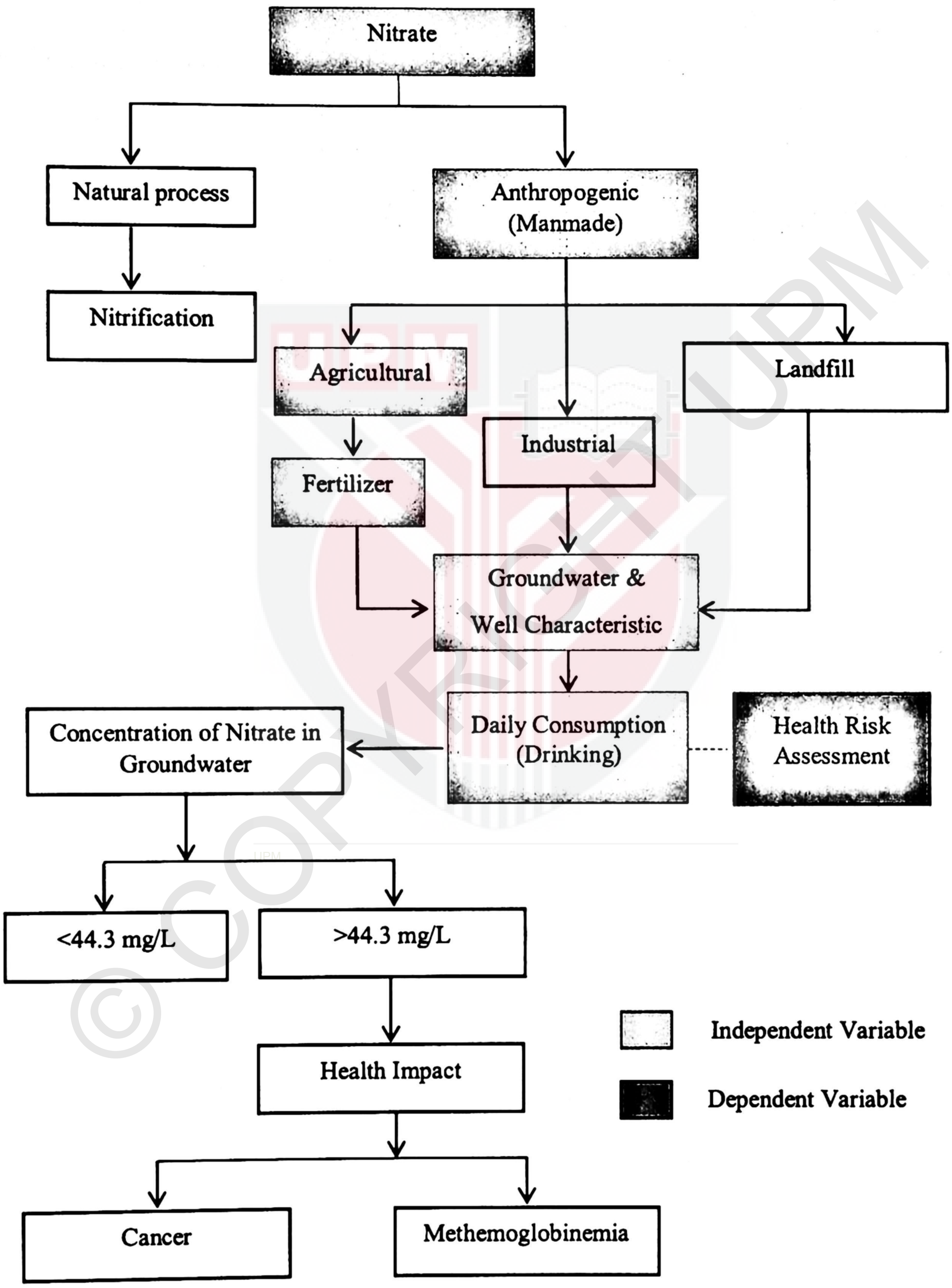

Figure 1.1 Conceptual Framework

1.7) Conceptual Definition

1) Anthropogenic

Anthropogenic is the activity that result directly from the influence of human being on environment and has significantly altered the function of ecosystems (Energy Education, 2015).

2) Agriculture

Agriculture is the science or practice of farming, cultivation of the soil, growing plants and raising animal livestock. It consists of the preparation of animal and plant products for humans to use and distribute to the markets (National Geographic Society, 2011)

3) Fertilizer

Fertilizers are chemical compounds that are added into the soil to give nutrients to make it more fertile. The chemicals in fertilizers comprise necessary elements required for plant growth (Science Learning Hub, 2013).

4) Groundwater

Groundwater is all the water held underground in the soil or pores and located under the Earth's surface. This water comes mainly from rain, melted snow, and different water that seeps via soil, sand, or the cracks in the sidewalk (Augustyn et al., 2019).

5) Health Risk Assessment

Health risk is an effect of health hazard and exposure to that hazard. In order for risk to be characterized, a health hazard must first be present. Qualitative risk assessment only characterizes or differentiate the hazard of a chemical relatives to others. In addition, Quantitative risk assessment measure the risk or safety of a chemical exposure by numerical and comprise 5 steps (Guidance Document on Health Impact Assessment (HIA) in Environmental Impact Assessment (EIA), 2012):

I. Issues Identification

II. Hazard Identification

III. Dose-Response assessment

IV. Exposure Assessment

V. Risk Characterization

1.8) Operational Definition

1) Drinking Water

Drinking water samples is collected from the groundwater sources for each respondent.

2) Nitrate Level

Nitrate concentration from each sample will be analyzed by using Portable PH/ORP/ISE Meter model HI98191 and Probe Model HI4113.

CHAPTER 2

LITERATURE REVIEW

2.1) Nitrate

Nitrate concentrations in natural waters come from non-point sources such as wildlife, atmospheric and degradation of naturally derived soil organic matter in most environments (Me nció et al., 2016). Nitrate is readily soluble in water, easily leaches through soil and then it accumulates in groundwater (Hallberg, n.d.). A study from Shuval and Gruener. (n.d.) mentioned that nitrogen accumulation in the environment can be occur due to massive use of inorganic nitrogenous fertilizers and increasing accumulation rate of organic wastes from the growing human and animal population. Over the last century, nitrogen cycle dramatically altered by human activities and causes nitrate (NO_3^-) accumulating in water resources such as in groundwater and surface water (Aida Soraya Shamsuddin et al., 2014).

Nitrate and nitrite are the form that come from oxidation process of ammonia that released by the decomposition of organic materials in soils. Nitrate is more familiar than nitrite although both of them can be found in soil (Ellis, 2010). In water and wastewater, the greatest concern forms of the nitrogen are inorganic nitrogen, ammonia, nitrite and nitrate. Nitrogen in soil is derived from various sources. In production agriculture it is dominantly from fertilizer, biological nitrogen fixation and mineralization of organic nitrogen from soil organic matter, crop residues and wastes (Division of Public Health, 2017).

Regarding Haber-Bosch process in 1909, most of the reactive nitrogen within the biosphere was generated by microorganisms. Although the Haber-Bosch process more than quadrupled the productivity of agricultural crops, chemical fertilizers and other anthropogenic sources of fixed nitrogen currently way exceed natural contributions, resulting in unprecedented environmental degradation (Stein & Klotz, 2016).

2.2) Nitrogen Cycle

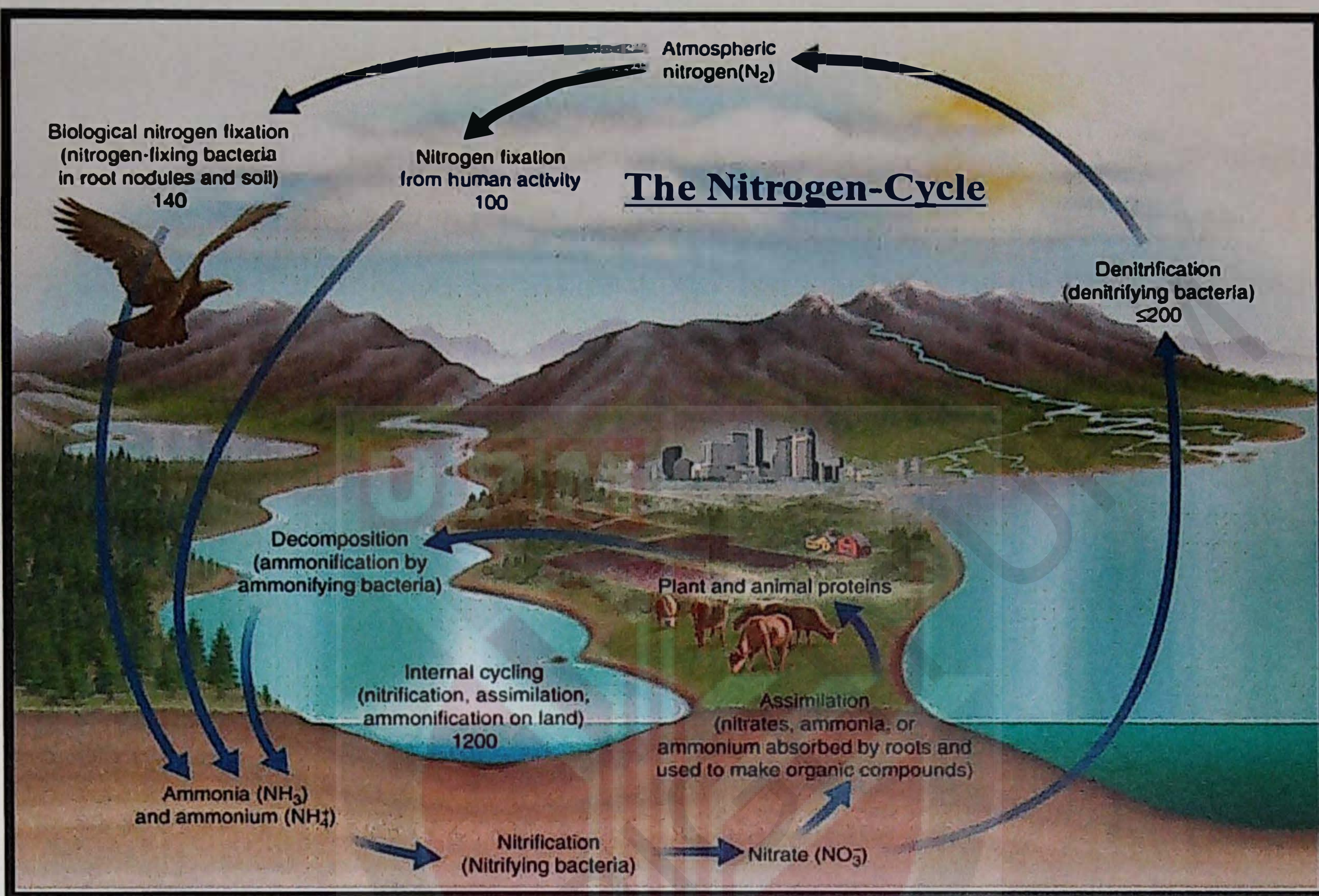

Figure 2.1 Nitrogen Cycle

Source Environment, 2004

Nitrogen is important for all organisms as it is a part of biological molecules in protein and nucleic acid. There is 78% of nitrogen gas (N_2) in atmosphere (Environment, 2004). Nitrogen is in stable condition at atmosphere and need to be separated in order for the nitrogen atoms combine with other elements to form proteins and nucleic acids. Five main steps in the nitrogen cycle: nitrogen fixation, nitrification, assimilation, ammonification, and denitrification.

Nitrogen fixation is the conversion of atmospheric nitrogen to ammonia, performed by nitrogen-fixing bacteria, including cyanobacteria in soil and aquatic environments. Biological nitrogen fixation is very important biological process as it is necessarily needed to sustain food supply to its inhabitants (Weisany et al., 2013).

Nitrification is the process of conversion of ammonia or ammonium to nitrate, performed by nitrifying bacteria. Nitrification was performed by soil bacteria, which is a two-step process (Environment, 2004). First, soil bacteria convert ammonia or ammonium to nitrite (NO_2^-). Then, another soil bacterium oxidizes nitrite and nitrate. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite (US EPA, 2002).

The reduction of nitrate to ammonium and then followed by ammonium assimilation into amino acid which is nutrient for plants is called nitrogen assimilation (Masclaux-Daubresse et al., 2010). When animals consume plant tissues, they also assimilate nitrogen by taking in plant nitrogen compounds (amino acids) and converting them to animal compounds (proteins).

Ammonification is specifically referring to the microbial conversion of organic-nitrogen into ammonia (NH_3) or ammonium (NH_4^-) (Ammonification, 2018). Ammonification begins when organisms produce nitrogen-containing waste products such as urea (in urine) and uric acid (in the wastes of birds). These substances, plus the nitrogen compounds that occur in dead organisms, are decomposed, releasing the nitrogen into the abiotic environment as ammonia. The ammonia produced by ammonification enters the nitrogen cycle and is once again available for the process of nitrification and assimilation (Environment, 2004).

Denitrification is the conversion of nitrate to nitrogen gas, performed by denitrifying bacteria. This process occurs when soil bacteria use oxygen in nitrate for respiration (International Plant Nutrition Institute, n.d.). Denitrifying bacteria reverse the action of nitrogen-fixing and nitrifying bacteria by returning nitrogen to the atmosphere as nitrogen gas.

2.3) Source of Nitrate

Nitrate contamination in groundwater induced due to anthropogenic activities such as use of nitrogen fertilizer, animal waste, organic manure and etc (Ako et al., 2014). From other study also mentioned that some of the possible sources of nitrate in groundwater including animal feedlots, nitrogen – containing fertilizers and improper disposal of sanitary wastes (Edokpolo et al., 2016). The hydrogeochemical behaviour of nitrate in groundwater is strongly influenced by the present of plants (organic load in soil) animal waste and anthropogenic input (fertilizer, industrial effluent, and animal excretion) (Sunil Kumar et al., 2018). In general, nitrate in groundwater originate from nitrogen sources on the land surface, where nitrogen-rich wastes are buried. As agriculture region was high in contribute to the nitrogen fertilizer used, population near this area was exposed to the high nitrate consumption from drinking water especially drinking water that taken from shallow wells and located near nitrogen source (Ward et al., 2018). The high use of nitrogenous fertilizer in agricultural areas can cause nitrate contamination and degrade the quality of groundwater (Chen, Wu, & Qian, 2016).

One study in India reported that, nitrate contamination is the priority concern among them as more than 70% of the population depend on the agriculture activities (Sunil Kumar et al., 2018). Sadler et al. (2016) also reported that, there were various source of nitrate in groundwater such as animal feedlots, improper disposal of sanitary wastes, agricultural land on which legumes are grown and nitrogen-containing fertilizers. According to Mina Parvizishad et al. (2016), nitrite and nitrate are also present in foods as food additives. These compounds were identified in the vegetables and processed meat and act as promoters of some food colours and preservatives in order to prevent growth of Clastridium botulinum bacteria. Another study also stated that nitrite is also a compound that used for the colour and flavor of cured meats (Bedale, Sindelar, & Milkowski, 2016).

2.4) Nitrate in Groundwater

Groundwater is one of the source of water for agricultural, industrial purposes, and domestic in many countries of the world (Hosseinifard et. al., 2015). Groundwater also is the world's most extracted raw material with the total volume of fresh groundwater stored on earth is believed to be in the region of 8–10 million km^3 and withdrawal rates in the range of 982 km^3/year and (Saurabh Shukla et al., 2018).

Groundwater contamination usually originate from chemical substance, commonly nitrate that originate from either fertilizers or raw sewage (Muhamad Nur Fakhri MR and Shaharuddin MS, 2017). Nitrate will contaminate the groundwater due to the fertilizer leaches into the soil and migrate to the level where most of the groundwater is present. This continue leaching process will induce the groundwater contamination due to the excessive use of fertilizer (Mahirah Kamaludin, Khalid Abdul Rahim & Alias Radam, 2013). Physical process such as rain and irrigation induce nitrate infiltration through the soil down to the water table before entering the groundwater (Vinod, Chandramouli, & Koch, 2015). Climates also play a role in nitrate infiltration into the underground. As stated by Mehdi, Ludwig & Lehner. (2015) where Total Phosphate (TP) and nitrate increased during wet climates compared to dry climates.

In addition, distance of groundwater from a point source of nitrate also play a role in contamination of groundwater (Aida Soraya Shamsuddin et al., 2016). High nitrate was identified in the well below than 50 meters to the livestock area in the agriculture area. Shallow and old wells typically have higher nitrate contents rather than deep and new wells (Juntakut et al., 2019).

2.5) Drinking Water Quality Standard

The Drinking Water Quality Surveillance Unit, Engineering Services Division, Ministry of Health Malaysia prepared a set of guidelines regarding safe and potable water supply throughout Malaysia in response to the need for an appropriate and realistic of guidelines. In addition, guideline values for nitrate in drinking water had derived from various organizations and countries such as United States Environmental Protection

Agency (US EPA), World Health Organization (WHO), as well as regulatory bodies in New Zealand, European Union, Australia, and Canada (Sadler et al., 2016). According to the Drinking Water Quality Standards, the maximum acceptable value in drinking water of nitrate-nitrogen (NO_3-N) are 10mg/L nitrate - N, or 44.3 ppm nitrate-NO_3.

2.6) Health Risk Assessment

High levels of nitrate in groundwater may pose a risk to human health. Health risk assessment is important in order to detect possible health effects in humans due to exposure to the chemical contaminants (US EPA, 2016). Hazard Quotient (HQ) might be used to determine the likelihood of adverse non-carcinogenic health impact. A hazard quotient (HQ) of 1 or greater signifies a hazardous condition, while for HQ of less than 1 is categorized as an acceptable risk for a non-carcinogenic health effect by the US EPA (Guidance Document on HIA in EIA, 2012). A reference dose for oral exposure (RfD) value that used in this study is 1.6mg/kg/day (US EPA, 2017).

2.7) Health Effect Due to Nitrate

2.7.1) Methemoglobinemia

Methemoglobinemia formed when nitrite binds to hemoglobin, which disrupt the oxygen capacity in blood (Ward et al., 2018). People that exposed to nitrates or nitrites prone to get methemoglobinemia (Mina Parvizishad et al., 2016). A study conducted by Ward et al. (2018) admitted that association between ingestion of drinking water nitrate and methemoglobinemia has strong evidence and after considering other studies. Those who have genetic birth defects in reeducates NADH cytochrome b5, fetuses and infants less than 6 months old are the susceptible group that imposed methemoglobinemia (Mina Parvizishad et al., 2016). Infants maybe exposed to the nitrate contamination due to feed through dried milk (prepared with drinking water contaminated by nitrate) or breastfeeding. Another study by Sadeq at al. (2008) also mentioned that older children (1-7 years) are significantly affected by blue baby syndrome (methemoglobinemia) if drinking water contaminated with nitrate (more than 50 mg/L). The most susceptible group to methemoglobinemia is infants under six months of age due to the enzyme in body inadequately change methemoglobin (nitrite oxidizes the iron in the hemoglobin of red blood cells to form methemoglobin and lack the ability of blood to carry oxygen) to oxyhemoglobin and thus, lead to methemoglobinemia or 'blue baby syndrome'. Moreover, methemoglobin usually low in human body, age one year and above, because they have the ability to rapidly convert methemoglobin back to oxyhemoglobin (Oram, n.d.)

2.7.2) Cancer

Long-term exposure of nitrate can lead to the formation of nitrosamine compounds and these compounds are carcinogenic (Mina Parvizishad et al., 2016). International Agency for Research on Cancer (IARC 2010) classified N-nitroso compounds (NOC) as a probable human carcinogen due to the ingestion of nitrate and nitrite. Jones et al. (2018) reported that increased risk of bladder cancer among postmenopausal women was associated with long-term ingestion of nitrate in drinking water. Long-term average nitrate level ingestion through drinking water had a greater relative risk of bladder cancer among postmenopausal women in Iowa with statistically significant associations for those with 4 or more years at levels > ½ MCL (5 mg/L NO_3-N). In addition, dietary digested nitrate regardless of the vegetable source or animal or menopausal status was not associated with breast cancer. However, breast cancer was significantly ($p<0.05$) increased with high red meat consumption among postmenopausal women (Espejo-Herrera et al., 2016). An ecological study from Slovakia found a positive association between Colorectal Cancer (CRC) and cancers in all digestive system with nitrate levels in drinking water (Schullehner et al., 2018). Another case-control study in Iowa reported that higher nitrate level in drinking water consumed among susceptible subgroup (high red meat and low vitamin C intake) with elevated endogenous nitrosation showed an increased colon cancer risk.

2.7.3) Other Health Effect

Mina Parvizishad et al. (2016) reported that the present study showed an association between consumption of food and drinking water that contain high concentration of nitrates and nitrites and diseases such as methemoglobinemia, cancer, diabetes mellitus, and enlargement of thyroid gland. In addition, a registry-based study in Finland found a positive correlation in type 1 childhood diabetes (T1D) incidence with ingestion of drinking water that contain nitrate. However, it is contrast in an ecological analysis in Italy that showed an inverse correlation with T1D rates and nitrate levels (Ward et al., 2018). Moreover, recent findings from the Framingham Offspring Study suggested that increased risk of death was associated with plasma nitrate when adjusted for glomerular function but no association was observed for incident cardiovascular disease (Maas et al., 2008). Quist et al. (2018) also conclude that there is no association between nitrate in drinking water with pancreatic cancer risk except for dietary intake from processed meat.

CHAPTER 3

METHODOLOGY

3.1) Study Design

The study design for this project was a cross-sectional study in order to determine the nitrate level in groundwater at Kampung Keting in Bachok, Kelantan.

3.2) Study Population

The total population that located in Kampung Keting are 1608 people and 262 houses with an area 1.64 km^2. The population in this study was respondents that live in Kampung Keting, Bachok, Kelantan who used groundwater as the main source for drinking and cooking purposes.

3.3) Study Sample

The study had been conducted on a respondent who meets the inclusion criteria only at Kampung Keting, Bachok, Kelantan. Below are the respondent's criteria for this research study. The inclusive and exclusive criteria will help to determine who is the participant in this study.

Inclusive Criteria	Exclusive Criteria
1. Age 18 years and above 2. Long life residents (at least 6 years). 3. Use groundwater as their main source of drinking water supply	1. Having drinking water source other than groundwater 2. Using water filtration system.

3.4) Study Location

The study location in this study is Kampung Keting, Bachok, Kelantan. Bachok district is one of the ten district in the state of Kelantan. It is situated at east and about 25 km from Kota Bharu. Kampung Keting located in Mukim Gunong, Bachok and has an area of 1.6 km^2.

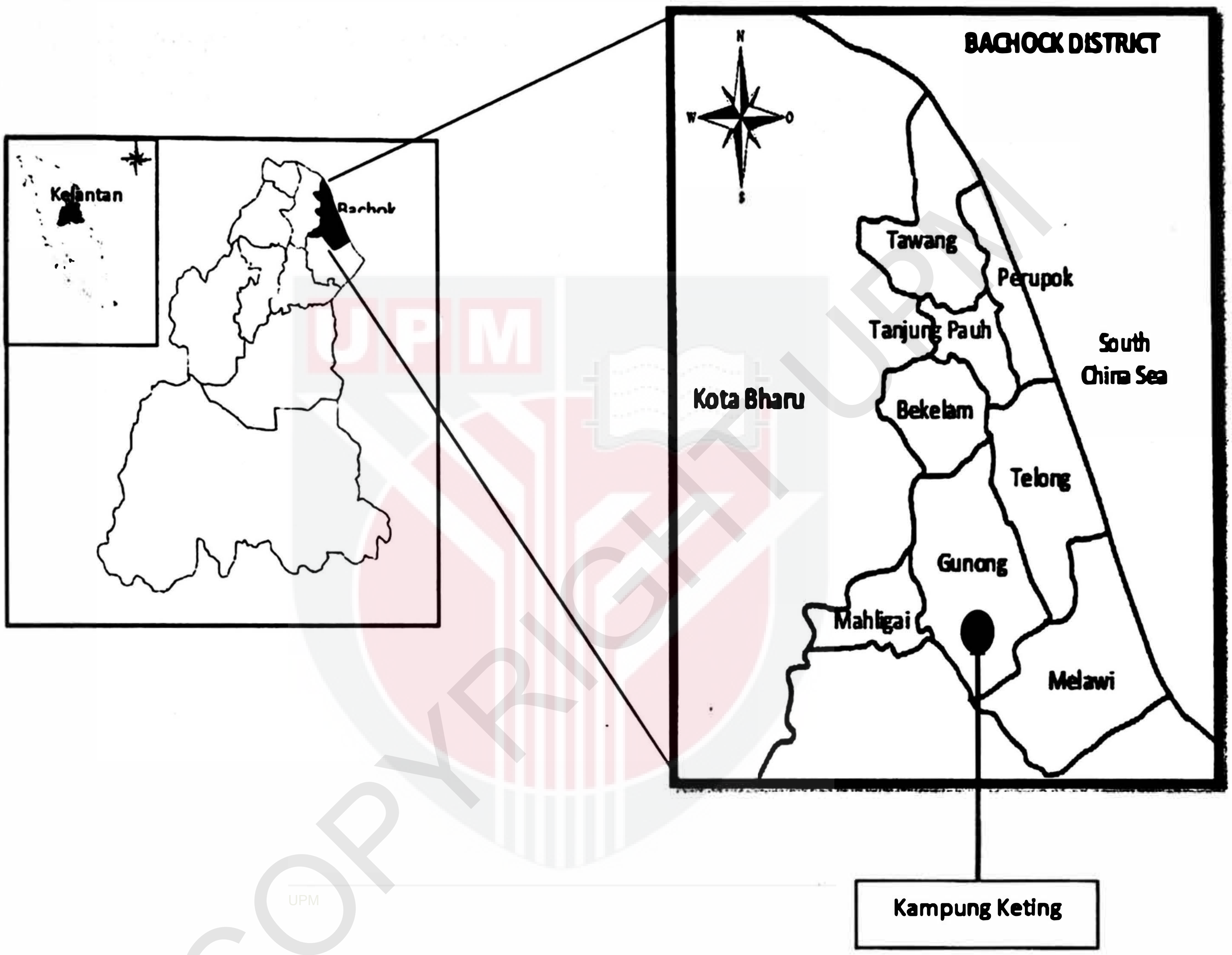

Figure 3.1 Study Location

3.5) Sample Size

The sample size was determined by using a formula establishes by Kirkwood and Sterne (2003):

$N = p(1-p) / e^2$

Where,

N = sample size

P = Prevalence

e = sampling error

Calculation:

$$N = \frac{0.087(1-0.087)}{0.05^2}$$

$$N = 32$$

The prevalence of nitrate level in groundwater which above than 10 mg/L is 8.7% was determined by previous study (Alif Adham Z and Shaharuddin MS 2014). The number of sample size required is 32. To ensure the study is statistically significant and taken into consideration damaged, the sample size was taken is 47 samples.

3.6) Sampling Method

The sampling method that was used in this study was purposive sampling. The respondent had been selected based on inclusive and exclusive criteria.

3.7) Study Instrument

3.7.1) Questionnaire

A set of questionnaires were given to respondent to gather information from the selected respondent. The questionnaires comprised of socio-demography data, water supply types, residential area information and health status of the respondents. The questionnaire was important to ensure the respondents comply with the inclusive criteria. This questionnaire was adapted from the Baseline, Descriptive and Time Activity used in the National Human Exposure Assessment Survey (NHEXAS).

3.7.2) Body Weight

The weight of the respondents was weigh using digital weighing machine. The reading was taken three times to get an accurate result in unit Kilogram (Kg).

3.7.3) Drinking Water Daily Intake

We used standard cup of 200 ml to measure daily water intake rate of water among respondents. Respondents also had to recall back their water consumption amount in a day due to calculate the Chronic Daily Intake (CDI) and Hazard Index (HI).

3.7.4) Global Positioning System (GPS) Mobile Application

Global Positioning System (GPS) Mobile Application was used in this study to determine the distance of well from paddy field area at the study location from each participant. The coordinate of each well was collected and pinned in the GPS and the nearest paddy field area was measured.

3.8) Water Sampling

3.8.1) Water Analysis

Water sample from each respondent's house was collected directly from well by using High Density Polyethylene (HDPE) bottle and replicated by two times. Then, the nitrate level in the water sample were analyzed by using Portable PH/ORP/ISE Meter model HI98191 and Probe Model HI4113 by following standard operating procedure that was given by the manufacturer (Hanna Instrument Company).

Figure 3.2 Portable PH/ORP/ISE Meter model HI98191
Source HANNA Instrument Website

Figure 3.3 Probe Model HI4113
Source HANNA Instrument Website

3.8.2) Analysis of Nitrate

Nitrate in groundwater was analyzed by using Portable PH/ORP/ISE Meter model HI98191 and Probe Model HI4113. First of all, the probe was assembled and attached to the portable meter. Then, the portable meter was calibrated by using buffer solution with concentration of 100ppm and 1000ppm. After that, 50 ml of the water sample was put into the beaker. 1 ml of Ionic Strength Adjuster (ISA) reagent was added into the beaker in order to elevate the nitrate level. Moreover, the probe was the inserted into the beaker in order to read the nitrate level in the water. These steps were repeated for other water samples that represent different well.

3.9) Risk assessment

Chronic Daily Intake (CDI) had been used to calculate the health risk of the population due to the exposure of nitrate contamination in groundwater, using following equation:

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

(Guidance Document on Health Impact Assessment (HIA) in Environmental Impact Assessment (EIA), 2012)

[Equation 1]

Where,

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

C = Nitrate level in groundwater (mg/L),

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

BW = body weight (kg)

For non-carcinogenic health effects posed by nitrate in drinking water, the Hazard Index (HI) was calculate by using the following equation:

$$HI = \frac{CDI}{RfD}$$ (Nazaroff & Alvarez-Cohen, 2001)

[Equation 2]

Where,

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

RfD = Reference dose (mg/kg/day)

If the HI value show more than 1 (HI>1), it is show that there is a significant risk level. The higher the value, the greater the likelihood of adverse non-carcinogenic health impact. RfD value for nitrate in this study is 1.6mg/kg/day (US EPA, 2017).

3.10) Data Analysis

The data obtained were analyzed by using the IBM SPSS (Statistical Package for the Social Sciences) Version 22. The type of analysis for this study is shown in the table below, based on the objectives of this study.

Objectives	Type of analysis
To determine the weight of respondent in order to calculate health risk assessment	Descriptive Analysis
To identify the age, depth, and distance of the well	Descriptive Analysis
To identify the nitrate level in groundwater	Descriptive Analysis
To determine the association between age of well, depth of well, and distance of well from paddy field area with nitrate level	Parametric: Pearson Correlation Non-Parametric: Spearman correlation
To determine the health risk associated with nitrate level in groundwater	Descriptive Analysis

3.11) Ethical Consideration

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

b. 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.

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

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

CHAPTER 4

RESULTS AND DATA ANALYSIS

4.1) Number of Respondents

Table 4.1 shows the total number of respondents who participated in this study was 47. From Figure 4.1, the number of male respondents was 15 (31.9%) while the number of female respondents was 32 (68.1%).

Table 4.1 Number of male and female respondents in Kampung Keting.

Variables	Respondent (%)
Respondents	
Male	**15 (31.9%)**
Female	**32 (68.1%)**

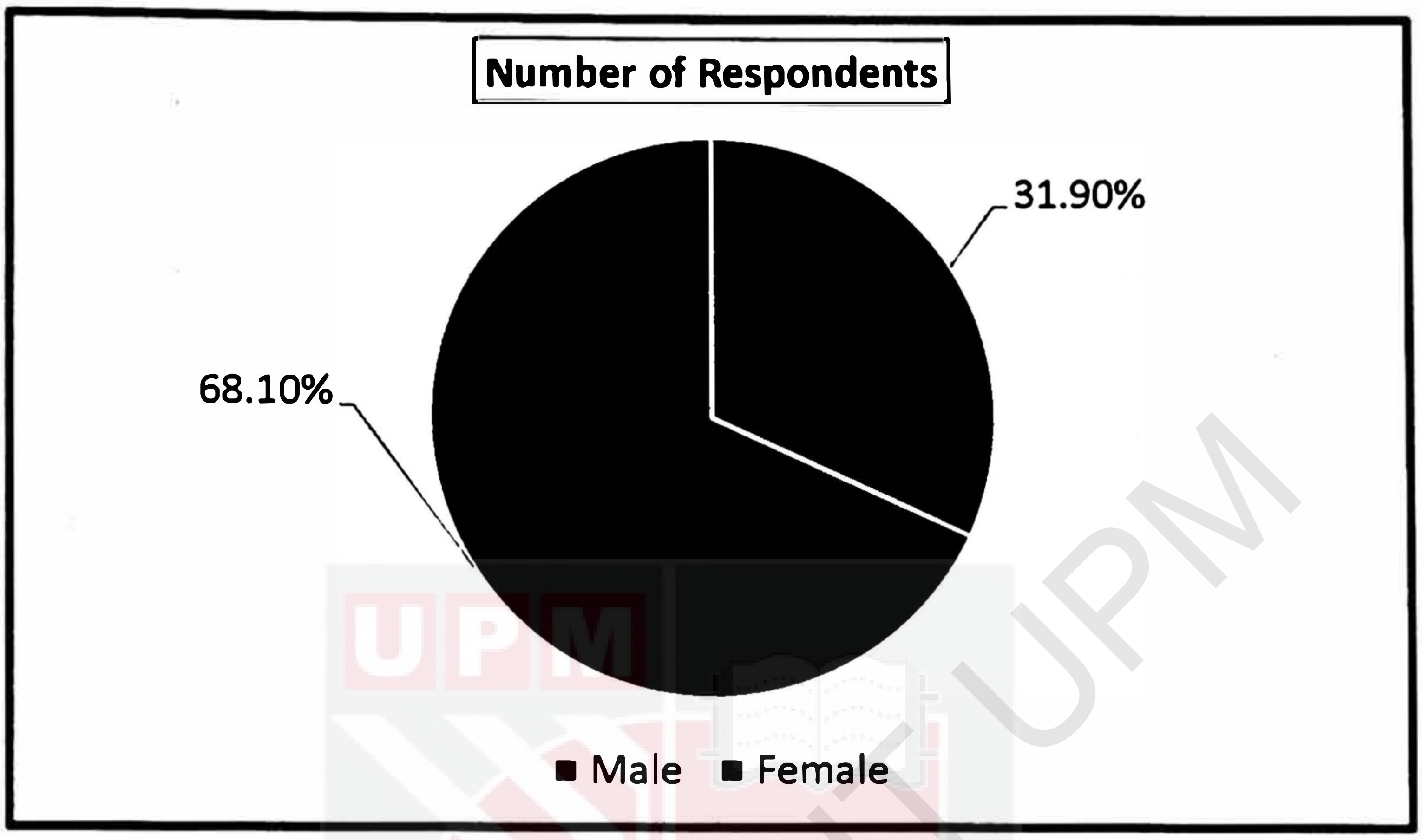

Figure 4.1 Percentage of Respondents

4.2) Weight of The Respondents

From the table below, it shows that Mean ± SD of the weight of the respondents which was 63.72 ± 12.59 (kg) and the respondents' weight range between 35.0 – 105.0 (kg).

Table 4.2 Weight of The Respondents

Variable	Mean ± SD (kg)	Range (kg)
Weight	**63.72 ± 12.59**	**35.0 – 105.0**

N = 47

4.3) Characteristics of The Well

Table 4.3 below shows the characteristics of the well that analyzed from the questionnaires. From Figure 4.2, most of the samples were collected from the well with age more than 20 years (>20) which is 19 (40.4%) of wells out of 47, while followed by 5 – 10 years, 16 – 20 years, below than 5 years (<5), and 11 – 15 years with the frequency 10 (21.3%), 9 (19.1%), 7 (14.9%), and 2 (4.3%) respectively.

In addition, information about the depth of the well also was obtained from the respondents. From Figure 4.3, most of the wells have a depth between 5 – 10 meters, which is 28 (59.6%) from 47 of the samples. Other readings are 11 – 15 meters by 16 (34%) of the respondents, meanwhile only 3 (6.4%) of the respondents had more than 15 meters (>15m) of the depth of the wells. None of them had the depth below than 5 meters (<5m).

After that, the distance of the wells to the nearest paddy field was measured by using GPS. It is shown that, the result for Mean ± SD is 157.3 ± 102.1 meters while the range obtained is 20.0 – 500.0 meters.

Table 4.3 The characteristics of wells of the respondents

Variables	Frequency(%)	Mean ± SD (meters)	Range (meters)
Age of Well			
< 5 years	7 (14.9%)		
5 – 10 years	10 (21.3%)		
11 – 15 years	2 (4.3%)		
16 – 20 years	9 (19.1%)		
> 20 years	19 (40.4%)		
Depth of Well			
< 5 meters	0		
5 – 10 meters	28 (59.6%)		
11 – 15 meters	16 (34%)		
> 15 meters	3 (6.4%)		
Distance of Well From Paddy Field (meters)	**47 (100%)**	**157.3 ± 102.1**	**20.0 – 500.0**

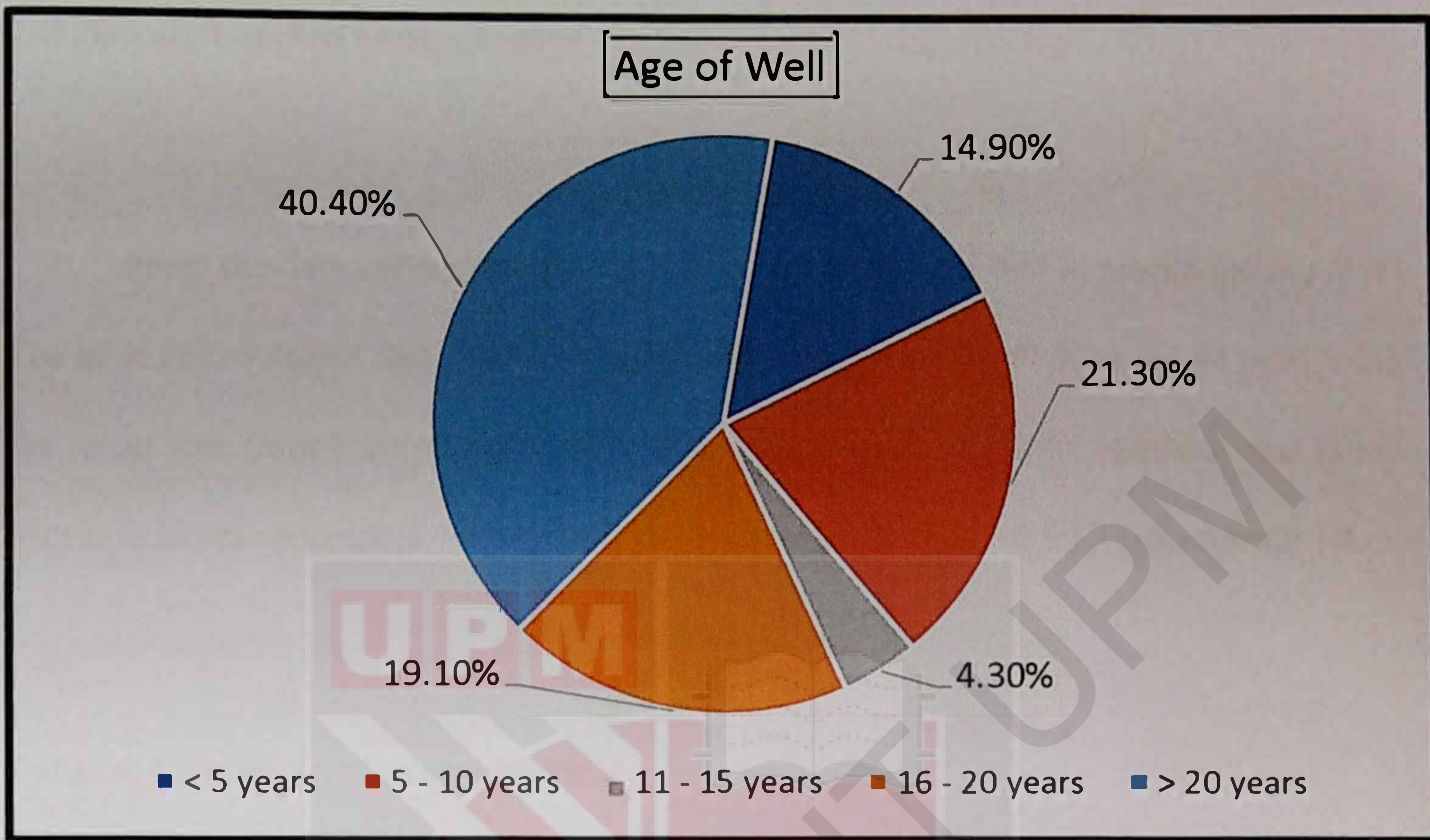

Figure 4.2 Percentage of age of well

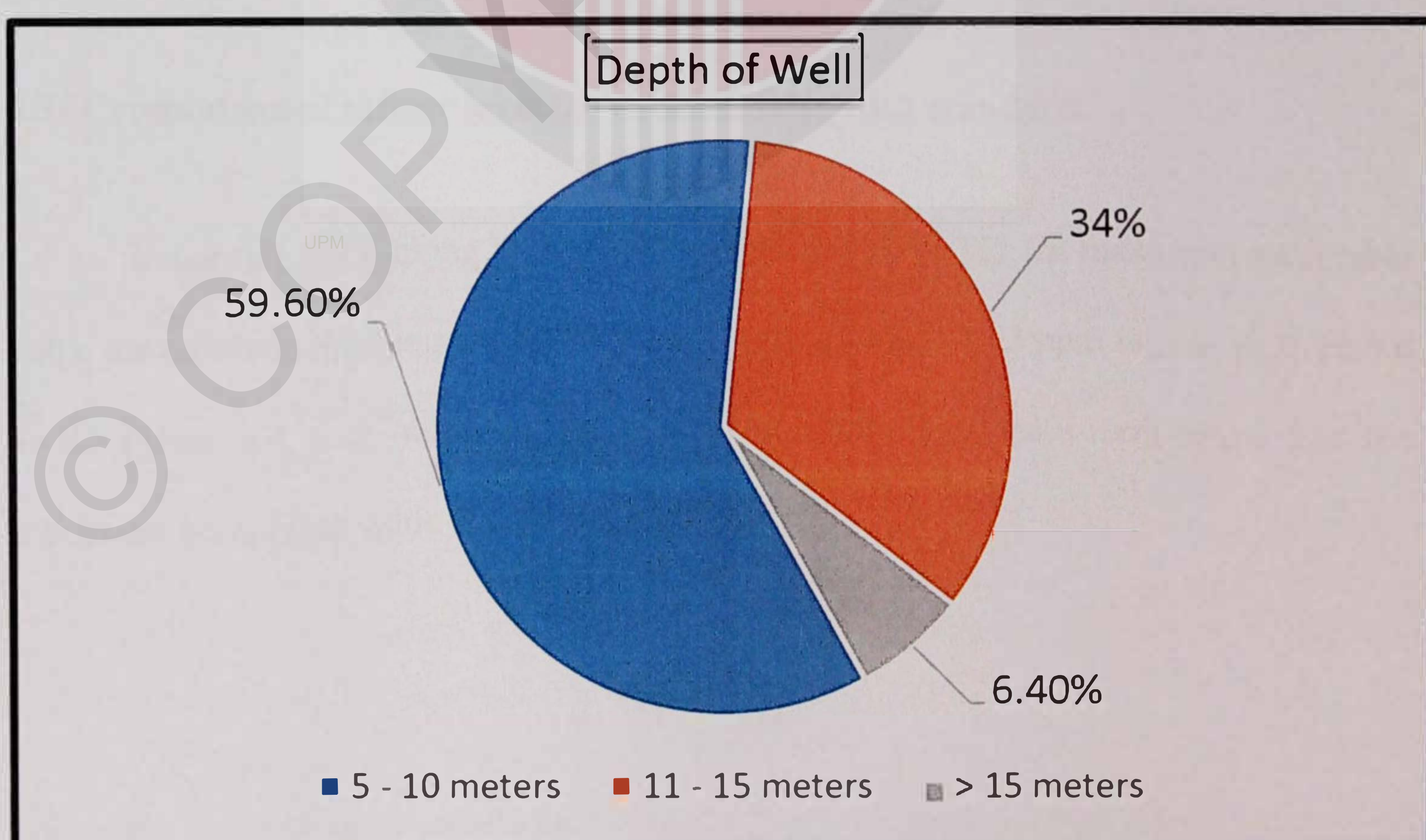

Figure 4.3 Percentage of depth of well

4.4) Nitrate Level in Groundwater

From the data collection, the value that had been used was in nitrate (NO_3^-) level. The table below shows that, the Mean ± SD of the nitrate level was 5.34 ± 4.94 ppm, while the range was from 0.40 ppm to 23.65 ppm. The amount of nitrate obtained was below than maximum acceptable value which is 10 mg/L nitrate - N, or 44.3 ppm nitrate-NO_3.

Table 4.4 Nitrate level in groundwater

Variable	Mean ± SD (ppm)	Range (ppm)
Nitrate	**5.34 ± 4.94**	**0.40 – 23.65**

N = 47

4.5) Comparison of nitrate level for each samples with standard.

Regarding to Drinking Water Quality Standard (DWQS), the maximum acceptable value for nitrate in drinking water is 10 mg/L nitrate - N, or 44.3 ppm nitrate-NO_3. Based on the Figure 4.4, it showed that nitrate level from all the samples were below than the maximum acceptable value (MAV) of DWQS.

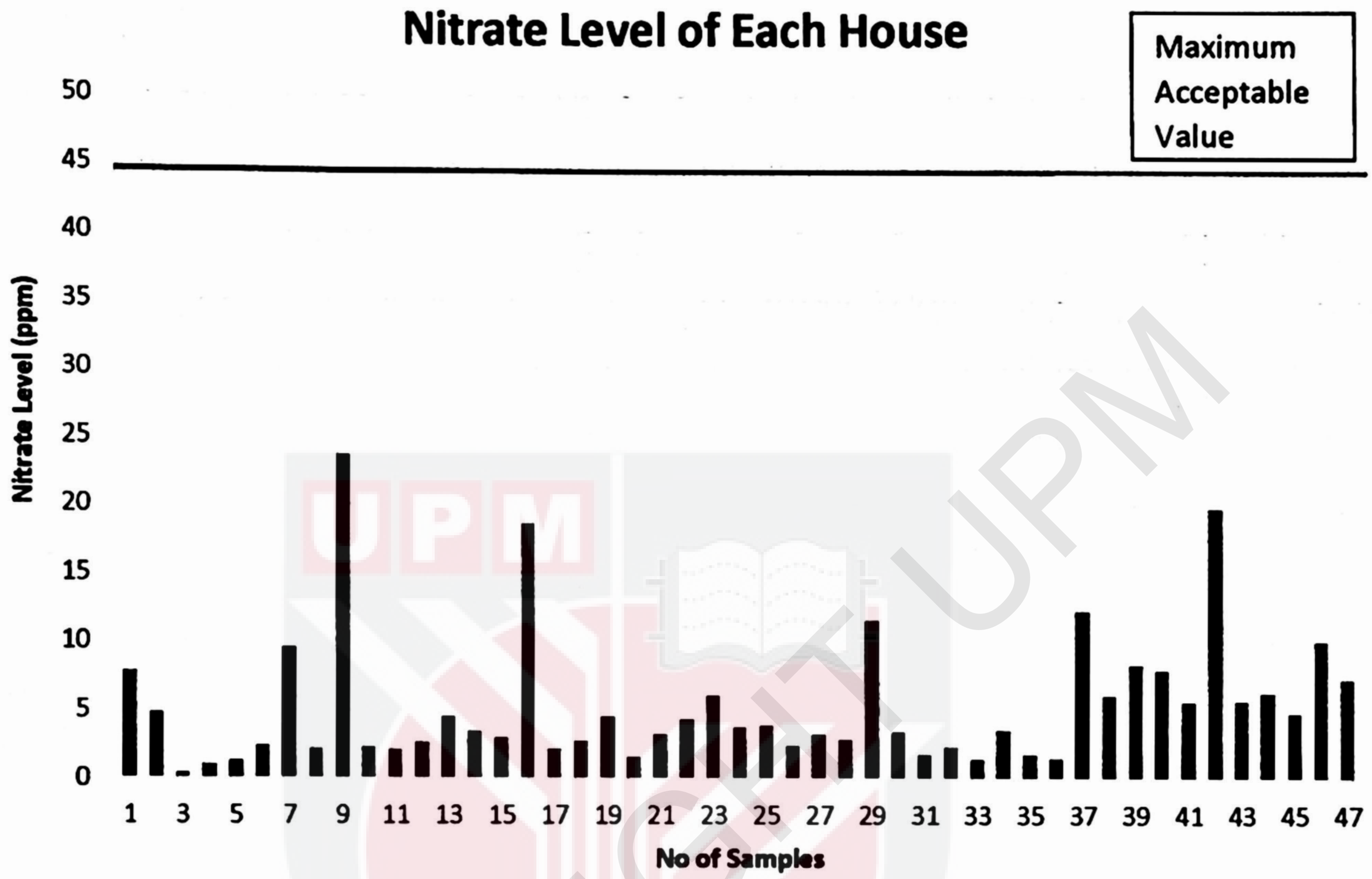

Figure 4.4 Comparison of Nitrate Level with Drinking Water Quality Standard (DWQS)

4.6) The association between age of well, depth of well and distance of well from paddy field with nitrate level (Spearman Rho Correlation test).

In this study, the test that was used to find the association between the age of well, the depth of well and the distance of well from paddy field with nitrate level is Spearman Rho Correlation test due to non-parametric value for nitrate level. Both age (years) and distance (meter) had no significant different with nitrate level ($p>0.05$). However, there is significant different between depth(meter) and nitrate level ($p < 0.05$) with correlation coefficient, $r = -0.348$.

Table 4.5 Spearman Rho Correlation test

Variable	**Nitrate Level**	
	r	**p - value**
Age(years)	0.03	0.84
Depth (meter)	-0.348	0.017
Distance from source (meter)	0.226	0.127

N=47

4.7) Chronic Daily Intake (CDI) estimation

Table 4.6 showed the result of Chronic Daily Intake (CDI) estimation of the respondents. For daily water intake, the Mean ± SD was 1.23 ± 0.25 (L/day) and had a ranged between 0.6 – 1.6 (L/day).

After that, CDI was calculated based on the equation stated in Chapter 3. The result showed that, the Mean ± SD for CDI was 0.11 ± 0.10 (mg/kg/day) and obtained a range between 0.01 – 0.41 (mg/kg/day).

Table 4.6 Chronic Daily Intake (CDI) estimation

Variable	N = 47			
	Mean ± SD	**Median**	**IQR**	**Range**
Nitrate Level (ppm)	5.34 ± 4.94	3.49	3.99	0.40 – 23.65
Daily Water Intake (L/day)	1.23 ± 0.25	1.2	0.4	0.6 – 1.6
Weight (kg)	63.72 ± 12.59	64	17	35.0 – 105.0
CDI (mg/kg/day)	0.11 ± 0.10	0.07	0.08	0.01 – 0.41

4.8) Hazard Index (HI) estimation

Hazard Index (HI) was calculated by using the equation in Chapter 3 in order to estimate non-carcinogenic risk that exposed by the respondents. The result in the Table 7 showed that the Hazard Index (HI) was less than 1 (HI < 1) for all respondents. This indicates that there is no risk from nitrate exposure to the respondents.

Table 4.7 Hazard Index (HI) estimation value

Hazard Index (HI)	*No. of Respondent*	*Percentage (%)*
HI < 1	47	100
HI > 1	*0*	*0*

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1) Background of Respondents

A total of 47 respondents from Kampung Keting had been involved in this study. Most of the respondents were female which was 32 (68.1%) respondents, while 15 (31.9%) respondents are male. All of them were selected based on inclusive criteria, which were 18 years and above and considered as an adult, lifelong resident in Kampung Keting at least 6 years and use groundwater as their main source of drinking water supply. The weight of the respondents also had been collected in order to conduct health risk assessments with Mean ± SD was 63.72 ± 12.59 (kg) and had ranged between 35 kg to 105 kg.

The selection of respondents was strictly based on inclusion and exclusion criteria but any exposure to nitrate contamination from other sources had not been controlled.

5.2) Characteristics of Wells

Three characteristics of the wells which had been collected were age, depth and its distances from the paddy field area. The data obtained for age of wells was shown in Figure 2. The lowest age was between 11- 15 years, which 2 (4,3%) wells only, while followed by below than 5 years (< 5), 16-20 year, 5-10 years, and higher than 20 years (> 20) with the frequency 7 (14.9%), 9 (19.1%), 10 (21.3%) and 19 (40.4%) respectively.

Next, the data obtained from the depth of wells was showed in Figure 3. From Figure 3, there was no data collected from the depth below than 5 meters (< 5). Most of the wells had a depth between 5-10 meters, which was 28 (59.6%) of wells from 47, while followed by 11 – 15 meters and more than 15 meters (> 15) with the frequency 16 (34%) and 3 (6.4%) respectively. In addition, the distance of the wells to the nearest paddy field area was measured by using GPS. The Mean ± SD obtained was 157.3 ± 102.1 meters while the ranged was 20.0 – 500.0 meters.

These three variables were identified in this study because they are potentially contributed to elevate nitrate level in the groundwater (Swistock, Clemens, & Sharpe, 2009). This study stated that older wells (usually shallow) are allowing nitrate in water to enter deeper groundwater and the nearest cornfield and other crop fields to the well typically end up with nitrate contamination in the wells.

5.3) Nitrate Level in Groundwater

Regarding to the results of nitrate level, the Mean ± SD of nitrate level was 5.34 ± 4.94 and the maximum level of nitrate was 23.65 ppm nitrate – NO_3 which is still below than maximum acceptable value. Although the concentration of nitrate was less than maximum acceptable value, consumption of excess nitrate through drinking water could pose serious health risk and toxicity in humans (Ahada & Suthar, 2018).

Data collection was conducted during main season, which was from August to February every year (Kemubu Agricultural Development Authority, 2018). Surface water was used as the main source of water to irrigate for paddy cultivation during this season. Noraziah Jamaludin et al. (2013) stated that nitrate level will increase in groundwater when the water table increased and also due to leachate of nitrogenous fertilizer into well water during rainy season.

In addition, two types of fertilizers with their chemical composition used during paddy cultivation in Kelantan are Urea (46% N, 20% P, 10% K) and Compound (17% N, 20% P, 10% K) (Skim Baja Padi Kerajaan Persekutuan, 1998). These fertilizers usages can induce nitrate level in groundwater, especially to the groundwater located near the paddy field area. Muhamad Nur Fakhri MR and Shaharuddin MS. (2017) also mentioned that nitrate fertilizers can contaminate the groundwater by leaching into the soil and enter the nearest groundwater.

Since all the nitrate level at each house below than the standard, this may be due to the data collection during pre-planting of paddy field. This is because, fertilizer was not yet applied during pre-planting phase and this phase had the lowest reading of nitrate level rather than other phases which is planting phase and harvesting phase (Amirah Ahmad Roslan, Shaharuddin Mohamad Sham, & Sharifah Norkhadijah Syed Ismail, 2014).

5.4) Comparison with Drinking Water Quality Standard (DWQS)

Each country has different standards for nitrate in groundwater in order to ensure the groundwater is safe to drink and consume by users. In Malaysia, standard for nitrate level as stated by Drinking Water Quality Standard (DWQS) is 10 mg/L nitrate - N, or 44.3 ppm nitrate-NO_3. Thus, nitrate level that had been collected on January 2019 was below than the standard.

From the previous study in district Bachok and Kota Bharu, Kelantan, nitrate levels were within acceptable limit (< 44.3 ppm) and the health risk also were acceptable (HI < 1) (Amirah Ahmad Roslan et al., 2014). Another study by Alif Adham Z and Shaharuddin MS. (2014) also stated that nitrate levels were below than the acceptable limit from all sampling sites in Pasir Puteh, Kelantan.

The nitrate concentration at different places were determined by various factors. Various anthropogenic activities such as chemical fertilizers used in agriculture, urban activity and industrial development may result in effluent disposal and increase nitrate and sulphate level in groundwater (Devic, Djordjevic & Sakan, 2014). Plus, level of nitrate contamination in groundwater also depends on geological background, land-use pattern, soil-drainage capacity, and type of aquifer (Jaturong et al., 2015).

5.5) The association between age of wells, depth of wells and distance of wells from paddy field with nitrate level (Spearman Rho Correlation test).

Based on the analysis, there was no significant correlation between age of wells and nitrate level. This indicates that age of the wells did not contribute to the nitrate contamination in this study. However, a study by Swistock et al. (2009) had different result where the age of wells was related to the nitrate level in groundwater. The study also stated that the older wells were typically shallow wells and lead to nitrate-rich in water.

From the analysis also shows that there was no significant correlation between the distance of wells from a paddy field with nitrate level. However, from the previous study showed that the agriculture activities potentially contaminate the groundwater. Aida Soraya Shamsuddin et al. (2016) had stated that the well below 50 meters to the livestock area in the agriculture area contained high nitrate level. Plus, groundwater flow direction also should be taken account which is an important factor in nitrate contamination (Koh & Kim, 2015). So, present of high nitrate level in groundwater in that area, possibly due to factors such as atmospheric deposition, leaking sewers and discharge from the septic tank (Pastén-zapata et al., 2014).

In addition, depth of the well was the only variable had significant correlation with nitrate level in this study. The result obtained between these two variables was a negative and fair correlation (r = -0.348). This indicates that nitrate level tends to decline with increasing depth of the wells. This result was consistent with previous studies where private and shallow depth wells were contained higher nitrate levels (Khademikia et al., 2013) and more liable to contamination compared to deep wells due to anaerobic environment (Sahele, Zewdie & Narayanan, 2018).

5.6) Health Risk Assessment (Hazard Index)

Based on the result obtained, Chronic Daily Intake (CDI) estimation for all of the respondents had 0.11 ± 0.10 (mg/kg/day) for Mean ± SD and acquired a range between 0.01 – 0.41 (mg/kg/day). After that, this result was used to calculate Hazard Index (HI) by using the equation that stated in Chapter 3. Hazard Index (HI) values for all respondents showed in this study were less than 1 (HI<1). Thus, this indicates that the risk was acceptable to all respondents.

This result is consistent with Noraziah Jamaludin et al. (2013) where the hazard index was less than 1 and indicated that the risk is not significant. Noraziah Jamaludin et al. (2013) did a study in three villages in district Bachok, Kelantan namely as; Kampung Kandis, Kampung Telong, and Kampung Aman. However, the result was different from Aida Soraya Shamsuddin et al. (2016) where the Hazard Index (HI) value that exceeds safe limit was 2.34% from study population. Discharge from the septic tanks and leaking sewers were identified as the factors that contribute to the high concentration of nitrate.

5.7) CONCLUSION

Water is one of the most important resources in our daily life. Everyone has to make sure the quality of water for our daily uses must always clean and safe for drinking and cooking purposes. Contamination of water from nitrate or any other contaminants should be avoided. This is because contamination of water can cause many health problems.

In this study, it shows that nitrate level from each houses was below than maximum acceptable value of nitrate-NO_3 (44.3 ppm). This value was still considered safe for drinking and cooking purposes. After conducted health risk assessment, the result obtained for Hazard Index was lower than 1 (HI < 1). This indicate that there was no adverse health effect to the villagers due to nitrate exposure. However, the condition of the nitrate in the groundwater will vary from time to time and will likely exceed the maximum acceptable value. Thus, effort to minimize any further exposure of nitrate towards human as well as ecosystem and environment should be put as vital concern.

5.8) LIMITATION

First of all, the information obtained from the respondents could not be determined to be 100% valid. This is due to recall bias may contribute to this study.

Next, the sample size in this study cannot represent the whole district Bachok, Kelantan. However, the data obtained can be guideline value for nitrate level in Kampung Keting for future research.

Last but not least, the data obtained for nitrate concentration only during pre-planting season due to time constraint. The data should be obtained for other phases which is planting phase and harvesting phase in order to observe significantly different from the nitrate level between phases.

5.9) RECOMMENDATION

First of all, this was a cross-sectional study. The data was collected at one point of time only and further studies are recommended where the data can be collected at different point of time.

Next, the study location in this study does not represent the whole district or state. Additional investigation should be done in a wider population and larger sample size so that the results of the study can be generalized to the population.

This study suggests that if villagers want to create a new well, it is advisable to make wells away from source of nitrate such as septic tanks, agricultural activities and animal feedlots. The well should be dug deeper than usual in order to get clean water and to reduce the water from becoming contaminated. This is important for residents who need to use groundwater as the main source of water in daily life.

Health authority should play their role to monitor nitrate level in the groundwater from time to time. The level of nitrate in the groundwater may vary at different time and place. Health authorities should take proactive step so that contamination of nitrate in groundwater can be prevented. Besides that, the villagers are recommended to use water filtration system in order to minimize the exposure to the water contamination.

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APPENDIX 1

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

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

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

1. STUDY TITLE :

Contamination Of Nitrate In Groundwater And Evaluation Of Health Risk In Bachok, Kelantan: A Cross-Sectional Study

2. INTRODUCTION:

Nitrate (NO_3^-) is one of the compounds of nitrogen. It exists together with different type of nitrogen in a complex cycle. Nitrogen in soil and water usually come from atmospheric deposition, uses of 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-demography data, water supply types, resindetial area information and health status of the respondents. Then, researcher will measure the level of nitrate in your groundwater by using Portable PH/ORP/ISE Meter. Researcher also will take a weight measurement of the respondents to calculate the risk assessment.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Participants who meet these criteria:

a. Having water source other than groundwater
b. Using water filtration system

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

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All the information and identify that are provided by the respondents will remain confidential

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

If you have any questions you can contact the researcher responsible for the study or supervisor of this research.

Muhammad Azri Bin Mat Yusof
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University Putra Malaysia,
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Dr. Shaharuddin Bin Mohd Sham (Supervisor)
Department of Environmental and Occupational Health,
Faculty of Medicine and Health Sciences,
University Putra Malaysia.
Tel: 03 8947 2407
Email: shaha@upm.edu.my

Please initial here if you have read and understood the contents of this page______

9. CONSENT

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)

Signature ………………………………… (Witness)

Date :……………………………………..

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 2

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

Research title	:	**Contamination of Nitrate in Groundwater and Evaluation of Health Risk in Bachok, Kelantan: A Cross-Sectional Study**
Study Site	:	**Bachok, Kelantan**
JKEUPM Ref No.	:	**JKEUPM-2018-387**
Researcher	:	**Muhammad Azri bin Mat Yusof**
Supervisor	:	**Dr. Shaharuddin bin Mohd Sham**

Documents received and reviewed with reference to the above study:

1. Ethics Application Form, Version 1 dated 29/10/2018
2. Respondent Information Sheet & Consent (English), Version 1 dated 29/10/2018
3. Respondent Information Sheet & Consent (Malay), Version 1 dated 29/10/2018
4. Proposal (English), Version 1 dated 29/10/2018
5. Questionnaires/ Interviews (Malay), Version 1 dated 29/10/2018
6. 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 16 NOVEMBER 2019**

Researchers should comply with the following:

I. Complete a Study Final Report upon study completion (Form 3.2).

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

III. Applicable for Clinical Trial Studies and Clinical interventional Studies only: Progress Report has to be submitted to JKEUPM at every 6 months from the date of approval (Form 3.1). Report

APPENDIX 3

BORANG SOAL SELIDIK

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

No ID : ☐☐☐☐☐☐☐☐☐☐

Umur : ☐☐ tahun

Jantina : ☐ Lelaki ☐ Perempuan

Pekerjaan : ....

Pendapatan Sebulan : RM ....

Taraf Pendidikan :

☐ Tidak bersekolah
☐ Sekolah rendah
☐ Sekolah menengah
☐ Universiti
☐ Lain-lain

Berat : .... kg

Bahagian B: Maklumat penggunaan air paip

Apakah punca air paip di rumah?

☐ Air Kelantan SB ☐ Bawah Tanah ☐ Kedua-duanya ☐ Lain-lain, sila nyatakan:

Jika kedua-duanya, yang manakah yang lebih digunakan: ☐ AKSB ☐ Bawah Tanah ☐ Sama rata

Berapa gelas air yang anda minum setiap hari? .... gelas (200 ml)

Penggunaan air dari dapur: ☐ Memasak ☐ Minum ☐ Kegunaan domestik ☐ Lain-lain,

sila nyatakan ....

Adakah anda berpuas hati dengan kualiti air paip yang digunakan? ☐ Ya ☐ Tidak ☐ Tidak Pasti

Adakah anda menggunakan sistem penapisan air persendirian di rumah? ☐ Ya ☐ Tidak

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

Bahagian C: Maklumat persekitaran tempat tinggal

Adakah terdapat kawasan pertanian berdekatan dengan tempat tinggal anda?

☐ Ya ☐ Tidak ☐ Tidak pasti

Apakah jenis perpaipan air di rumah? ☐ Logam ☐ PVC ☐ Tidak pasti

Umur Telaga ☐ < 5 Tahun ☐ 5-10 Tahun ☐ 10-15 Tahun ☐ 16-20 Tahun ☐ > 20 Tahun

Kedalaman telaga (m) ☐ < 5 m ☐ 5-10 m ☐ 11-15 m ☐ >15 m

Jenis telaga ☐ Terbuka ☐ Tertutup ☐ Boring ☐ Lain-lain, sila nyatakan ....

Bagaimana air diambil daripada telaga? ☐ Menggunakan baldi ☐ Pam elektrik ☐ Lain-lain, sila nyatakan................

Bahagian D: Maklumat Kesihatan

Adakah anda mempunyai masalah kesihatan serius: ☐ Ya ☐ Tidak

TERIMA KASIH ATAS KERJASAMA ANDA
-TAMAT-

NAMA PENUH RESPONDEN	
NOMBOR RUMAH	
NAMA JALAN	
NAMA KAMPUNG	
JARAK DARIPADA SAWAH/SISTEM SEPTIK (M)	
KOORDINAT GEOGRAFI	
MAKLUMAT TAMBAHAN	
TANDATANGAN ENUMERATOR & TARIKH	

APPENDIX 4

On-site work pictures