Contamination of Nitrate in Groundwater and Evaluation of Health Risk in Bachok, Kelantan: A Cross-Sectional Study

Aida Soraya Shamsuddin, Sharifah Norkhadijah Syed Ismail, Emilia Zainal Abidin, Ho Yu Bin and Hafizan Juahir

Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia

East Coast Environmental Research Institute (ESERI), Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, 21300 Kuala Terengganu, Terengganu, Malaysia

Abstract: High concentrations of nitrate through drinking water have been associated with health problems. This cross sectional study sought to determine the level of nitrate concentrations in private well water and the association to the disease caused by nitrate among population of Bachok, Kelantan. The concentrations of nitrate in 256 wells were sampled from September to October 2015. About 126 respondents from the agricultural area and 130 respondents from the non-agricultural area were participated in the study. The human health risk associated with ingesting nitrate were assessed by Hazard Quotient (HQ) and Odd Ratio (OR). The physicochemical characteristics of well water in both areas (i.e., pH, ammonia, Dissolved Oxygen (DO), conductivity, turbidity, Total Dissolved Solid (TDS) and salinity) were within the acceptable limits of Malaysian Drinking Water Quality Standard. The mean ± SD levels of nitrate in the agricultural area was 13.04±14.39 mg L⁻¹ NO₃⁻-N, exceeding the maximum acceptable limits of Malaysia NDWQS (10 mg L⁻¹ NO₃⁻-N) and were two fold higher than the non-agricultural area (6.31±5.22 mg L⁻¹ NO₃⁻-N). 52 wells (41.27%) in the agricultural area and 35 wells (26.9%) in the non-agricultural area had nitrate level above the maximum acceptable nitrate (10 mg L⁻¹ NO₃⁻-N). The HQ associated with the potential non-carcinogenic risk of drinking nitrate contaminated groundwater ranged from 0.007 to 1.143×10⁻⁶ in the agricultural area, slightly higher than in the non-agricultural area (0.002 to 0.468×10⁻⁶). The OR for disease such as diabetes, goitre and gastric were not significant with high levels of nitrate in the well water. The results of the present study showed that there was no statistically significant association between nitrate in well water and the risk of related health disease such as diabetes, goitre and gastric in this study.

Keywords: Nitrate, Groundwater, Agricultural Area, Health Risk, Drinking Water

Introduction

Nitrate contamination is one of the most widespread groundwater problems worldwide (Almasri, 2007; Gupta et al., 2008). Nitrate contamination in groundwater arise from point sources such as livestock facilities, sewage disposal systems, including septic tanks and non-point sources such as fertilized cropland, or naturally occurring sources of nitrogen (Manassaram et al., 2006; Yang et al., 2007; Gupta et al., 2008). Nitrate is soluble and negatively charged and thus has a high mobility and potential for loss from the unsaturated zone by leaching and can remains in groundwater for decades (Manassaram et al., 2006). Many studies showed high correlation and association between agriculture activities and nitrate concentration in groundwater due to the extensive use of nitrate-nitrogen fertilizers (Chowdary et al., 2005; Dunn et al., 2005; Jordan and Smith, 2005; Liu et al., 2005; Almasri, 2007; Yang et al., 2007).
Many factors influence the occurrence and concentration of nitrate in groundwater. Water from shallow wells (less than 100 feet below land surface) in areas with well-drained soils and high nitrogen inputs (e.g., proximity to agricultural areas) possibly have nitrate-rich groundwater (Manassaram et al., 2006). Private wells are usually shallower and are closer to sources of nitrate contamination, whereas public supply wells are usually in deeper groundwater aquifers where contamination is less likely (Manassaram et al., 2006). High nitrate concentration in drinking water causes health problems such as methemoglobinemia (blue baby syndrome) in infants, thyroid disorders, spontaneous abortions and birth defects and cancer in adults (Almasri, 2007; Gupta et al., 2008; Chang et al., 2010). As such, the U.S. Environmental Protection Agency (EPA) has established Maximum Contaminant Level (MCL) for nitrate in drinking water of 10 mg L\(^{-1}\) nitrate-nitrogen (\(\text{NO}_3^-\text{N}\)) and the World Health Organization (WHO) guideline of 11 mg L\(^{-1}\) as \(\text{NO}_3^-\text{N}\) were promulgated to protect against methemoglobinemia (Ward et al., 2005). However, this standard applies only to public water supplies, not to private wells and it was not based on the estimates of cancer risk (Ward et al., 2003). The effectiveness of this regulatory limit for preventing other health risks such as cancer has not been adequately studies (Yang et al., 2007).

In Malaysia, the demand for water has increased tremendously and groundwater has been identified as one of the alternatives to new sources. In Kelantan, almost 70% of people consumes groundwater in their daily lives (Zawawi et al., 2010). However, due to uncontrolled development and human activities, groundwater is subjected to pollution. The shallow aquifer system in the Kelantan river delta constitutes an important source of water not only for public water supply, but also for domestic and agricultural purposes. Being shallow and relatively unprotected, the aquifer is generally exposed to higher risk of nitrate contamination due to anthropogenic activities at the surface. In Kelantan, the climate changes are obvious and produce significant impacts on groundwater levels which contribute towards the nitrate contamination of groundwater in the shallow aquifers.

The objectives of this study were to determine the level of nitrate in the well water and the association to the disease caused by nitrate among population of Bachok, Kelantan. This study provides baseline data on the level of nitrate in the groundwater and the evaluation of health hazard risk by hazard quotient method and the possible association with the health symptoms among the population. The results of this study may be beneficial in establishing a groundwater protection plan to support the sustainable utilization of groundwater resources in Kelantan.

Materials and Methods

Study Area

A cross-sectional study was performed in Bachok, Kelantan (6° 4’ 0” North and 102° 24’ 0” East), a north-eastern state in Peninsular Malaysia (Fig. 1). The study was conducted from September to October 2014. Paddy cultivation is the main activity of Bachok population. Almost 70% of community in Kelantan used groundwater for drinking (Zawawi et al., 2010). Problems such as dirty and smelly water supply, low coverage performance and frequent water disruption had caused the population to use groundwater as their alternative water sources (Kamaludin et al., 2013). However, groundwater is subjected to pollution due to uncontrolled development and human activities such as agriculture. Two areas were selected for this study, agricultural area (focus on paddy field) and non-agricultural area for comparison.

Data Collection

This study utilised questionnaire as a tool. A set of structured questionnaires were used in this study which comprised of three sections. The first section contained questions regarding respondent’s background information such as the age, gender, education, monthly income and duration of residence. The second section contained questions regarding water consumption and physical characteristics of the respondent’s well while in the third section contained the questions about the health status of respondents. Consent forms were obtained from each respondent before interview prior to the interview. The respondents were informed that the questionnaires could be answered voluntarily, anonymously and the information would be treated confidentially. The interviews took approximately 10 min.

Well water was sampled from 256 private wells (126 wells in the agriculture site and 130 wells in the non-agriculture site) during wet season of 2014 (September to October). The well water was collected using the High-Density of Polyethylene (HDPE) bottles.

The bottles were cleaned by soaking them in diluted HNO\(_3\) for 24 h and they were washed thoroughly with deionised water and dried before water sampling. Chemical properties of well water such as pH, temperature, dissolve oxygen, conductivity, turbidity, TDS and salinity were measured using YSI Professional Plus handled multiparameter meter. The samples were stored in an ice-box and transported to the lab immediately. The analysis of nitrate was carried out using a Cadmium Reduction Method (Method 8171) with a HACH brand of DR/2500 spectrophotometer.
Fig. 1. Study area and distribution of agricultural area in Bachok
**Human Health Risk Assessment**

The non-cancer health risk associated with drinking nitrate contaminated water was assessed herein. A method for estimating the Target Hazard Quotient (THQ) by the US EPA Regional Risk-Based Concentration Table was used (Liu et al., 2011). The risk associated with the non-cancer effects of nitrate through drinking water is expressed as follows:

\[
THQ = \frac{C \times EF \times ED \times IR}{(RfD \times BW \times AT)}
\]

Where:
- \( C \) = The nitrate concentrations (mg/L)
- \( EF \) = The exposure frequency (350 days/year)
- \( ED \) = The duration of exposure (30 years)
- \( IR \) = The amount of water ingested by an adult, (2L/day)
- \( RfD \) = The oral reference dose (1.6 mg/L/day of NO₃⁻N)
- \( BW \) = The body weight of an adult (60 kg)
- \( AT \) = The averaging time for non-carcinogens (30 years \times 365 days/year = 10,950 days)

An acceptable standard human health risk by drinking water is a THQ value of under unity (Liu et al., 2011).

**Statistical Analysis**

Data from questionnaire, nitrate and in-situ parameters concentrations in the well water of both areas were analysed using IBM Statistical Package for Social Sciences (SPSS) version 21.0. Kolmogorov-Smirnov, Shapiro Wilk and Skewness tests were used to check the normality of distribution of the interval/ratio scale variables. Data in this study were non-normally distributed (p<0.05), thus nonparametric statistics were used. Chi-square, Mann-Whitney and Kruskal-Wallis Test were used to test for differences between the variables. The Spearman correlation analysis was used to determine whether definable statistical relationships exist between the observed nitrate concentration and several variables. The Spearman correlation coefficient \( r_s \) shows the strength of the relationship and whether the relationship is positive or negative; the \( p \) value shows the significant level of test. Odd Ratios (ORs) and their 95% confidence interval (95% CI) were calculated using the respondents in the non-agricultural area as the reference group. Values of \( p<0.05 \) were considered statistically significant.

**Results**

**Respondent’s Background**

A total of 126 respondents from the agricultural area and 130 respondents from the non-agricultural area were participated in the study (Fig. 1). Majority of the respondents are female where 67 (53.6%) of them in agricultural area and 71 (54.6%) in the non-agricultural area (Table 1). Most of respondents in both areas are married (N = 109 in the agricultural area; N = 114 in the non-agricultural area). The respondents aged ranged from 18-91 years old. All of the respondents are Malays. Majority of respondents in these areas had the highest qualification from secondary school (N = 53 in the agricultural area, N = 50 in non-agricultural area). Most of them are self-employed (61 (48%) in the agricultural area and 56 (43.1%) in the non-agricultural area). About 37.0% respondents in the agricultural area and 40.0% respondents in non-agricultural area do not have permanent income. The average income for the population is less than RM 500 per month (25.2% in the agricultural area and 25.4% in the non-agricultural area) and between Malaysia Ringgit (RM) 500 to RM 1500 (22% in the agricultural area and 20.8% in the non-agricultural area). Majority of respondents had lived in the study area for more than 30 years (59.5% in the agricultural area and 58.5% in the non-agricultural area). The chi square test indicates no significant difference in socio-demographic background of respondents in this study (Table 1).

**Physicochemical Characteristics of Well Water**

Table 2 shows the physicochemical characteristics of well water in this study. The mean ± SD of pH values of well water in the agricultural area was significantly lower (5.64±0.32) compared to the non-agricultural area (6.32±0.76) (Z = -8.30; p<0.001). The mean pH of well water in both areas do not fall within the Malaysia National Drinking Water Quality Standard (NDWQS) (6.5 to 9.00). The well water in the agriculture area has significantly lower (5.64±0.32) compared to the non-agricultural area (6.32±0.76) (Z= -7.82; p<0.001). The mean pH of well water for both areas was within the maximum level of NDWQS (7.0).

The mean ± SD of ammonia in the agricultural area was significantly higher (0.84±1.32 mg L⁻¹) compared to the non-agricultural area (0.24±0.41 mg L⁻¹) (Z= -7.82; p<0.001). The ammonia level in the well water for both areas was within the maximum level of NDWQS (1.5 mg L⁻¹).

The mean ± SD of conductivity in the well water in agriculture area was significantly lower (94.67±60.80 µS cm⁻¹) than the non-agricultural area (205.14±209.11 µS cm⁻¹) (Z= -4.81, p<0.001). The NDWQS has no guideline value for conductivity however, WHO has set a limit to 500 µS cm⁻¹. The mean of conductivity values in both areas in this study were below than the WHO acceptable limit.

The well water in the agriculture area has significantly higher turbidity compared to the non-agriculture area with the Mean ± SD of 5.06±9.65 NTU and 2.94±3.78 NTU respectively (Z = -3.96, p<0.001) (Table 2).
Table 1. The socio-demographic background of respondents in the agricultural and non-agricultural areas (n = 256)

| Variables               | Agricultural area | Non-agricultural area | $\chi^2$ |
|-------------------------|-------------------|-----------------------|---------|
|                         | (N = 126)         | (N = 130)             |         |
| n (%)                   | n (%)             |                       |         |
| **Gender**              |                   |                       |         |
| Male                    | 58 (46.4)         | 59 (45.4)             | 0.011   |
| Female                  | 67 (53.6)         | 71 (54.6)             |         |
| **Marital status**      |                   |                       |         |
| Single                  | 16 (12.6)         | 16 (12.3)             | <0.000  |
| Married                 | 109 (85.8)        | 114 (87.7)            |         |
| **Education**           |                   |                       |         |
| None                    | 17 (13.4)         | 17 (13.1)             | 0.908   |
| Primary school          | 39 (30.7)         | 44 (33.8)             |         |
| Secondary school        | 53 (41.7)         | 50 (38.5)             |         |
| Certificate             | 3 (2.4)           | 3 (2.3)               |         |
| Diploma                 | 8 (6.3)           | 8 (6.2)               |         |
| Bachelor’s degree       | 5 (3.9)           | 8 (6.2)               |         |
| **Employment**          |                   |                       |         |
| Government sector       | 9 (7.1)           | 9 (6.9)               | 1.651   |
| Private sector          | 9 (7.1)           | 14 (10.8)             |         |
| Self-employed           | 61 (48.0)         | 56 (43.1)             |         |
| Unemployed              | 43 (33.9)         | 47 (36.2)             |         |
| Student                 | 3 (2.4)           | 4 (3.1)               |         |
| **Income (RM)**         |                   |                       |         |
| <500                    | 32 (25.2)         | 33 (25.4)             | 2.299   |
| 500–1500                | 28 (22.0)         | 27 (20.8)             |         |
| 1501–3500               | 17 (13.4)         | 14 (10.8)             |         |
| >3500                   | 1 (0.8)           | 4 (3.1)               |         |
| Do not have permanent income | 47 (37.0) | 52 (40.0)             |         |
| **Period of residency (year)** |             |                       |         |
| ≥10–20                  | 12 (9.5)          | 22 (16.9)             | 3.578   |
| >20–30                  | 38 (30.2)         | 32 (24.6)             |         |
| >30                     | 75 (59.5)         | 76 (58.5)             |         |

Chi Square Test, * Significant at level p<0.05

Table 2. The physicochemical characteristics of well water in the agriculture and non-agriculture area (n = 256)

| Parameters               | Agricultural area (N = 126) | Non-agricultural area (N = 130) | Z value |
|-------------------------|-----------------------------|----------------------------------|---------|
| pH                      | 5.64±0.32                   | 6.32±0.76                        | -8.30** |
| Median                  | 5.68                        | 6.48                             |         |
| Range                   | 4.63-6.37                   | 3.67-7.71                        |         |
| Ammonia (mg/L)          | 0.84±1.32                   | 0.24±0.4                         | -7.82** |
| Median                  | 0.28                        | 0.12                             |         |
| Range                   | 0.05-6.81                   | 0.02-2.42                        |         |
| Dissolved Oxygen (mg/L) | 6.37±1.07                   | 9.63±1.71                        | -12.75**|
| Median                  | 6.21                        | 9.22                             |         |
| Range                   | 3.75-9.96                   | 6.13-13.67                       |         |
| Conductivity (µS/cm)    | 94.67±60.80                 | 205.14±209.11                    | -4.81** |
| Median                  | 73.47                       | 147.84                           |         |
| Range                   | 25.40-287.50                | 16.20-1251.67                    |         |
| Turbidity (NTU)         | 5.10±9.65                   | 2.94±3.78                        | -3.96** |
| Median                  | 2.63                        | 1.33                             |         |
| Range                   | 0.28-69.80                  | 0.15-16.60                       |         |
| Total Dissolved Solids (TDS) (mg/L) | 69.34±46.09                  | 165.08±173.65                  | -5.16** |
| Median                  | 56.55                       | 126.43                           |         |
| Range                   | 15.60-214.5                 | 12.35±1007.50                    |         |
| Salinity (ppt)          | 0.05±0.04                   | 0.13±0.17                        | -5.28** |
| Median                  | 0.04                        | 0.09                             |         |
| Range                   | 0.01-0.16                   | 0.01-1.25                        |         |

Mann-Whitney U Test, ** Significant at p<0.001
The mean turbidity value in agricultural area was fairly above the WHO acceptable limit of 5.00 NTU. The Mean ± SD of Total Dissolved Solid (TDS) in the non-agricultural area was significantly higher (165.08±173.65 mg L\(^{-1}\)) compared to the agricultural area (69.34±46.09 mg L\(^{-1}\)) (Z = 5.16, p<0.001). The TDS for both areas do not exceed the Malaysia NDWQS (1000 mg L\(^{-1}\)).

The mean turbidity value in agricultural area was significantly higher than the non-agricultural area (69.34±46.09 mg L\(^{-1}\) compared to the agricultural area with the mean± SD of 0.05±0.04 ppt and 0.13±0.17 ppt respectively (Z = -5.28; p<0.001).

**Nitrate Concentration in Well Water**

Table 3 shows the concentration of nitrate in the well water. The Mean ± SD of nitrate in agricultural area was significantly higher (13.04±14.39 mg L\(^{-1}\) NO\(_3\)-N) than the non-agricultural area (6.31±5.22 mg L\(^{-1}\) NO\(_3\)-N) (Z = -3.55, p<0.001). The nitrate level in the agricultural area has exceeded the Malaysia NDWQS (10 mg L\(^{-1}\) NO\(_3\)-N). The natural levels of nitrate in groundwater are usually less than 3 mg L\(^{-1}\) NO\(_3\)-N and nitrate concentration exceeding the threshold of 3 mg L\(^{-1}\) NO\(_3\)-N is considered as contaminated due to human activities (the so-called human affected value) (Babiker et al., 2004; Pastén-Zapata et al., 2014). The maximum acceptable levels of nitrate in drinking water is set at value 10 mg L\(^{-1}\) NO\(_3\)-N according to Malaysia NDWQS. This value is the limit to protect against methemoglobinemia in infants (Ward et al., 2005). Therefore, the concentration of nitrate in well waters in this study were categorised into three categories which is < 3 mg L\(^{-1}\) NO\(_3\)-N, between 3 to 10 mg L\(^{-1}\) NO\(_3\)-N and > 10 mg L\(^{-1}\) NO\(_3\)-N.

There were 49 wells (38.89%) in agricultural area and 44 wells in non-agricultural area had nitrate levels exceeding 3 mg L\(^{-1}\) NO\(_3\)-N indicating a contamination due to human activities. About 52 wells (41.27%) in the agricultural area and 35 wells (26.9%) in the non-agricultural area had nitrate levels above 10 mg L\(^{-1}\) NO\(_3\)-N which is not suitable for being drinking water. 25 wells (19.84%) in the agricultural area and 44 wells (39.23%) in the non-agricultural area had nitrate value less than 3 mg L\(^{-1}\), indicating a natural levels of nitrate in groundwater.

**The Relationship between Physical Characteristics of Well and Nitrate Concentration**

Table 4 shows the well characteristic in this study. Majority of the wells are 20 years of age with a depth 1 to 5 m. Majority of the wells are located between 5 to 10 m from the septic tank (N = 123 in agricultural area and 90 in non-agricultural area) and 20 to 30 m from their neighbours septic tank. Most of the well is located within the range of 50 to 200 m from the agricultural area and less than 50 m from the livestock farm.

There was no significant relationship between nitrate level and well characteristics. In general, the Mean ± SD of nitrate in the well of less than 20 years was higher (18.79±19.77 mg L\(^{-1}\) NO\(_3\)-N) than well aged >20 years (12.41±13.32 mg L\(^{-1}\) NO\(_3\)-N). High nitrate was detected in shallow well (depth <1-5 m) in the agricultural area with the Mean ± SD of 13.41±13.84 mg L\(^{-1}\) NO\(_3\)-N. Nitrate was also high in the well with high turbidity during rainy day especially in the agricultural area (13.61±14.02 mg L\(^{-1}\) NO\(_3\)-N).

Elevated nitrate concentrations also were detected in the well located 200 m from the paddy field. The Mean ± SD of nitrate was 13.15±13.11 mg L\(^{-1}\) NO\(_3\)-N for 0-50 m distance, 10.46±10.62 mg L\(^{-1}\) NO\(_3\)-N for 50-100 m and 15.15±17.09 mg L\(^{-1}\) NO\(_3\)-N for 100-200 m. High nitrate also was determined in the well < 50 m to the livestock area with the Mean ± SD of 15.92±11.42 mg L\(^{-1}\) NO\(_3\)-N in the agricultural area.

Nitrate was high in a well located near to septic tank. For example, the Mean ± SD of nitrate in the well with distance of 5 to 10 m to septic tank was 13.31±14.45 mg L\(^{-1}\) NO\(_3\)-N, 14.54±14.54 mg L\(^{-1}\) for 20 to 30 m from septic tank and 17.40±19.36 mg L\(^{-1}\) NO\(_3\)-N for 30 to 40 m from a septic tank in the agriculture sites. High nitrate also was detected in the well located 15-20 m to the septic tank (10.56 mg L\(^{-1}\) NO\(_3\)-N) in the non-agriculture sites. The highest nitrate also was detected in the well located 30 to 40 m (17.40±19.36 mg L\(^{-1}\) NO\(_3\)-N) from neighbour’ septic tank in the agricultural area.

**Correlation between Chemical Parameters**

High positive correlation was found between conductivity with TDS and salinity with the r value = 0.980 (p<0.001) (Table 5). Nitrate also showed significant positive relationship with ammonia (r = 0.411, p<0.001), conductivity (r = 0.502, p<0.001), TDS (r = 0.480, p<0.001) and salinity (r = 0.485, p<0.001).

**Human Health Risk Assessment and Health Status of Respondents**

The level of nitrate ingestion from drinking water was ranged from 0.007 to 1.143×10\(^{-6}\) in the agricultural area and 0.002 to 0.468×10\(^{-6}\) in the non-agricultural area. The highest nitrate concentration (57.23 mg L\(^{-1}\) NO\(_3\)-N) in the well water has THQ value of 1.143 which exceeds the acceptable standard, indicating a potential non-cancer risk for Bachok residents.

Table 6 shows the odd ratio of diseases caused by nitrate in the agricultural and non-agricultural area in this study. There was no significant association between nitrate exposure and health. Out of 256 respondents in this study, only 1 (0.8%) of them has diabetes type I and 8 (6.3%) have diabetes type II in the agricultural area. While in the non-agricultural area, only 2 of the respondents (1.5%) have diabetes type I and 7 (5.4%) have diabetes type II. Only one respondent (0.8%) from each group has goitre.
disease. In addition, 50% (n = 63) of respondents in the agricultural area and 43.8% (n = 57) of respondents in the non-agricultural area have gastric. The ORs for diseases such as diabetes, goitre and gastric were not significant related to nitrate. The ORs for the studied areas were 0.512 (CI = 0.04-5.72) for diabetes type I, OR = 1.191 (CI = 0.42-3.39) for diabetes type II, OR = 1.03 (CI = 0.06-16.68) for goitre and OR = 1.28 (CI = 0.78-2.09) for gastric.

Table 3. Comparison of nitrate concentration in the agricultural and non-agricultural area (mg/L NO\textsubscript{3}-N) (n = 256)

| Descriptive statistics          | Agricultural area (N = 126) | Non-agricultural area (N = 130) | Z value |
|---------------------------------|----------------------------|---------------------------------|---------|
| Mean ± SD                        | 13.04±14.39                | 6.31±5.22                       |         |
| Median                          | 6.695                      | 4.907                           |         |
| Range                           | 0.36-57.23                 | 0.11-23.45                      |         |
| <3 mg L\textsuperscript{-1} NO\textsubscript{3}-N (N) | 19.84% (25)                 | 39.23% (51)                     | -3.554**|
| ≥3–10 mg L\textsuperscript{-1} NO\textsubscript{3}-N (N) | 38.89% (49)                 | 33.85% (44)                     |         |
| >10 mg L\textsuperscript{-1} NO\textsubscript{3}-N (N) | 41.27% (52)                 | 26.92% (35)                     |         |

Mann-Whitney U Test. **Significant at p<0.001

Table 4. Physical properties of well and nitrate concentration (n = 256)

| Well information                  | Agricultural area (N = 126) | Non-agricultural area (N = 130) | Sig.  |
|-----------------------------------|----------------------------|---------------------------------|-------|
| Age of well (year)                |                            |                                 |       |
| >10-15                            | 6                          | 5.39±3.59                       |       |
| >15-20                            | 19                         | 18.79±19.77                     |       |
| >20                               | 101                        | 12.41±13.32                     |       |
| Depth of well (m)                 |                            |                                 |       |
| <1-5                              | 88                         | 13.41±13.84                     |       |
| >5-10                             | 33                         | 12.44±16.29                     |       |
| >10-30                            | 5                          | 10.33±12.91                     |       |
| Turbidity during rainy day        |                            |                                 |       |
| Yes                               | 53                         | 13.61±14.02                     |       |
| No                                | 73                         | 12.62±14.73                     |       |
| Distance of septic tank to well (m) |                            |                                 |       |
| >5-10                             | 123                        | 13.31±14.45                     |       |
| >10-15                            | 6                          | 5.39±3.59                       |       |
| >15-20                            | 1                          | 1.62                            |       |
| Distance of neighbour’s septic tank to well (m) |                  |                                 |       |
| >10-20                            | 11                         | 10.96±12.10                     |       |
| >20-30                            | 12                         | 14.54±14.54                     |       |
| >30-40                            | 12                         | 17.41±19.36                     |       |
| >40                               | 2                          | 2.69±1.51                       |       |
| Not sure                          | 1                          | 0.36                            |       |
| Distance of agricultural area to well (m) |            |                                 |       |
| >50-100                           | 35                         | 10.46±10.62                     |       |
| >100-200                          | 46                         | 15.15±17.09                     |       |
| >200-500                          | 10                         | 4.41±2.11                       |       |
| Distance of livestock area to well (m) |           |                                 |       |
| >50-100                           | 6                          | 12.37±10.43                     |       |
| Not sure                          | 4                          | 8.94±6.88                       |       |

Table 5. The Spearman correlation coefficients of the different groundwater parameters (n = 256)

| pH   | Nitrate | Ammonia | DO  | EC   | Turbidity | TDS   | Salinity |
|------|---------|---------|-----|------|-----------|-------|----------|
| 1.000| 0.007   | 0.010   | 0.451** | 0.646** | 0.057     | 0.658** | 0.660**  |
| Nitrate | 1.000 | 0.411** | 0.211** | 0.502** | 0.181** | 0.480** | 0.485**  |
| Ammonia | 0.010 | 1.000 | 0.290** | 0.419** | 0.151** | 0.345** | 0.421**  |
| DO   | 0.451** | 0.411** | 1.000 | 0.226** | -0.151** | 0.291** | 0.301**  |
| EC   | 0.646** | 0.502** | 0.419** | 1.000 | -0.096   | 0.990** | 0.980**  |
| Turbidity | 0.057 | 0.181** | 0.151** | 0.226** | 1.000 | 0.900** | 0.900**  |
| TDS  | 0.658** | 0.480** | 0.345** | 0.291** | 0.900** | 1.000  |          |
| Salinity | 0.660** | 0.485** | 0.421** | 0.301** | 0.980** | 0.980** | 1.000    |

Correlations are significant at p<0.05, **Correlations are significant at p<0.001
The groundwater with high TDS concentration is helpful in the accumulation of acidity, resulting in a decrease in pH of the groundwater (Xun et al., 2007).

Ammonia may be present in groundwater as a result of the degradation of naturally occurring organic matter or manmade sources such as nitrogen-fertilizer application, livestock operations, industrial processes, sewage infiltration and cement mortal pipe lining. High conductivity of well water in the non-agricultural area was possibly due to the location of this area which situated near to coastal area. The addition of salt water from the coastal which is often mixed with groundwater greatly increases the conductivity (Yan et al., 2015).

The well water in the agriculture area was slightly acidic compared to the non-agricultural area. This possibly due to the present of dissolved carbon dioxide (CO₂) and biocarbonates in the aquiferous rocks (Igboekwe et al., 2011). Under natural condition, H⁺ in the groundwater may be derived from dissociation of H₂CO₃ as well as from the acidity of rainwater. The H₂CO₃ in the groundwater is formed by dissolution of CO₂, which comes mainly from the biological contribution (Xun et al., 2007). Lack of alkaline substances in the groundwater system is also helpful in the accumulation of acidity, resulting in a decrease in pH of the groundwater (Xun et al., 2007).

The well water in the agriculture area in this study was possibly due to extensive use of nitrogen fertilizers and organic matter transported into the groundwater by percolation from rainfall or from irrigation (Gao et al., 2012). Excessive rainfall also would tend to leach nitrate below the root zone and ultimately to groundwater (Babiker et al., 2004; Hussain et al., 2013). In the non-agriculture area, 35 wells had nitrate values exceeded 10 mg L⁻¹ standard. This possibly due to factors of atmospheric deposition, discharge from septic tanks and leaking sewers which also can contribute to the high concentration of atmospheric nitrate (Pastén-Zapata et al., 2014). Besides, soil characteristics and hydrogeologic variables such as depth of well, depth below the water table, aggregated thickness of clay above the well screen and thickness of clay in the unsaturated and saturated zones were also considered potential factors influence nitrate contamination in the study area (Yang et al., 2007; Kuo et al., 2007; Khademikia et al., 2013).

High nitrate in shallow well and well of less than 20 years was consistent with the literatures. According to previous studies, nitrate pollution is more common in old and shallow wells (Hu et al., 2005; Rutkoviene et al., 2005). Nitrate contamination generally decreases with increasing depth to the groundwater. A close distance between water table and the land surface in shallow well may cause high concentration of nitrate and a potential sources pf contamination, such as fertilizers and septic system (Rutkoviene et al., 2005; Gao et al., 2012).

Agriculture activities close to well potentially contaminate the groundwater. This is consistent with the finding in this study where high nitrate was determined in the well < 50 m to the livestock area in the agricultural area. In general, short distance to a point source caused higher nitrate content.

Furthermore, ammonia and nitrate has positive relationship as ammonia can be oxidized to nitrate from nitrogen-fertilizer application. Nitrate concentration in groundwater generally increases with the increasing of ammonia. Nitrate is dissolved inorganic solids and act as conductors in the water while conductivity is the ability of water to conduct an electrical current. Therefore, the presence of nitrate in groundwater can affect the conductivity. Besides, because of nitrate is dissolved inorganic solids, nitrate also has correlation with TDS and salinity (Igboekwe et al., 2011; Annapoorani et al., 2014; Yan et al., 2015).

Nitrate in drinking water may pose a health risk when the levels exceed the MCL of 10 mg L⁻¹ (Coss et al., 2004; Liu et al., 2011). Agriculture uses large amounts of fertilizer and manure compost on farming lands, causing the significant nitrate pollution in groundwater. The hazard quotient assumes there is a level of exposure below which it is unlikely for even sensitive population to

### Table 6. Diseases caused by nitrate in the agricultural and non-agricultural area (n = 256)

| Disease          | Agricultural area | Non-agricultural area | Odd ratio (95% CI) |
|------------------|-------------------|-----------------------|-------------------|
| Diabetes type I  | 1 (0.8%)          | 2 (1.5%)              | 0.51 (0.05-5.72)  |
| Diabetes type II | 8 (6.3%)          | 7 (5.4%)              | 1.19 (0.42-3.39)  |
| Goitre           | 1 (0.8%)          | 1 (0.8%)              | 1.03 (0.06-16.68) |
| Gastric          | 63 (50.0%)        | 57 (43.8%)            | 1.28 (0.78-2.09)  |
experience adverse health effects. There may be a concern arising for the potential non-carcinogenic effects if the HQ exceeds 1×10^−1 (Unity).

Elevated nitrate in drinking water have been associated with several adverse health impacts such as diabetes, Age-related Macular Degeneration (AMD), gastric and thyroid dysfunction (Coss et al., 2004; Kuo et al., 2007; Ruckart et al., 2008; Aschebrook-Kilfoy et al., 2012; Klein et al., 2013).

Previous studies have confirmed that exposure to nitrate is strongly associated with various diseases. For example, infants below 6 months could become seriously ill and may cause fatality due to nitrate exposure. The symptom of nitrate toxic is shortness of breath and blue-baby syndrome (Liu et al., 2011). Besides, long term exposure to low levels of nitrate presents a non-carcinogenic risk to human (Majumdar and Gupta, 2000). Some studies have investigated the relationships between risks of pancreatic and bladder cancers and nitrate exposure caused by drinking water (Ward et al., 2003; Coss et al., 2004). However, no relationship was observed in this study.

Conclusion

The results of this study show nitrate was higher in the agricultural area compared to the non-agricultural area which exceeds the acceptable level of nitrate in drinking water (10 mg L\(^{-1}\) NO\(_3\)-N). This possibly related to the high usage of fertilizer and pesticide. Nitrate also was determined as high in older well, with shallow depth, high turbidity and close by to septic tank and agricultural area. In this study, the possible association between nitrate concentrations and health diseases such as diabetes, goitre and gastric was not significant.

The strength of this study was it managed to compare the nitrate level in the private well waters in two different areas of agricultural and non-agricultural area in Kelantan. This study has considered the physical characteristics of the well in the analysis such as well age and depth which is possible to be associated with high nitrate in the groundwater. The distance of the well to a point source such as agricultural area, septic tank and livestock area were also taken into account in this study. This information is important as predictive factors for nitrate levels in study area.

There are several limitations in this study such as we did not include the geological information in the discussion due to limited data available. This information contribute to the variability in nitrate levels in groundwater and would likely result in substantial misclassification of nitrate exposure (Ismail et al., 2013). Our study is also limited by the fact that we did not have data and evaluate on other contaminants such as pesticides in the well water where the presence of other drinking water contaminants may correlated with high nitrate concentrations. Dietary nitrate intake information such as types of vegetables consumed, the levels of nitrate in the vegetables (including the nitrate content of fertilizers) and the amount of vegetables consumed were not evaluated. Approximately 80% of dietary nitrate are derived from vegetable consumption. However vegetables contain vitamin C and other antioxidants that might prevent nitrosamines formation and therefore, is most likely to attenuate association. Besides, the results also cannot be generalized to represent the whole population in the country as the sample was restricted to only certain area in Kelantan.

Future studies should consider widening the sampling area, where the participation of respondents from other states in the country may increase the precision of nitrate exposure. Study also could be improved by increasing the precision of the estimation of the individual’s intake of nitrate, through both food and water and controlling for confounding factors such as occupational exposure to chemicals. Additional studies of population with higher exposure level would be also informative. The uses of biomarker such as urine and saliva to confirm the effect of nitrate to human health may be useful.

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Author’s Contributions

Aida Soraya Shamsuddin: Designed the study, conducted the survey, interview and data analysis of this study.

Sharifah Norkhadijah Syed Ismail: Designed the study, prepared the manuscript, analyzed and interpreted the data. She also supervised and analyzed the edited manuscript.

Emilia Zainal Abidin: Discussed the results and implications and commented on the manuscript at all stages.

Ho Yu Bin: Discussed the results and implications and commented on the manuscript at all stages.

Hafizan Juahir: Provided the instruments during the data collection.
Ethics

Universiti Putra Malaysia/Ethics Committee for Research Involving Human Subjects (JKEUPM).

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