The chemical, heavy metal and microbial quality of well water in an urbanised village in the Klang Valley

Stephen Ambu[1], Stacey Foong Yee Yong[2], Yvonne Ai Lian Lim[3], Mak Joon Wah[1], Donald Koh Fook Chen[1], Soo Shen Ooi[1], Sau Peng Lee[1], Ti Myen Tan[1], Mei Yen Goh[1], Danapridha Nyanachendram[1]

Abstract

IeJSME 2014 8(3): 28-44

Background: The public health issue of consuming groundwater is a major concern because people often extract groundwater directly from the aquifers either through wells or boreholes without treating it with any form of filtration system or chlorine disinfection. Based on the Malaysian National Drinking Water guidelines the current study was designed to provide a better understanding on the variable factors that are influencing the quality of well-water in an urbanised village in Malaysia. Well water quality assessment of heavy metals, chemicals, microbial and physical parameters were carried out for Sungai Buloh Village in the Klang Valley to ensure it was safe for human consumption.

Materials and Methods: Water samples were collected from wells at four sites (Sites A,B,C,D), a river and a tap inside a house in Sungai Buloh village. Soil was sampled from the riverbed and area surrounding the wells. Samples were collected every two months over a one year duration from all sites. The water samples were processed and examined for viruses, coliforms and protozoa as well as for heavy metal contaminants.

Results: The turbidity and colour ranged in the average of 0.57-0.13 Nephelometric Turbidity (NTU) and 4.16-5.00 Total Conjunctive Use (TCU) respectively for all sites except Site C. At Site C the turbidity level was 2.56 ± 1.38 NTU. The well-water was polluted with coliforms (1.2 to 2.4 x 10^3 CFU/100 ml) in all sites, *E. coli* (0.12 - 4 x 10^2 CFU/100 ml CFU/ 100 ml) and *Cryptosporidium* oocysts (0.4 cysts/100 ml). All the heavy metals and chemical parameters were within the Malaysian Guidelines' limits except manganese. The average pH ranged from 5.44 - 6.62 and the temperature was 28 ºC.

Conclusion: In summary, the well water at Sungai Buloh is considered unsafe for consumption due to pollution. Therefore the major thrust will be to provide better quality of drinking water to the residents of the village.

Keywords: well water, microbial, heavy metal, chemical, urbanised village, public health

Introduction

Groundwater is an alternative water source in many countries where sanitised water supply is scarce. The public health issue of consuming groundwater is a major concern because people often extract groundwater directly from the aquifers either through wells or boreholes without treating it with any form of filtration system or chlorine disinfection.[1,2,3] Many studies have reported on contaminated groundwater with microbial and toxic heavy metals as not being suitable for drinking.[4,5,6] The sources of groundwater pollution are often due to anthropogenic activities that include discharged waste from wastewater treatment plants, manufacturing industries, construction activities and animal farming.[7,8,9,10] The weathering of soils and rocks have also indirectly contributed to the addition of contaminants to the groundwater systems.[1,12] The quality of Malaysian drinking water is governed by the Malaysian National Drinking Water Guidelines and the WHO Guidelines for Drinking-Water Quality.[34,44] Based on these guidelines the current study was designed to provide a better understanding on the variable factors that are influencing the quality of well-water in an urbanised village in Malaysia.

Bioaccumulation of heavy metals in the food chain such as vegetables and livestock are potentially carcinogenic, damaging to the kidney, liver and nervous systems and reduces cognitive development in children and neonates.[9,13,14,15,16,17] The deleterious impact on human health after drinking nitrogen polluted groundwater for long term has a high carcinogenic risk to communities.[18] Therefore, it is the interest of this project to assess the level of heavy metals and chemical contaminations in the groundwater that has been consumed by residents in an

---

[1]International Medical University, Kuala Lumpur, MALAYSIA, [2]School of Biosciences, Taylor's University, MALAYSIA, [3]Department of Parasitology, Faculty of Medicine, University of Malaya

Address for Correspondence:

Stephen Ambu, School of Postgraduate Studies, Institute of Research, Development and Innovation, International Medical University, Malaysia
E-mail: stephen_ambu@imu.edu.my Tel.:+603 2731 7291 Fax: +603 8656 7229

urbanised village in the Klang Valley. Heavy pollution of groundwater with lead, manganese, iron, cadmium, zinc, sodium, chloride, nitrates, ammoniacal nitrogen, tin and arsenic due to run-offs from landfills have been reported in some parts of the Klang Valley.[10,19] Nevertheless, the extensiveness of heavy metal pollution in groundwater also depends on the geographical soil conditions and properties of the heavy metals.[11,12]

Microbial contaminants such as bacteria, viruses and parasites in groundwater can result in immediate health consequences such as dysentery, diarrhoea, vomiting and anorexia. Cases of food-borne diseases due to enteric viruses and bacteria have shown association with vegetables irrigated with contaminated groundwater.[20,21] Even though the microbial contamination of groundwater is not frequently seen as in surface water but microbial pollutants in groundwater could be highly concentrated by the slow filtration of water through many layers of soil and rocks in the ground.[22,23,24] Some microbes are more prevalent and concentrated in groundwater. Tracking the microbial source of contamination of groundwater is equally important for detecting toxic heavy metals.

The four genogroupings of male-specific RNA coliphages (FRNA) have been recognised to be the effective microbial source tracking indicators for surface and groundwater water systems.[25,26,27] The genogroups of I and IV FRNA coliphages are associated with animal faecal matters, whereas the genogroups of II and III are associated with human sewages.[28] The pinpointing of the sources of microbial contamination will help to reduce and eradicate further pollution of the groundwater.

In this project, the chemical, heavy metal and microbial qualities of well water at Sungai Buloh Village (3.1996 N, 101.5760 E) were assessed to evaluate if it is safe for human consumption. This village with an estimated population of 466,163[29] covering an area of 243 square kilometres was chosen for this study because it is urbanised and consists of residential homes, poultry farming and a high density of small and medium sized industries involved in furniture and food manufacturing. A majority of the residents here have opted to use water from wells located in their houses even though they have access to treated water supply because of economic constraints. Furthermore, the migrant labourers living within this locality do not have sufficient understanding of the health risks that will emerge as a consequence of polluting the groundwater. This study ultimately would provide insight of the complex environmental issues that are impacting the quality of well water in this urbanised village. Therefore, the environmental factors such as the physical and chemical characteristics of well water, the inputs of chemicals, heavy metals and sources of microbial pollutants from the nearby river water and riverbank into the well water were included in this study to provide better understanding on the variable factors that are influencing the quality of well-water. The tap water sample was included to show the quality of drinking water supplied to the village. The collection of samples for tap water analysis was limited to Site B as it was difficult to get tap water from other sites due to economics of cost and unwillingness on the part of residents to participate.

Materials and Methods

Sampling sites

A total of four sampling sites of groundwater and soil samples located at Sungai Buloh Village were identified (Figure 1: Site map). Site A (3.1866N 101.5560E) has a well with the depth of 7.03 m but 8 m away from a self-constructed toilet and surrounded by furniture and marble tile factories. Site B (3.1903N 101.5576 E) has a well that is 5.68 m deep with small-scale poultry rearing (approximately 50 chickens) and a car workshop nearby (3 m). Site C (3.1890 N 101.5760 E) has a bore-well situated on lower land that is surrounded by food manufacturers, steel and furniture factories and some squatter houses. Site D (3.1996 N 101.5675 E) has a well 7.03 m deep and is covered. Samples from the River Hampar that runs across these four sampling sites were collected. The tap water was sampled from a house at

Site B.

Collection and processing of water samples

Water samplings were conducted over a period of 12 months on alternate months. One hundred litres of groundwater were collected from Sites A, B, C and D for microbiological testing. Ten litres of groundwater samples were collected separately for chemical testing. For river water, 25 L were collected for both microbiological and chemical testing. Water samples were collected in 25 L sterile jerry cans and delivered to the laboratory for immediate processing. The Continuous Flow Centrifugation Velpro CFC-200 system (CFC) (Scientific Methods, Inc) at 10,000 rpm and peristaltic pump inflow rate of 500 mL/min concentrated bacteria and protozoa from the 100 L groundwater samples to 250 mL in the CFC bowls. The outflow of water from CFC was continuously passed through the ViroCap electropositive membrane filter (Scientific Methods, Inc) for capturing the coliphages.

Eluting viruses from the ViroCap electropositive membrane filter

Five hundred ml of OptimaRE elution buffer (pH 9.2) were used to elute coliphages from the ViroCap filter at 200 mL/min using the peristaltic pump (Cole-Parmer). The final eluent volume was adjusted to pH7.2 ± 0.2 using HCl. To further concentrate coliphages in the eluent, 0.4 M NaCl and 8% polyethylene glycol (PEG) 8000 (Sigma) was added to the eluent and stirred overnight at low speed at 4 ± 1°C. The mixture was then centrifuged at 3700 rpm for 45 minutes at 4°C to pellet coliphages down and re-suspended in 2 mL of phosphate buffer.

Plaque analysis

The plaque analysis was performed using the DAL assay Method 1601 with slight modification.[30] The number of plaque-forming units was counted and calculated according to the following formula:

$$\text{Undiluted phage suspension} = \frac{(\text{pfu}_1 + \text{pfu}_2 + \ldots\text{pfu}_n)}{(\text{v}_1 + \text{v}_2 + \ldots\text{v}_n)}$$

PFU : number of plaques forming units from countable sample dilutions plates

v : volume used × dilution factor

n : number of counts

RNA was extracted from isolated plaques using the QIAamp® MinElute® Virus Spin Kit (QIAGEN).

Genotyping of FRNA coliphages

Four pairs of primer representing the four genogroups of FRNA coliphages (MS2, V00642; GA, X03869; Qß, AY099114 and SP, X07489) were designed using Primer 3 version 0.4.0 (Table 1).[31] Duplex-RT-PCR was carried out in 20 µl reaction mix containing 1 x AMV/*Tfl* reaction buffer, 0.3mM dNTP mix, 3mM Mg^{2+}, 1U AMV reverse transcriptase, 1U *Tfl* DNA polymerase, 0.5µM of each forward and reverse primers and 20 ng of RNA. The amplification was initiated with reverse transcription at 45°C for 45 minutes, followed by heat inactivation and denaturation of cDNA at 94°C for 2 minutes in a thermal cycler (MyCycler™, Bio-Rad). Duplex RT-PCR consisted of 35 cycles of 94°C for 30s, 59°C for 1 minute and 68°C for 2 minutes with a final extension of 7 minutes at 68°C. The sensitivity of duplex RT-PCR was evaluated from 0.0001 ng to 2 ng of RNA template.

Detection of *E. coli* and coliform using Easygel® Coliscan

Enumeration of total and faecal coliforms was performed on the 250ml concentrated water sample using the Easygel® coliscan (Scientific Methods, Inc) according to the manufacturer's instruction. Fifteen ml of Easygel® coliscan liquid medium were mixed with 5 ml of groundwater sample thoroughly and poured into a pre-treated Easygel® petri dish, then incubated at 37°C for overnight. The *E. coli* (purple) and coliforms (pink) colonies were counted and recorded in CFU/100 ml. The total bacterial count was calculated according to the formula, (Number of Colonies/5 ml) x 100 = CFU/100ml.

Detection of *Cryptosporidium* and *Giardia*

The water concentrate was subjected to immunomagnetisable separation (IMS) (Dynal, Cat. No. 730.02, Oslo, Norway) according to the manufacturer's instructions. The concentrate containing isolated protozoa was deposited onto a microscope slide and stained with a commercial fluorescein isothiocyanate (FITC)-labelled monoclonal antibody kit reactive with exposed epitopes on *Giardia* cysts and *Cryptosporidium* oocysts and the nuclear fluorogen 4'6-iamidino-2-phenyl indole (DAPI) according to the manufacturer's instructions (Cellabs Pty Ltd., Cat. No. KR2, Brookvale, Australia; Sigma Chemical Co., Cat. No. 32670-5MG-F Louis, Missouri, USA). Stained samples were examined by epifluorescence microscopy (400x) and putative cysts were confirmed by viewing at 1000x using Nomarski differential interference microscopy to confirm their internal morphologies.[32] The number of observed cysts was enumerated three times based on the sampled volume. Enumeration of (oo)cysts density in water samples was based on the following formula:

$$\text{No. of (oo)cysts per litre} = \frac{N \times C}{A \times F}$$

N = number of (oo)cysts observed on the slide

A = analysed volume (L)

C = concentrated volume (mL)

F = filtered volume (L)

Measuring physical and chemical parameters

The temperature, pH, turbidity, colour and hardness of water samples were measured. Concentrations of metals (Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Se, Ag, Zn) and cations (Na, Mg) were quantified using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), ELAN 6100, Perkin Elmer. Ammonium–nitrogen (NH^+_4–N), nitrate–nitrogen (NO^-_3 –N), Chloride (Cl) and Sulphate (SO_4) values were determined according to the standard method.[33] All analysis was conducted in triplicates.

Soil sampling and processing

Soil samples were collected using the hand auger kit that was pushed into the ground by a turning process to the depth not exceeding 3.5 m. The soil samples were observed to determine the difference in soil profiles. The soil samples from the top surface and the deepest part in the auger hole were selected for laboratory analysis. The samples were microwave digested according to EPA 3051-Environmental Test prior to Inductively Coupled Plasma Mass Spectrometry (ICPMS) analysis for aluminium, arsenic, cadmium, chromium, cobalt, iron, lead, magnesium, manganese, selenium, silver, sodium and zinc.

Statistical Analysis

The relationships between each parameter that were not normally distributed were examined using Spearman's rho correlation whilst those with normal distribution were calculated using Pearson's means (± standard deviation, SD) in the SPSS version 18 (SPSS Inc. Chicago, IL). The qualitative variables were estimated and presented as present and absent. Values of $p< 0.05$ were considered statistically significant.

Results

Microbial Analysis

Coliforms were detected in all well water samples with an average of 1.2 to 2.4 x 10^3 CFU/100 ml throughout the six months of sampling (Table 1). For Site B, coliforms were only detected in the months of August and October. For the month of April, coliforms were not detected in all well water samples except Site A with 2.04 CFU/100 ml. In some months, *E. coli* was only found in Site A and C with an average of 0.12 - 4 x 10^2 CFU/100 ml CFU/ 100 ml. The river water as expected was highly contaminated with coliforms which was too numerous to be counted and *E. coli* was detected in the range of 1.0 x 10^4 -4.1 x 10^5 CFU/100 ml throughout the six months of sampling. The *Cryptosporidium* oocysts were sporadically detected in the well-water at Sites A, B and C and river water with a concentration of 0.4 oocysts/100 ml (Table 2). However, *Giardia* cysts were only found in river water in June with the highest count of 25 cysts/100 ml, followed by the months of April (7.6/100 ml) and March (5.2 cysts/100 ml). The plaque analysis did not detect any F-specific coliphages in all well water samples except the river water in the range of 9.8 x 10^2 to 4.04 x 10^4 PFU/100 ml (Table 3). However, RT-PCR detected FRNA coliphages of genogroups I, II and IV in well-water and river water in some months (Table 3). The FRNA coliphage of genogroup I was also detected in the tap water in January, March and April. However, the tap water was free of other microbial contaminants.

Correlation analysis of microbes with physical parameters, chemical compounds and heavy metals

Pearson's correlation analysis indicated that the majority of the microbes except *E. coli*, F specific coliphages and *Cryptosporidium* are significantly associated ($p < 0.01$) with colour, turbidity, ammoniacal nitrogen, arsenic and iron (Table 4). Coliform was shown to have significant positive correlation with iron. *E. coli* is highly correlated with coliforms, F specific coliphages and *Giardia* cysts. F specific coliphages and *Giardia* cysts are significantly correlated. Coliforms and *Cryptosporidium* are not correlated with any of the tested microbes.

Soil sample profiles

The soil profiles in Sungai Buloh Village were heterogeneous. At Site A, the solid type at the top layer (0.00-0.50 meter depth) was brown silt sand with gravel and the bottom soil (0.15-0.80 meter depth) was medium brown silt with gravel. At Site B, the top layer (0.00-0.15 meter depth) was dark brown silt with fine sand and traces of gravel and the bottom layer (0.15-3.50 meter depth) was pale brown silts with gravel. At Site C, the top layer (0.00-0.15 meter depth) was brown sandy silt with gravel and the bottom layer (0.15-3.50 meter depth) was pale brown sand with some gravel. At Site D, the top layer (0.00-0.15 meter depth) was brown silt with traces of gravel and the bottom layer (0.15-3.50 meter depth) was yellowish brown to dark brown silts and with gravel. At the riverbank, the top layer (0.00-0.20 meter depth) was brown sand with traces of gravel and the bottom layer (0.20-1.20 meter depth) was brown to medium grey sand with gravel.

The majority of the trace elements were detected in higher concentrations at the topsoil as compared to the bottom soil layer. In particular, manganese was found to be high in concentration at the topsoil layer with the range of 6.48-0.10 ppm except at Site D. At Site D, the concentration of manganese was found 58% higher in the bottom soil layer (Table 5).

As shown in Table 5, Sites B, C and D were contaminated with more heavy metals at the top soil layer than the bottom soil layer. At Site B, aluminium, chromium, manganese and zinc were found to be 59%, 50%, 86% and 97% higher in the top soil layer than in bottom soil layer, respectively. The concentrations of iron (54%) and magnesium (66%) were relatively higher at the bottom soil layer than the top soil layer. Similarly, at Site C, the concentrations of aluminium, arsenic, iron, lead, magnesium, manganese and zinc at the top soil layer were 39%, 99%, 96%, 97%, 94%, 99% and 85% respectively which were higher than at the bottom soil layer. Site D also found higher levels of arsenic, chromium, iron, and magnesium at the top soil layer with 96%, 65%, 76% and 76% respectively compared to the bottom layer.

On the contrary, at Site A, the elements of aluminium, arsenic, chromium and iron were found in higher concentrations at the bottom soil layer compared to the top layer at 39%, 72%, 84% and 55% respectively (Table 5). However, magnesium and manganese were 96% and 75% more in the top soil layer than the bottom soil layer respectively. At the river, the concentrations of all elements present at top and bottom soil layers were consistent except for zinc that was found to be 81% higher at the bottom soil layer than the top layer.

Heavy metal and chemical compounds

The heavy metal and chemical compound analysis showed that all well water and river water samples met the water quality standard requirements[34] except for manganese and iron compounds. Manganese exceeded the standard requirements of 0.001 ppm in all water samples with an average range of 0.007 – 0.157ppm (Table 6). Site A had the most contaminated well water with manganese in the range of 0.110-0.242 ppm, followed by Sites C, B and D (Table 6). The tap water sample was found contaminated with manganese in the range of 0.004-0.051 ppm that was slightly above the standard limit. However, the iron content in the river and well water of Site C were found to exceed the standard limit of 0.3 ppm in January and March with 1.046 and 0.298 ppm respectively.

The well water at Site A was found to be polluted with most of the tested heavy metals in greater amount than the well water at Sites C, B, D and tap water. However, arsenic, cadmium, chromium, copper, lead, selenium and silver compounds were not always found in well water at Site A. Indeed, several heavy metals such as cadmium, lead and silver were not found in the well water at Sites B, C and D, tap and river water samples. Arsenic, selenium and zinc were also absent or present in small amounts during some months in all the tested water samples. An average amount of 0.001-0.005 ppm of arsenic was detected in the well water at Sites A, C, river and tap water. The river water had the highest average amount of heavy metals as compared to other water samples but with the absence of cadmium, lead and silver.

All water samples except the tap water and site D were contaminated with ammoniacal nitrogen with the average range of 1.22 – 8.01 mg/L that exceeded the standard requirements of 0.5 mg/L (Table 7). The river water was heavily contaminated with the average concentration of 8.014 ± 3.001 mg/L of ammoniacal nitrogen. The well water at Site C was exceptionally contaminated with ammoniacal nitrogen at 15 times higher than the standard limit in the month of October.

On the other hand, Site A is not heavily contaminated with chemical compounds as compared to the Sites B, C and D except that it had the highest chloride at 19.50 ± 2.21 mg/L (Table 7). Site C has the highest average concentration of hardness (74.98 ± 30.36 mg/L) compared to other water samples whereas Site D had the highest average nitrate level (2.445 ± 4.549 mg/L) amongst all water samples. The river sample had 88% more contamination with ammoniacal nitrogen, 16% hardness and 14% chloride then the average of the well-water samples. However, the river water had 87% and 47% less contamination with nitrite and sulphate compared to the average of well water samples.

Physical parameters

All the well water samples have a slight acidic pH ranging from 5.44-6.62 and constant temperature of

28ºC. The water at Site C as expected has a mean higher turbidity of 2.56 ± 1.38 NTU that correlated well with the high TCU of 7.16 because it is situated at the lower land elevation. Other well water samples had similar turbidity and colour levels with the range of 0.57-0.13 NTU and 4.16-5.00 TCU respectively which is within the standard water quality index. The tap water had similar physical parameters as the well water samples. As expected, the river water sample had the highest turbidity of 18.66 ± 7.71 NTU and intense colour of 45.83 ± 24.16 TCU.

Discussion

The well water from all sampling sites in Sungai Buloh Village met the majority of Malaysia's standard drinking water requirements such as arsenic, cadmium, chromium, copper, lead, selenium and silver compounds but certain types of heavy metals (manganese and iron) and chemicals (chloride and nitrate) exceeded the standard limits. However major public health risks have been found that are related to those that do not meet the standards. The long-term exposure to heavy metals and chemicals will create an unhealthy population and affect every level of human development.[35] In particular, manganese exceeded more than 100% of the standard level in all water samples (Table 5). The deleterious impact on human health after ingestion of constant high dosage of manganese for long term will result in adverse neurological effects such as neurobehavioural and neuropsychological conditions as seen in occupation studies.[35] Site A had the highest average amount of manganese of 0.157 ± 0.045 ppm, followed by Sites C, B and D. This data is consistent with the soil profiles at Site A where the level of manganese at the top layer soil was the highest at 3.23 ppm and it showed a significant correlation with the level of manganese in wellwater at 0.52 (data not shown). Manganese occurs naturally in soil and it may have eroded into the surface water and groundwater by rain-wash off. Heavy metals and chemicals such as arsenic, cadmium, lead and zinc are likewise naturally found in the soil and rocks that may seep into well water.[7,36,37] However, industrial activities such as furniture, marble tile and motor spare parts manufacturing that are found in the surrounding areas of Site A may have contributed to the high levels of manganese and acidic well water. A similar pattern of industrial waste discharge has contributed to heavy metal contamination of the groundwater in India.[7] Overall, the well water at Site A was more heavily contaminated with heavy metals than other sites.

The soil profile analysis revealed that the bottom layer soil with a depth of 3.5 m at all sites contained a variety of heavy metals and chemicals. Thus it is expected that heavy metals and chemicals are present in well water samples. A study has shown a strong interaction between soil and water impacted the level of heavy metal contamination in groundwater.[11] For example, the hardness in water is usually due to the dissolved calcium and magnesium ions present in the sedimentary rocks.[16] This is observed in the well water at Site C where the level of magnesium is slightly skewed higher and this may have contributed to the increased hardness of the water as compared to other wells.

Strong associations between the soil contents and wellwater with the elevation of the land was demonstrated at Site C. Site C is located at a lower level to capture runoff water that carries heavy metals and chemicals easily as compared to other sites that are located at higher ground. The well water at Site C thus had the highest level of turbidity and stronger colour with solid suspensions such as sand, mud and dirt. The high contents of solid suspension may trap microorganisms that may explain the significantly strong correlation between turbidity with *E. coli*, F-specific coliphages and *Giardia* at p>0.1 at Site C's well water. The possible faecal contamination of Site C's well water was most likely indicated by the high level of ammoniacal nitrogen. In this project, the source of ammoniacal nitrogen is most likely contributed by the sewage discharged since the correlation analysis between *E. coil*, F-specific coliphages and *Giardia* with ammoniacal nitrogen is significant at $p<0.1$. Similarly, the Site A's well water had high content of ammoniacal nitrogen.

The RT-PCR also detected FRNA genogroup in both Sites A and C, indicating animal faecal contamination. Microbial source tracking using RT-PCR was shown to be highly sensitive to detect low populations of FRNA coliphages in water matrixes.[6,38] However, the human sewage originated genogroups of FRNA coliphages were not detected in Site A's well-water even though it is situated next to the self-contained toilet. The survival of phages in the environment depends on their resistances against inactivation by temperature, pH level of soil and sedimentation loses.[39,40,41] The fate of the human originated phages may have been inactivated by one of these environmental factors.

The levels of heavy metals and chemicals in the wellwater at Sites B and D did not trigger any public health concern. Even though aluminium and iron levels are considerably higher in the soils at both sites, nevertheless no significant amount was seen in the well water. Both Sites B and D were not surrounded by industrial activities hence trace elements and heavy metals were low in the well-water. The level of ammoniacal nitrogen was not significantly high in the well water at Site B even though it was next to poultry farming activities. This may explain the absence of *E. coli* in the well water at Site B. However, the traces of FRNA coliphage genogroup I and IV that are associated with animal faecal matters and genogroup II that is associated with human sewage were detected in the well water in all months except at Site B in March. These results revealed that the microbial risk assessment of water quality is essentially important to incorporate several reliable detection methods for microbe indicators to validate each data obtained. This is to reassure the quality of water is at the acceptable level for human consumption and usage.

Microbial contamination of drinking water is absolutely not acceptable as the related health consequences in the affected population can emerge as a major public health problem. In this study, the well water at all sites was found not safe for drinking, washing, bathing and for other human activities. The microbial polluted well water with coliforms (1.2 to 2.4×10^3 CFU/100 ml) and occasionally with *E. coli* (.12 - 4×10^2 CFU/100 ml CFU/100 ml) and *Cryptosporidium* oocysts (0.4 cysts/100 ml) indicates that urgent and immediate remedial measures must be taken to provide clean portable water. The presence of these microbial indicators in well water could indicate the presence of highly infectious bacteria, viruses and protozoa that may lead to diarrhoeal diseases.[42] *Cryptosporidium* oocyts and *Giardia* cysts are commonly derived from animals with occasional human origins.[5] As shown in our study, the correlation between *E. coli*, F specific coliphages and *Giardia* is highly significant at $p > 0.01$. These three microbes also have high correlation with iron and arsenic for the fact that they are able to metabolize these elements in their metabolism.[35] Further studies will be required to evaluate the possibility of using these elements as indicators for the presence of microbe pollutants in our water systems. Through this study there are indications that the residents had some knowledge of the quality of ground water they were consuming but there was lack of concern regarding the impact it will have on their health. Therefore there is a need for research in this area to create awareness of the health consequences.

Water-borne diseases cause major economic and public health burdens to individuals and society. Heavy metal contamination can similarly cause severe health consequences and financial burden to health services. Groundwater pollution with heavy metals due to run-offs from landfills has been reported in Malaysia.[43] Our study shows that the river water has unhealthy levels of ammonical nitrogen, iron, cadmium as well as turbidity which could be attributed to leachates from the soil. Some of these pollutants can be potentially carcinogenic and hazardous to various human organ systems especially when they affect the health of growing children. These hazardous impacts on human health can be long term and permanent, thus it is important to implement appropriate monitoring and treatment systems to ensure that the quality of groundwater meets the drinking water regulatory requirements of the country. In this study the tap water in the village was found to be free of ammonical nitrogen and other microbial contaminants. Even though the manganese content in the tap water was found to be slightly higher than the standard limit, it is recommended that tap water be made available to the population at this urbanised village at an affordable cost to mitigate the effects of pollutants on their health. Appropriate consultation can be given to the water purveyor to reduce the manganese content and improve the quality of tap water.

Acknowledgements

The authors acknowledge the Ministry of Science Technology and Innovation for providing the research fund (Project No.: 06-02-09-SF0008). Appreciation to Heads of the various institutions such as the International Medical University, Monash University Malaysia, Taylors University and University of Malaya, all other technical staff and colleagues for supporting and helping us to carry out this study and Dr. Amalraj Fabian Davamani for his valuable comments. Mr Jim Ireland kindly tightened the flow of our English expression.

REFERENCES

1. Savichtcheva O, Okabe S. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. J Water Res. 2006; 40: 2463-76.
2. Prüss-Üstün A, Bos R, Gore F, Bartram J. Safer water, better health: costs, benefits and sustainability of interventions to protect and promote health, World Health Organization, Geneva; Switzerland. 2008.
3. Turky AS, Morsy WS, Awad NM, El Fakharany ZM. Microbial Community in Rural Shallow Groundwater Affected by Surface Contaminated Soil. Int J Academic Res. 2012; 4(4): 89-198.
4. Basturea GN, Harris TK, Deutscher MP. Growth of a bacterium that apparently uses arsenic instead of phosphorus is a consequence of massive ribosome breakdown. J Biol Chem. 2012; 287:28816-9.
5. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev. 2011; 24: 110-40.
6. Jung EL, Lee H, You HC, Hor GH, Ko G. F+ RNA coliphage-based microbial source tracking in water resources of South Korea.Sci Total Environ. 2011; 412:127-31.
7. Bhagure GR, Mirgane SR. Heavy metal concentrations in groundwaters and soils of Thane Region of Maharashtra India. Environ Monitoring Assess. 2011; 173: 643-52.
8. Fianko JR, Osae S, Adomako D, Ache DG. Relationship between land use and groundwater quality in six districts in the eastern region of Ghana. Environ Monitoring Assess. 2009; 153: 139–46.
9. Lai HY, Hseu ZY, Chen TC, Chen BC, Guo HY, Chen ZS. Health risk-based assessment and management of heavy metals-contaminated soil sites in Taiwan. Int J Environ Res Public Health. 2010; 7: 3595–614.
10. Mohamed AF, Teng WS. Assessment of water quality status for the Selangor River in Malaysia. Water Air Soil Pollut. 2010; 205: 63-77.
11. Namaghi HH, Karami GH, Saadat S. A study on chemical properties of groundwater and soil in ophiolitic rocks in Firuzabad, east of Shahrood, Iran: with emphasis to heavy metal contamination. Environ. Monitoring Assess.2011; 174: 573-583.
12. Batayneh A, Ghrefat H, Zaman H, Mogren S, Zumlot T, Elawadi E, Laboun, A, Qaisy S. Assessment of the physicochemical parameters and heavy metals toxicity: application to groundwater quality in unconsolidated shallow aquifer system. Res J Environ Toxicol. 2012; 6(5): 169-83.
13. Mukhopadhyay S. Arsenic in food chain. Indian Dairyman. 2008; 60(2): 17-26.
14. Vidya R, Chandrasekaran N. Ecotoxicological studies on the bioaccumulation of the heavy metals in the Vellore population, Tamilnadu, India. Recent Res Sci Tech. 2010; 2(2): 60-65.
15. Chung BY, Song CH, Park BJ, Cho JY. Heavy metals in brown rice (*Oryza sativa* L.) and soil after long-term irrigation of wastewater discharged from domestic sewage treatment plants. Pedosphere 2011; 21(5): 621-7.
16. World Health Organization. Hardness in Drinking-water. WHO Press: Geneva, Switzerland. 2011.
17. Yang H, Huo X, Yekeen TA, Zheng Q, Zheng M, Xu X. Effects of lead and cadmium exposure from electronic waste on child physical growth.Environ Sci Pollut Res (2013) 20:4441–4447DOI 10.1007/s11356-012-1366-2.
18. Gao Y, Yu G, Luo C, Zhou P. Groundwater nitrogen pollution and assessment of its health risks: A case study of a typical village in rural-urban continuum, China. 2012; PLoS ONE 7(4): e33982. doi:10.1371/journal.pone.0033982.
19. Fatimah A, Zakaria-Ismail M. Notes on the water quality of the Hulu Selai River, Endau-Rompin National Park, Johor, Malaysia. *The Forests and Biodiversity of Selai*, Endau-Rompin. Institute of Biological Sciences: University of Malaya, Kuala Lumpur. 2005; 27 – 30.
20. Cheong S, Lee C, Sung W S, Weon CC, Chan HL, Sang JK. Enteric viruses in raw vegetables and groundwater used for irrigation in South Korea. Appl Environ Microbiol. 2009; 75(24): 7745-51.
21. Gelting RJ, Baloch MA, Zarate BM, Selman C. Irrigation water issues potentially related to the 2006 multistate *E. coli* O157:H7 outbreak associated with spinach. Agri Water Manag. 2011; 98(9): 1395-402.
22. Ogorzaly L, Bertrand I, Paris M, Maul A, Gantzer C. Occurrence, survival, and persistence of human adenoviruses and F-Specific RNA phages in raw groundwater. Appl Environ Microbiol. 2010; 76: 8019–25.
23. Tufenkji N, Emelko MB. Fate and transport of microbial contaminants in groundwater. Encyclopedia of Environmental Health, Five-Volume

Set. Elsevier: USA. 2011; 715-26.

24. Zhuang J, Jin Y. Virus retention and transportas influenced by different forms of soilorganic matter. J Environ Quality 2003; 32: 816-23.
25. Stewart-Pullaro J, Daugomah JW, Chestnut DE, Graves DA, Sobsey MD, Scott GI. F+RNA coliphage typing for microbial source tracking in surface waters. J Appl Microbiol. 2006; 101(5): 1015-6.
26. Haramoto E, Otagiri M, Morita H, Kitajima M. Genogroup distribution of F-specific coliphages in wastewater and river water in the Kofu basin in Japan. Lett Appl Microbiol. 2012; 54(4): 367-73.
27. Jones TH, Johns MW. Assessment of F-RNA coliphage as a potential indicator of enteric virus contamination of hog carcasses. J Food Prot. 2012; 75(8): 1492-500.
28. Hsu FC, Shieh CYS, Duin JV, Beekwilder MJ, Sobsey MD. Genotyping male-specific RNA coliphage by hybridization with oligonucleotide probes. J Appl Environ Microbiol. 1995; 6: 3960-6.
29. Malaysia 2010: Total Population by ethnic group, mukim and group. http://www.statistics.gov.my/portal/download_Population/files/population/05Jadual_Mukim_negeri/Mukim_Selangor.pdf 2010.
30. Salter RS, Durbin GW. Modified USEPA Method 1601 to indicate viral contamination of groundwater. Journal of American Waterworks Association. 2012 http://dx.doi.org/10.5942/jawwa.2012.104.0115
31. Rozen S, Skaletsky H. J. Primer3 on the WWW for General Users and for Biologist Programmers. Bioinformatics Methods and Protocols: Methods in Molecular Biology.Totowa, NJ: Humana Press. 2000; 365–86.
32. Anonymous. Isolation and identification of *Giardia* cysts, *Cryptosporidium* oocysts and free living pathogenic amoebae in water, etc. Methods for the examination of waters and associated materials. Department of the Environment, Standing Committee of Analysts: HMSO, London. 1990; 6-14.
33. APHA 1999: Standard Methods for the Examination of Water and Wastewater. © Copyright 1999 by American Public Health Association, American Water Works Association, Water Environment Federation.
34. World Health Organization. Guidelines for drinking water quality first addendum. Vol.1, Recommendations. 3rd ed. WHO Press: Geneva, Switzerland. 2006.
35. Santamaria AB. Manganese exposure, essentiality and toxicity. Indian J Med Res. 2008; 128: 484-500.
36. Brammer H, Ravenscroft P. Arsenic in groundwater: a threat to sustainable agriculture in South and South-east Asia.Environ Int. 2009; 35: 647-54.
37. Radloff KA, Zheng Y, Michae HA, Stute M, Bostick BC, Mihajlov I, Bounds M, Huq MR, Choudhury I, Rahman MW, Schlosser P, Ahmed KM, Geen A van. Arsenic migration to deep groundwater in Bangladesh influenced by adsorption and water demand. Nat Geosci. 2011; 4: 793–8
38. Friedman SD, Cooper EM., Casanova L, Sobsey MD, Genthner F.J. A reverse transcription-PCR assay to distinguish the four genogroups of male-specific (F+) RNA colihages. J Virol Meth. 2009; 159: 47-52.
39. Dowd SE, Pillai SD, Wang, Corapcioglu MY. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. J Appl Environ Microbiol. 1998; 64(2): 405-10.
40. Brookes JD, Antenucci J, Hipsey M, Burch MD, Ashbolt NJ, Ferguson C. Fate and transport of pathogens in lakes and reservoirs. Environ Int. 2004; 30: 741-59.
41. Ji HJ, Chang HY, Eung SK, Hak MK, You JN, Weon HJ, Yong SJ. Occurrence of Norovirus and other enteric viruses in untreated groundwaters of Korea. J Water Health. 2011; 9(3): 544-55.
42. Wu J, Long SC, Das D, Dorner SM. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J Water Health. 2011; 9: 265-78.
43. Rahman MM, Hassan N, Latif PA, Daud M, Bardaie MZ. Heavy metal pollution in soil, groundwater and surface water of seven selected landfill sites of Kuala Lumpur. Accessed 18 February 2014 from the website:www.cprm.gov.br/pgagem/Manuscripts/rahmanm.htm

Table 1: Primer sequences

FRNA geno-group	Primer	Sequences 5'- 3'	Targeted strains
I	MS2-F	CGGGTAAGTCCATCATAAGC	MS2, JP501, M12, ZR, fr, R17
	MS2-R	GACCCCGTTAGCGAAGTTG	
II	GA-F	GTCGTTCGTTGTTGACTGGTT	GA, JP34, KU1, TL2, SD, TH1, JP500
	GA-R	CATTGCTAACAGGAACGACAG	
III	Qß-F	AATCCGCGTGGGGTAAATC	Qß, M11, MX1
	Qß-DEG	CAAGKGGTRGGGTTCTGGATCTT	
IV	SP-F	CACCGCACTACAGAGGAGAA	SP, NL95, F1
	SP-R	ACCACAGGTCACTCGCACTA	

Table 2: Coliform and *E. coli* counts (per 100 ml) detected in water samples collected for six months.

Sites	Coliform (CFU/100 ml)						*E. coli* (CFU/100 ml)						Cryptosporidium Oocysts (100 ml)				Giardia cysts (100 ml)				
	J	M	A	J*	A*	O	J	M	A	J*	A*	O	J	M	A	J-O	J	M	A	J*	A-O
A	2.4 x 10^2	1.02	2.04	4 x 10^2	2.4 x 10^3	4 x 10	0	0	0.24	0	1.2	4 x 10^2	0	0	0.4	0	0	0	0	0	0
B	0	0	0	0	2.1 x 10^3	1.1 x 10^3	0	0	0	0	0	0	0.4	0	0	0	0	0	0	0	0
C	2.0 x 10	1.9 x 10^2	0	6 x 10	4.6 x 10^2	9.8 x 10^2	0	0	0	2 x 10	0	0	0	0	0.4	0	0	0	0	0	0
D	1.6 x 10^3	1.5 x 10	NA	7.8 x 10^2	NA	1.8 x 10^2	0	0.12	NP	0	NP	0	0	0	NP	0	0	0	NA	0	0
Tap water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
River water	TNTC	TNTC	TNTC	TNTC	TNTC	7.28 x 10^3	4.1 x 10^5	9.4 x 10^5	4.3 x 10^5	1.0 x 10^4	7.6 x 10^4	3.3x 10^4	0	0.4	0	0	0	5.2	7.6	25	0

A = April; A*= August; J = January; J*=June; M = March; O = October; J - O = June – October; A - O = August – October

Table 3: F-specific coliphages counts (per 100 ml) and RT-PCR genotyping of F-FRNA coliphage isolates in water samples collected for six months.

Sites	Genogrouping of F-specific coliphages					
	J	M	A	J*	A*	O
A	ND	GP I	GP I	ND	GP I	GP I
B	GP II	ND	GP I	GP IV	GP I	GP I
C	ND	GP I	GP I	ND	GP I	GP I
D	GP I	GP I	GP I	ND	GP I	GP I
Tap water	GP I	GP I	GP I	ND	ND	ND
	F-specific coliphages (PFU/100 ml) and Genogrouping of F-specific coliphages					
River water	1.22 x 10^4	4.04 x 10^4	3.32 x 10^4	9.8 x 10^2	1.3 x 10^3	3.55 x 10^3
	GP I	GP I	GP I	GP IV	GP I	GP I

A, April; A*, August; J, January; J*, June; M, March; O, October; GP, Genogrouping; ND, non detected

Table 4: Pearson's correlation matrix of Microbial contaminants with physical parameters, chemical compounds and heavy metals

Parameter	Coliform	*E. coli*	F specific coliphages	*Cryptosporidium*	*Giardia*
pH	-0.060	0.186	0.147	-0.040	0.215
Temperature	0.018	-0.164	-0.047	-0.074	-0.273
Colour	-0.135	0.887**	0.583**	0.077	0.852**
Turbidity	0.470*	0.882**	0.694*	0.192	0.818**
Aluminium	-0.123	0.174	0.147	0.150	0.205
Ammoniacal Nitrogen	0.170	0.485**	0.414*	0.022	0.436*
Arsenic	0.230	0.730**	0.688**	0.061	0.754**
Cadmium	-0.045	-0.061*	-0.053	-0.064	-0.06
Chromium	0.255	0.361	0.076	0.235	0.234
Chloride	-0.038	0.370*	0.186	0.290	0.259
Copper	-0.050	-0.087	-0.055	0.253	-0.100
Hardness	0.165	0.173	0.147	0.098	0.180
Iron	0.501**	0.444**	0.605**	0.057	0.418*
Lead	-0.096	-0.122	-0.047	0.325	-0.052
Magnesium	-0.006	0.143	0.202	0.287	0.128
Manganese	0.046	0.457**	0.255	0.128	0.181
Nitrate	-0.075	-0.114	-0.095	-0.122	-0.114
Selenium	0.074	-0.043	-0.043	-0.006	-0.106
Silver	-0.045	-0.061	-0.053	-0.064	-0.06
Sodium	-0.069	0.398*	0.431*	0.288	0.319
Sulphate	-0.017	-0.110	-0.223	0.020	-0.133
Zinc	0.018	0.316	0.602**	0.333	0.469**
Coliform	1.000	-0.047	.a	-0.084	.a
E. coli	0.813	1.000	0.692**	0.223	0.798**
F specific coliphage	0.000	0.000	1.000	0.306	0.782**
Cryptosporidium	0.670	0.204	0.078	1.000	0.142
Giardia	0.000	0.000	0.000	0.422	1.000

*Indicate significant relationship with p <0.05; ** indication significant relationship with p <0.01

Table 5: Concentration of elements (ppm) detected in the soil samples.

Sites	A		B		C		D		Riverside	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Aluminum	69.56	114.64	63.21	25.69	42.36	25.69	60.5541	72.2272	26.7521	26.7586
Arsenic	0.35	1.26	0.08	0.18	0.88	0.0016	0.9848	0.0365	0.0274	0.0274
Cadmium	0.003	0.0004	0.002	0.0002	0.002	0.0001	0.0003	0.0024	0.0012	0.0008
Chromium	0.89	5.67	0.30	0.15	0.11	0.002	0.2751	0.0954	0.0109	0.0853
Cobalt	0.01	0.006	0.005	0.0006	0.007	0.0001	0.0021	0.0031	0.0019	0.0028
Iron	640.48	1444.24	121.50	262.81	46.78	1.51	280.5007	62.5692	40.8553	33.3699
Lead	0.16	0.15	0.13	0.21	0.29	0.009	0.1147	0.1245	0.1112	0.1471
Magnesium	3.23	0.11	0.18	1.38	1.53	0.08	1.7905	0.4359	1.0628	0.6817
Manganese	0.75	0.19	0.69	0.10	6.49	0.01	0.2476	0.5841	0.1026	0.1310
Selenium	0.01	0.006	0.001	0.003	0.0001	0.008	0.0079	ND	0.0001	0.0004
Silver	0.004	0.005	0.0008	0.0004	0.001	0.0001	0.0003	0.0104	0.0004	0.0004
Sodium	0.12	0.02	0.11	0.11	0.13	0.04	0.1253	0.0606	0.0551	0.0573
Zinc	0.70	0.24	2.88	0.07	1.59	0.24	0.1149	0.3414	0.6198	3.1814

Table 6: Range of heavy metal concentrations (ppm) of water samples collected for six months.

Parameters	Site A		Site B		Site C		Site D		Tap water		River Water	
	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average
Aluminum	0.04-0.315	0.136 ± 0.103	0.0-0.036	0.010 ± 0.138	0.00-0.015	0.006 ± 0.006	0.005-0.010	0.073 ± 0.002	0.032-0.165	0.060 ± 0.059	0.004-0.107	0.055 ± 0.050
Arsenic	0.00 -0.005	0.001 ± 0.002	0.000-0.001	0.000 ± 0.001	0.00 – 0.002	0.001 ± 0.000	0.000	0.000 ± 0.000	0.000-0.001	0.001 ± 0.000	0.004-0.006	0.005 ± 0.001
Cadmium	0.000 - 0004	0.001 ± 0.002	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000
Chromium	0.00-0.008	0.004 ± 0.003	0.002-0.006	0.005 ± 0.002	0.005-0.010	0.008 ± 0.002	0.002-0.008	0.003 ± 0.001	0.00 – 0.001	0.001 ± 0.000	0.004-0.012	0.007 ± 0.003
Copper	0.00-0.007	0.003 ± 0.002	0.001-0.006	0.002 ± 0.002	0.00-0.001	0.001 ± 0.000	0.001-0.007	0.004 ± 0.003	0.00-0.001	0.001 ± 0.001	0.001-0.002	0.012 ± 0.004
Iron	0.02-0.081	0.043 ± 0.027	0.051-0.132	0.079 ± 0.033	0.05-0.298	0.200 ± 0.093	0.006-0.035	0.052 ± 0.033	0.006-0.072	0.054 ± 0.056	0.049-1.046	0.450 ± 0.347
Lead	0.000-0.001	0.002 ± 0.002	0.000-0.001	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000-0.001	0.001 ± 0.001	0.000	0.000 ± 0.000	0.000-0.001	0.000 ± 0.001
Magnesium	0.140-1.887	1.209 ± 0.373	1.596-3.586	2.804 ± 0.757	1.300-3.38	2.211 ± 0.808	1.221-2.936	1.459 ± 0.598	0.546-1.131	0.807 ± 0.192	1.616-2.850	2.073 ± 0.412
Manganese	0.110-0.242	0.157 ± 0.045	0.011-0.025	0.018 ± 0.004	0.063-0.12	0.077 ± 0.023	0.006-0.008	0.007 ± 0.001	0.004-0.051	0.015 ± 0.018	0.050-0.326	0.140 ± 0.107
Selenium	0.000-0.007	0.002 ± 0.003	0.002-0.004	0.003 ± 0.001	0.00-0.002	0.001 ± 0.001	0.000-0.001	0.001 ± 0.001	0.000-0.002	0.001 ± 0.001	0.00-0.002	0.001 ± 0.001
Silver	0.000-0.002	0.000 ± 0.001	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000
Sodium	6.92-40.492	16.73 ± 12.68	8.877-88.063	42.69 ± 34.00	4.172-9.809	7.31 ± 2.29	5.488-71.660	41.39 ± 35.25	1.418-3.963	2.89 ± 1.13	9.262-71.661	43.19 ± 28.10
Zinc	0.020-0.048	0.303 ± 0.106	0.006-0.024	0.012 ± 0.006	0.000-0.035	0.016 ± 0.011	0.001-0.036	0.012 ± 0.016	0.000-0.008	0.003 ± .003	0.002-0.066	0.025 ± 0.026

Table 7: Range of chemical concentrations (mg/L) of water samples collected for six months.

Parameters	Site A		Site B		Site C		Site D		Tap water		River Water	
	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average	Range of Concentration	Average
Ammoniacal Nitrogen	0.535-1.921	1.200 ± 0.496	0.085-0.890	0.561 ± 0.307	0.86-7.30	2.011 ± 2.652	0.086-0.512	0.196 ± 0.224	0.187-0.512	0.263 ± 0.270	4.56-9.18	8.014 ± 3.001
Chloride	16.63-21.41	19.50 ± 2.21	14.60-18.35	17.40 ± 2.54	9.71-12.60	11.64 ± 1.33	5.88-14.28	12.00 ± 4.20	8.33-11.70	10.14 ± 1.38	10.40-5.24	17.58 ± 5.89
Hardness	14.14-32.50	28.77 ± 8.40	56.57-79.50	69. 67 ± 9.56	28.00-119.30	74.98 ± 30.36	46.94-56.90	51.69 ± 5.38	8.08-78.00	37.71 ± 22.97	43.30-68	61.60 ± 9.19
Chromium	0.05-6.83	1.265 ± 2.736	<0.01-0.78	0.133 ± 0.317	<0.01-1.43	0.241 ± 0.582	<0.01-9.26	2.445 ± 4.549	<0.01-1.36	0.235 ± 0.551	0.02-0.78	0.137 ± 0.315
Sulphate	12.35-16.88	15.36 ± 1.79	55.66-62.51	58.43 ± 3.83	17.2-42.09	26.54 ± 8.98	25.99-43.68	38.03 ± 8.14	2.22-27.30	11.73 ± 8.44	11.19-5.53	19.07 ± 9.06

Figure 1: Site Map of Sungai Buloh (Source Google Maps)