

## FECAL COLIFORM BACTERIA AND FACTORS RELATED TO ITS GROWTH AT THE SEKOTONG SHALLOW WELLS, WEST NUSA TENGGARA, INDONESIA

Doni Marisi Sinaga<sup>1,2,3</sup>, Mark Gregory Robson<sup>1,4</sup>, Beatrix Trikurnia Gasong<sup>2,5</sup>, Adonia Getse Halel<sup>2</sup>, Dian Pertiwi<sup>2</sup>

<sup>1</sup>International Program in Hazardous Substance and Environmental Management, Graduate School, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

<sup>3</sup>DL-Enterprise (Organizer and International Education Cooperation). Nakhon Pathom, Thailand

<sup>4</sup>School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA

<sup>5</sup>Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

Accepted: 24 May 2016

\*Correspondence:

Doni Marisi Sinaga

<sup>1</sup>International Program in Hazardous Substance and Environmental Management, Graduate School, Chulalongkorn University, Bangkok, 10330, Thailand

<sup>2</sup>Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

<sup>3</sup>DL-Enterprise (Organizer and International Education Cooperation). Prachakorn International House, 135/71-75 Salayaniwet, Phutthamonthon 4 Road, Nakhon Pathom, Thailand (73170)

Email: dms.sinaga@gmail.com, doni.m.sinaga@mail.ugm.ac.id

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### ABSTRACT

**Background:** The poor sanitation and small numbers of households who own toilet in Sekotong regency may relate to the diarrheal events due to the fecal coliform contamination in drinking water.

**Aim:** This paper aims to provide the concentrations of fecal coliform bacteria in shallow well waters and the factors associated to its growth.

**Method:** Fifteen groundwater samples were collected from 5 shallow wells to provide the concentrations of total fecal coliform bacteria (FC), mercury concentration, inorganic nitrogen compounds (represent as ammonia, nitrate, and nitrite), total phosphorus (TP), dissolved oxygen (D), pH, and salinity. The concentration of the parameters was then compared to the safe limit set by World Health Organization (WHO).

**Results:** The results indicated that the drinking water resources at the Sekotong regency were contaminated by coliform and mercury. One location with low mercury concentration was recorded with *E. coli* contamination. Residence, agriculture, and animal livestock were subjected as the sources of coliform contamination. Mercury concentrations may inverse the growth of FC. No apparent relationship was found between total phosphorous and inorganic nitrogen compounds to FC growth. However, we recognized the FC growth responded positively to the level of phosphorous in waters, but associated negatively to nitrate concentration. An inverse correlation was also found between coliform survival and salinity in this study. The pH range at 6.05 – 6.50 supported FC survival.

**Conclusion:** The drinking water resources at the Sekotong shallow wells were contaminated by coliform and mercury. It is important for local government to inform drinking water protection and treatment.

**Key Words:** Fecal coliform bacteria, nutrient load, physicochemical properties

## INTRODUCTION

Worldwide, in 2012, around 21 of 100,000 people die of diarrheal disease.<sup>1</sup> In 2013, this infectious disease contributed to 6% of mortality among children under-5 in Indonesia.<sup>2</sup> As consent of Ministry of Health of Indonesia, diarrheal prevalence in West Nusa Tenggara exceed the national prevalence, reached to 13.2%.<sup>3</sup> Study found poor sanitation was recognized as the cause of diarrhea in developing country.<sup>4</sup> With only 49,10% of the total households in Sekotong regency owns toilet, it is not surprising the District Health Office estimated 1,046 of 25,450 population in Sekotong suffered from diarrhea in 2013, or 10.8% increased than the previous year.<sup>5,6</sup>

The diarrheal episodes in Sekotong regency are perceived to be associated with fecal coliform bacteria. As it has been found in other developing countries, the infectious diarrhea was significantly related with contamination of fecal coliform bacteria in drinking waters.<sup>7-9</sup> Land use and livestock-related management practices, such as grazing and manure applications, may relate to fecal coliform indicator concentrations in waters.<sup>10</sup> This paper aims to measure the concentration of total coliform bacteria in drinking water resources and to find out the factors associated to its growth.

## METHODS

### *Groundwater samples*

There were 15 groundwater samples collected from 5 shallow wells at villages in Sekotong regency (S 08° 45-46' 51.1-2" and E 115° 56' 39.0-41.7"), as shown in Figure 1 with detail description available in previous study.<sup>11</sup> At the sampling locations, the land may be used for residence, agriculture, or animal live stocks as shown in Table 1. The elevation at subjected areas was noted arranged from 2 to 26.5 m asl. During sample collection,

the temperature of shallow well waters was recorded ranged 28.75 – 29.65°C. The samples were collected during rainy season in December 2014 with precipitation falls about 1,557 mm. The shallow well waters which drawn manually using an acid-washed plastic were preserved to a corning®50mL poly ethylene terephthalate (PET) centrifuge tube and stored in an insulated ice box, as the previous study.<sup>12</sup> Single-use glove was used during sample collection.

### *Mercury measurement*

Digestion procedure for waters as the Standard National Indonesia (SNI) 6989.78:2011 regarding to mercury determination in water samples.<sup>13,14</sup> Mercury concentration was measured at Laboratorium Penelitian dan Pengujian Terpadu (LPPT) – Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia, and the inter and intra-observer variations, and the quality control (QC) were controlled following the SNI 06-6992.2-2004 method, accredited to IKU/5- 4//MA-01. The laboratory has been assessed and accredited by SNI ISO/IEC 17025:2005 for conducting calibration and analytical test. The limit of detection (LOD) and the limit of quantification (LOQ) calculated in was 0.03 and 0.10 ppb, respectively, while the recovery of mercury concentration determination in water samples (with 6 replications) was 103%.<sup>11</sup>

### *Water property measurement*

Groundwater samples were transferred to Balai Budidaya Laut Lombok Laboratory for measurement of total organic matter (TOM), total phosphorous (TP) and total inorganic nitrogen by following the national standard of measurement SNI 06-6989: 2005. The pH, dissolved oxygen (DO), and salinity of the freshly collected non-filtered samples were measured *in-situ*

using a pH/DO/Salinity meter, respectively. The device was calibrated 3

times before the measurement.

**Figure 1. Sampling locations represent the 5 shallow wells in Sekotong reGENCY, West Nusa Tenggara, Lombok, Indonesia (Google Earth).**



#### *Fecal coliform measurement*

The fecal coliform bacteria were counted by plate count method at Tryptone Bile X-glucuronide agar 3 days after inoculation.

#### *Data analysis*

The data obtained from the field was analyzed using Microsoft Excel for calculation to obtain the mean value and standard error. The concentration of the observed parameters was represented as mean or mean  $\pm$  SE.

## **RESULTS AND DISCUSSIONS**

#### *Fecal coliform bacteria: concentrations and sources*

Results exhibited that during rainy season the fecal coliform (FC) was detected on all shallow wells at Sekotong's Gold Mining village and one of those five sampling locations for fecal *E. coli*. The colony forming unit (CFU) assessed in the shallow well water samples varied from 1 to 4,000 CFU/100 mL for coliform and measured at 300 CFU/100 mL for *E. coli*. Several numerous sources can be subjected as the

sources of coliform bacteria contamination in water resources including fecal contamination either from humans or animals, or runoff from agricultural field, rainfall, or sewage. Table 1 confirmed that the fecal coliform may enter the drinking water system from the nearby residence, agriculture, and animal stocks; but, by the data, only residence contributed to *E. coli* contamination to the drinking water resources. The highlighted sources of fecal coliform bacteria at the subjected locations are animal grazing and open defecation. The depths of water surface in the observed well waters may reflect the accessibility of coliform bacteria to the shallow wells,<sup>15</sup> but it is not associated to the number of TC in the shallow wells.

There is no doubt that the shallow well water at Sekotong's Gold Mining village should be treated. The total coliform bacteria measured in the subjected areas above the safe level by WHO guidelines that recommend the drinking water should have no detectable amounts of coliform bacteria. From the

previous study the authors highlighted that the local people drink the fresh water without boiling.<sup>11</sup> Drinking untreated water will impact to the diarrhea morbidity in Sekotong Lombok Barat.

#### *Mercury roles to fecal coliform bacteria*

This study shows a low concentration of fecal coliform bacteria was measured at location with mercury concentration above the safe limit for drinking water (2 ug/L), at the average ( $\pm$  SE) of  $2.370 \pm 0.653$  ug/L. An increase of coliform concentration was found with a decreasing

level of total mercury. The coliform may resist to the heavy metal,<sup>16</sup> but the growth can be deduced. Mercury contamination in Sekotong shallow wells was caused by the artisanal small-scale gold mining nearby as reported by the previous study.<sup>11</sup> The *E. coli* isolated at sampling location 5 resisted to elemental mercury at 10 ug/L (data not shown). Found at location 4, the concentration of mercury at the average of 0.115 ug/L may not relate to coliform suppression. We believe mercury should be existed in the water at certain level to inverse the growth of coliform bacteria.

**Table 1. Description of sampling locations and the level of the observed parameters including coliform bacteria, nutrient load, and physicochemical properties of the groundwater**

Parameters	Unit	Sampling Locations					Safe Limit <sup>a</sup>
		Location 1	Location 2	Location 3	Location 4	Location 5	
Elevation	m asl	5	26.5	19.5	2	21	
Depth to water surface	m	1.38	5.10	3.10	1.63	2.5	
<b>Land Uses</b>							
Residence		Yes	Yes			Yes	
Agriculture		Yes		Yes	Yes		
Animal Livestock		Yes		Yes			
Total Fecal Coliform	CFU/100 mL	1	20	365	1,120	4,000	0
Fecal <i>E. coli</i>	CFU/100 mL	-	-	-	-	300	0
Mercury concentration	ug/L	$2.370 \pm 0.653$	$0.042 \pm 0.012$	$0.054 \pm 0.006$	$0.115 \pm 0.085$	$0.070 \pm 0.011$	
<b>Nutrient Load</b>							
<i>Nitrogen Compounds</i>							
• Nitrate	mg/L	$1.95 \pm 0.071$	$2.75 \pm 0.071$	$2.05 \pm 0.071$	$1.70 \pm 0$	$0.90 \pm 0.141$	10
• Nitrite	mg/L	$0.02 \pm 0$	$0.03 \pm 0$	$0.02 \pm 0$	$0.02 \pm 0$	$0.02 \pm 0$	1
• Ammonia	mg/L	$0.004 \pm 0.001$	$0.045 \pm 0.001$	$0.015 \pm 0.001$	$0.049 \pm 0.004$	$0.006 \pm 0.001$	1.5
• Total Nitrogen	mg/L	1.97	2.83	2.09	1.77	0.93	
Total Phosphorous	mg/L	$0.060 \pm 0.001$	$0.016 \pm 0.001$	$0.011 \pm 0.001$	$0.036 \pm 0.001$	$0.037 \pm 0.001$	n.a
Total Organic Matter	mg/L	$104.87 \pm 0.06$	$51.87 \pm 0.06$	$13.90 \pm 0.01$	$7.86 \pm 0.39$	$150.42 \pm 0.01$	n.a
Dissolved Oxygen	mg/L	$1.43 \pm 0.01$	$2.82 \pm 0.01$	$2.51 \pm 0.05$	$1.59 \pm 0.01$	$1.66 \pm 0.05$	> 2.8
pH	-	7.25	7.55	6.05	6.50	7.35	6.5-8.5
Salinity	%	$0.25 \pm 0.029$	$0.05 \pm 0$	$0.20 \pm 0$	$0.18 \pm 0.001$	$0.05 \pm 0$	0.1

<sup>a</sup> WHO's guidelines<sup>17</sup>

#### *Concentrations of inorganic nitrogen compounds*

During the sampling time, the concentrations of inorganic nitrogen compounds (represented as mg of nitrogen per liter in the nitrate, nitrite or ammonia) were very low varied from 0.926 to 2.825 mg/L in the shallow well water samples.

The three assessed inorganic nitrogen species were found met the safe limit for drinking water by WHO. Nitrate concentrations were varied from 0.90 to 2.75 mg/L. The nitrite levels at the five sampling locations were almost similar ranged 0.02 – 0.03 mg/L. The peak level of nitrite was found at Location 2 where the

nitrate was found at the highest level among the other sampling locations. Ammonia concentrations were reported between a low of 0.004 mg/L and a high of 0.049 mg/L.

In environment, ammonia originates from agricultural and industrial processes, and indicates the possible pollution of sewage and poultry. However, from the result, the ammonia concentration assessed in the shallow groundwater was modest. The contamination may be caused by the human activity, but seems the ammonia was oxidized while infiltrated to the groundwater to nitrate and nitrite.<sup>18</sup> Therefore, the level of ammonia in the groundwater system may indicate either nitrogen compounds contaminated the water or nitrification process existed in the system. The high levels of nitrate which following with low concentrations of ammonia and dissolved oxygen may indicate the nitrification process on the observed shallow wells. At location 5, used as residence, we found that the low level of ammonia, nitrate, and dissolved oxygen may indicate the presence of nitrification, but low ammonia input. Contrary to the data assessed in location 2, the residence may contribute to an increasing of ammonia flow at the water system. By the data, it is not clear which activity contributed the most in an increasing ammonia concentrations in the shallow groundwater system.

*Total Phosphorus (TP), Total Organic Matter (TOM), Dissolved Oxygen, and pH* Phosphorus may be resulted from the decomposition of organophosphate pesticides, and it then runs off to the nearby water reservoirs.<sup>19</sup> The mean concentrations of total phosphorus (TP) load were between 0.11 and 0.60 mg/L. Referred to the criteria by OECD,<sup>20</sup> the trophic state index (TSI) of shallow well waters in Sekotong village fell into

mesotrophic and eutrophic. It may explain the low DO of the shallow well waters. From the Table 1, only one of five sampling locations satisfied the DO level standard for drinking water (2.8-12.5 mg/L), and interestingly at the wells which categorized in eutrophic the DO levels were measured less than 2 mg/L. The study shows the conditions may lead to eutrophication and this condition will promote anaerobic process in the groundwater system. As review before, an excess contamination of phosphate to the receiving water will grow algal and aquatic plants greatly, and consumed large amounts of oxygen,<sup>19</sup> and it fosters a decreasing of pH.<sup>21</sup> However, the pH of groundwater was at a low of 6.05 and a high of 7.55, and this pH supported FC survival.

The highest concentration of TP was found at location 1 where the combination of residence, agricultural activity, and animal livestock were taken place. The depth to water surface was also recognized to explain the high level of TP at the sampling locations, but not the dissolved oxygen and pH. Surprisingly, the results exhibited a negative correlation between DO and TP, as shown in location 2 and 3. It may indicate the low TP was a result of inadequate input of TP at those two locations. In addition, we found relatively low inputs of total organic at location 2 and 3, but high at location 1. By the data, there is weak evidence to show which activity increases or decreases the nutrient load to the shallow wells, but it is necessary to highlight an importance of direct access of contamination to the groundwater system through the wells.

#### *Influence of inorganic nitrogen and phosphorus on total fecal coliform*

During the observation, the lowest concentration of inorganic nitrogen compounds, measured at location 5, was

followed by a very high number of total coliform. By the result, the level of ammonia and nitrite cannot show an apparent association to total coliform, as it shows the small number of TC at location 1 where ammonia found at the lowest level and nitrite found at the relatively similar level with the location 5. We recognized a negative association between nitrate concentration and total coliform in this study. The previous study also exhibited the same pattern revealing the wells contaminated by fecal coliform bacteria had measured with low nitrate concentrations.<sup>22</sup> However, in a close system as the septic-tank system, where the high concentrations of nitrate was recorded, there was no suppression of TC.<sup>23</sup>

Among the five shallow wells, no apparent relationship was found between total coliform and total phosphorous. However, we highlight a positive association between total coliform and total phosphate when the location used only for agriculture and livestock. Our study supports the previous experiment revealing the fecal coliform and phosphorus released from land-applied manure are transported similarly in surface runoff.<sup>24</sup> In this study we found that the correlation between phosphorus and total coliform in residential areas somehow unclear. The possible explanation perhaps was due to the difference of defecation behavior and sanitation of the residence.

#### *Effects of salinity and pH on fecal coliform*

The roles of salinity and pH have been considered by previous study to identify the causes of dynamic coliform growth in water.<sup>25,26</sup> Our study underlined a location measured with 0.25% salinity was assessed with very low number of total coliform. An inverse correlation was also found between coliform survival and salinity by other authors.<sup>25</sup> However, at location 3 and 4

measured respectively with 0.20% and 0.18% the total coliform was tremendously higher than the number in location 1. The pH of shallow well water at location 3 and 4, respectively measured at 6.05 and 6.50, are still in optimum range of coliform bacteria to grow.

## CONCLUSION

The results conclude that the drinking water resources at the Sekotong shallow wells were contaminated by coliform and mercury. Animal grazing and open defecation are likely correlated to coliform contamination to the groundwater. For this reason, it is important for local government to inform drinking water protection and treatment. This research also presents the role of other contamination and physicochemical properties to the growth of fecal coliform bacteria in the shallow well system. An inverse growth of coliform may associate to mercury concentration and nitrate level in the groundwater. Should be realized that phosphorous will increase the concentration of fecal coliform in waters. The prevention actions of direct access of contaminants to the groundwater system through the wells are necessary.

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**Cite this article as:** Sinaga DM, Robson MG, Gasong BT, Hael AG, Pertiwi D. Fecal coliform bacteria and factors related to its growth at the Sekotong shallow wells, West Nusa Tenggara, Indonesia. *Public Health of Indonesia* 2016; 2(2): 47-54