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# Assessment of microbiological safety of ground water used in rainbow trout farms

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ABSTRACT

Ground water is the main type of water used in aquaculture farming in Armenia. Ground water arrives under pressure from a depth of about 100–180 m.

Its temperature and pH values varied between 13 °C and 15 °C and 6.5–7.42 respectively. This study was conducted to determine the frequency of occurrence of total, fecal coliform bacteria and bacteria from the genus *Pseudomonas* in the ground water samples from two aquaculture farms.

There were analyzed 100 samples of ground water in two fish farms. Total coliform bacteria were detected in 86% of analysed water samples. In 60% of analysed samples taken from wells of "SIS" farm number of total coliform bacteria exceeded 300 cfu/100 ml. Thermotolerant coliform bacteria were recovered from 46% of water samples. Among coliform bacteria the highest frequency of recovery shown by the following species: *Hafnia alvei*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella oxytoca*. Bacteria from the genus *Pseudomonas* have been recovered from 100% of analyzed water samples.



## 1. Introduction

Ground water is commonly considered the most desirable water source for aquaculture because, at a given site, it is usually consistent in quantity and quality, and free of toxic pollutants and contamination with predator or parasitic living organisms.

This type of water is becoming increasingly relied upon as a major source of water and the security of water quality for groundwater in developing nations is a major issue. Contamination of groundwater by microbial pathogens has been documented also in developed countries due to failures in well head protection, inadequate off-set and diffuse contamination sources (Bockelmann et al., 2009; Borchardt, Haas, & Hunt, 2004; Goss & Richards, 2008).

The quantity of fish farms in Armenia exceeds 200. 60% of mentioned fish farms are located in Ararat region. All these fish farms have their own wells (with a capacity of 50–100 L/s), through which water arrives under pressure (SYA, 2010). Estimations based on the reported quantity of produced fish per year in Armenia indicate that between 2005 and 2008 annual per capita consumption increased sharply from 0.3 kg to 1.8 kg (FAO, 2011,p. 59).

One of the main factors of ground water pollution is the microbial contamination by pathogenic microorganisms. Enteric pathogens are typically responsible for waterborne sickness (Karaboze, Ucar, Eltem, Ozdmir & Ates, 2003).

Despite the common assumptions that all enteric pathogens are a contamination risk for groundwater (John & Rose, 2005) the majority of studies of pathogens in groundwater has focused on the presence of indicator microorganisms (Emmanuel, Pierre, & Perrodin, 2009; Wall, Pang, Sinton, & Close, 2008).

Currently, coliforms and *Escherichia coli* are of great importance among bacterial indicators used in water quality definition and health risk (Giannoulis, Maipa, Konstantinou, Albanis & Dimoliatis, 2005).

Bacterial flora of fish would reveal the bacteriological conditions of the water where fish inhabit. Thus, contaminated ground water can be source of contamination of fish by pathogenic bacteria.

Faecal coliform in fish demonstrates the level of pollution of their environment because coliforms are not the normal flora of bacteria in fish (Cohen & Shuval, 1973).

However, a few reports on the bacterial flora of ground water used for aquaculture are available.

Ground water can be considered as a critical point during production of cultured rainbow trout, thereby quantitative and qualitative aspects of bacterial flora associated with this type of water must be studied to develop a risk management strategy and prevent possible cross contamination between water and fish.

Therefore the present study was designed to determine the frequency of occurrence of total, fecal coliform bacteria and

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bacteria from the genus *Pseudomonas* in the ground water used in "SIS" and "Gyumri" aquaculture farms.

## 2. Materials and methods

### 2.1. Sampling

Sampling was carried out in spring and summer months of 2010. Groundwater samples have been taken directly from the outlet points of each well. Ground water was collected from "SIS" and "Gyumri" fish farms which are located in Ararat region. Sterile glass sampling screw-cap bottles with capacity of 500 ml were used for collecting the water samples. The bottles were kept unopened until the moment of collection. Duplicate samples were brought to the laboratory within 1 h of collection and immediately analysed. All samples (each 100 ml) were filtered under vacuum (Vacuum Filtration System, Millipore®, USA) using 0.45 mm Millipore membrane filters (HiMedia Laboratories, India). After filtration, each filter was removed aseptically from the apparatus and placed onto Endo, Brilliant green, HiCrome EC 0157:H7, Xylose Lysine Desoxycholate, Bismuth sulphite and *Pseudomonas* Cetrimide agar media (HiMedia Laboratories, Mumbai, India).

### 2.2. Detection and enumeration of bacteria

#### 2.2.1. Total coliform

Plates with Endo and Brilliant green media (M029, M016, HiMedia Laboratories, Mumbai, India) were incubated aerobically at 37 °C and examined after 24 h of incubation.

#### 2.2.2. E. coli

For detection and enumeration of *E. coli* Endo and HiCrome EC 0157:H7 selective agar media (M1575, HiMedia Laboratories, Mumbai, India) have been used. Plates with Endo agar were incubated at 44 °C. For confirmation of *E. coli* after 24 h colonies were transferred on HiCrome Coliform Agar (M1300, HiMedia Laboratories, Mumbai, India). Dark blue colonies were considered as *E. coli*. Final confirmation was done using HiE. coli™ Identification Kit (KB010, HiMedia Laboratories, Mumbai, India).

#### 2.2.3. Salmonella

After filtration filters were placed on Xylose Lysine Desoxycholate and Bismuth Sulphite agars (M031 and M027, HiMedia Laboratories, Mumbai, India). Plates were incubated at 37 °C. For confirmation of the obtained colonies HiCrome Salmonella Agar and HiSalmonella™ Identification Kit (M1296 and KB011, HiMedia Laboratories, Mumbai, India) have been used.

#### 2.2.4. Pseudomonas

For enumeration and detection of bacteria from genus *Pseudomonas* Cetrimide agar (M024, HiMedia Laboratories, Mumbai, India) was used. Plates with Cetrimide agar were incubated at 30 °C. For identification of *Pseudomonas* species it has been used HiFluoro Pseudomonas Agar Base (M1469, HiMedia Laboratories, Mumbai, India).

### 2.3. Statistical analysis

Colony forming units (cfu)/ml was transformed into log cfu/ml. Data were analysed statistically using Descriptive Statistics for each type of microorganism and differences in counts determined by Least Square difference test. Prevalence was compared correlation test. All significant differences were determined at $P < 0.05$. All microbiological tests were replicated two times.

## 3. Results and discussion

This study was undertaken to assess the presence of Gram-negative bacteria in groundwater intended for aquaculture farming. 15 species of bacteria related to 11 genera have been isolated from 100 samples of groundwater.

In ground water intended for aquaculture farming the number of total coliform bacteria and thermotolerant coliforms should not exceed 1000 cfu/100 ml and 10 cfu/100 ml limits respectively in accordance with International Standards (WHO,1989).

Number of total coliform bacteria exceeded maximum permissible levels in wells #1 and #2 of "SIS" farm, in accordance with mentioned standard. In wells #1, 2, 3 and 4 numbers of *E. coli* have exceeded 10 cfu/100 ml limit.

Results of analysis of water samples from "Gyumri" farm have shown exceeding of the number of total coliform bacteria in Well #3. In water samples taken from Wells # 1, 2 and 3 relatively high numbers of *E. coli* have been occurred. Species from genera *Aeromonas*, *Pseudomonas*, *Flavobacter* are widespread and typical for water ecosystems.

The frequency of occurrence of the isolated bacterial species form ground water is given in Table 1.

Gram negative, oxidase positive bacteria for genus *Pseudomonas*, *Aeromonas* and *Flavobacterium* possessed high numbers and frequency of occurrence in samples of ground water. There was no occurrence of bacteria for genus *Vibrio* in samples of groundwater. There was noticed high prevalence of P*seudomonas aeruginosa* and *Pseudomonas putida* from ground water samples of "SIS" farm. From isolated coliform bacteria *Enterobacter aerogenes* had highest frequency of occurrence. It was detected in 45% of analysed samples of ground water in SIS farm.

According to (Aydin, 2007) total coliforms, thermotolerant coliforms, *E. coli*, *Enterococcus* spp., *Salmonella* sp., *Staphylococcus* spp. and *P. aeruginosa* were detected in 25%, 17.5%, 15%, 47.5%, 15%, 27.5% and 15% of the groundwater samples, respectively.

The prevalence of the bacteria tested in ground water of "SIS" and "Gyumri" farms is given in Table 2 and Table 3.

The overall prevalence observed for *Pseudomonas*, *E. coli* and total coliform, the difference across farms for the selected bacteria not being statistically significant.

A statistically significant difference ($P = 0.004$) was noted for the prevalence of *E. coliO157:H7* across farms which ranged from 0% to

**Table 1**
Frequency of occurrence of bacterial species in ground water taken from two different farms.

| Species | Farms | |
|---|---|---|
| | SIS (frequency of occurrence %) | GYUMRI (frequency of occurrence %) |
| *Pseudomonas aeruginosa* | 80 | 60 |
| *P. putida* | 20 | n.d. |
| *P. fluorescens* | 30 | 22 |
| *P. diminuta* | n.d. | 1 |
| *Flavobacterium psychrophilum* | 17 | n.d. |
| *Aeromonas hydrophila* | 2 | n.d. |
| Coliforms bacteria | | |
| *Edwarsiella tarda* | 1 | n.d. |
| *Enterobacter cloacea* | 45 | 15 |
| *Citrobacter freundii* | 3 | n.d. |
| *E. coli* | 22 | 7 |
| *Hafnia alvei* | 12 | 10 |
| *Yersinia ruskeri* | 5 | n.d. |
| *Serratia* spp. | 4 | 2 |
| *E. coliO157:H7* | 10 | 6 |
| *Salmonella* spp. | 2 | n.d. |

n.d. = not detected.