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USE OF IDEXX COLILERT AND ENTEROLERT AS A RAPID AND SIMPLE METHOD FORGROUNGWATER QUALITY MONITORING MANAGEMENT

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Abstract

Groundwater quality monitoring management requires the implementation and/or expansion of routine groundwater quality monitoring programs in all villages in the counties of Romania. While the standardization of sample collection and laboratory testing protocols is essential, the ability to provide timely public notification is an equally important element. In preparation for the implementation of the groundwater quality monitoring management programe we investigated the use of IDEXX Colilert-18, IDEXX Enterolert E, Quanti-Tray/2000 (IDEXX) as a means to reduce the time necessary for public notification of freshwater quality while still providing accurate and consistent test results. The fact that IDEXX decreases the time from sample collection to public notification by over six hours coupled with its ease of use and consistent results make this method an attractive choice for the implementation of groundwater management programs.

Keywords: *Escherichia coli, Enterococcus faecium, E. faecalis, ground water quality monitoring management.*

INTRODUCTION

In the past, fecal coliform bacteria were recommended for monitoring the microbiological quality of freshwaters and groundwaters. Many states adopted this standard for the testing of both fresh and marine environments. More recent studies have indicated that E. coli and enterococci may be better indicators of fecal contamination than the fecal coliforms. The USEPA recommends E. coli (freshwater, groundwater) or enterococci (freshwater, groundwater or marine) as indicators of fecal contamination because high densities of these microorganisms are linked to an increase in gastrointestinal illness (Kinzelman et al., 2005).

Implementation of the groundwater quality monitoring programs in all villages in the counties of Romania requires authorities to develop monitoring and notification programs based on water quality criteria and standards for their groundwaters consistent with published criteria for pathogen indicators. Routine monitoring, employing appropriate indicator organisms, allows for successful protection of public health but the regulation of groundwaters can suffer several limitations including a turn around time in excess of 24 hours, unavailability of trained staff, lack of laboratory facilities and a potential increase in costs associated with implementation.

The membrane filtration technique for the recovery of E. coli from groundwater or surface water samples has disadvantages, including the necessity of a traditional laboratory staffed by personnel proficient in basic microbiological techniques and a turn-around time in excess of 24 hours (Kinzelman et al., 2005). Environmental modeling and other analytical methods that could significantly reduce the turn around time are currently under investigation (fiber optics, flow cytometry, real-time PCR) but are not yet available to testing laboratories and are very expensive. In this study we tried to investigate simple and more rapid methods for the detection of E. coli and fecal enterococci in groundwater as a means for more timely public notification regarding drinking water quality.

Previous studies have verified the use of IDEXX Colilert (18-hour & 24-hour) as an acceptable alternative to other test methods for

the recovery of E. coli from drinking water, source water, and wastewater. Few studies worldwide explored the use of IDEXX as an alternative to traditional membrane test methods for the recovery of microorganisms from bathing waters. Of those studies, the major focus has been on marine recreational waters rather than fresh (Kinzelman et al., 2005). By utilizing the 18-hour formulation of this test in drinking and ambient water quality method for the detection of E. coli and fecal enterococci we sought to protect the public by providing a consistent monitoring program while working within the resources (both monetary and technical personnel) of local authorities.

MATERIAL AND METHODS

Sample Collection

A total of seven water samples from six different counties Buzău, Călăraşi, Ilfov, Ialomița,Prahova, RâmnicuVâlcea and one from Bucharest were analyzed during the period four years for the presence of E. coli and fecal enterococci. Groundwater samples were collected from Gura Teghii - Buzău, Drajna – Călăraşi, Tamași – Ilfov, Ciochina – Ialomiţa, Adunați – Prahova, Bogdănești - Râmnicu Vâlcea,2 3 August district – Bucharest in sterile screw-top bottles. Samples wereobtained from wells of varying depths between 6 and 12 meters and taking a 500 ml sample of the water. Samples were transported in a cooler on ice packs to the Ecology and Microbiology laboratory from Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest. All samples were received at the laboratory and processed immediately. Split samples from each individual monitoring site were simultaneously tested, within one hour of arrival, using the IDEXX defined substrate methods.

IDEXX Colilert-18®

Colilert simultaneously detects total coliforms and E. coli in water. It is based on IDEXX's proprietary Defined Substrate Technology. When total coliforms metabolize Colilert's DST nutrient-indicator, ONPG, the sample turns yellow. When E. coli metabolize Colilert's DST nutrient-indicator, MUG, the sample also fluoresces. Colilert can simultaneously detect these bacteria at 1 cfu/100 mL within 24 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

The IDEXX most probable number (MPN) test was performed according to procedures recommended in the IDEXX package insert. The chemical reaction of this test is based on Defined Substrate Technology™ (DST). Chemical substrate containing 4-methyl-umbelliferyl β-D-glucuronide (MUG) was added to a 100-ml poured into a multi-well tray, and sealed Quality control was run daily to validate test performance and included both positive (E. coli ATCC 25922) and negative (Pseudomonas aeruginosa ATCC 27853) controls. E. coli possesses the enzyme β-glucuronidase, which metabolizes MUG, releasing the 4-methyl-umbelliferyl dye. This dye has the ability to fluoresce under long wave ultra-violet light (365 nm). After incubating for 18 hours at 35°C, the samples were examined for fluorescence. A MPN table provided with the kit converts the number of wells producing fluorescence to MPN/100 ml.

IDEXX Enterolert - E

Enterolert-E detects enterococci, such as E. faecium and E. faecalis, in groun water, fresh and marine water. It is based on IDEXX's proprietary Defined Substrate Technology (DST). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert-E's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces. Enterolert-E detects enterococci at 1cfu per 100 mL sample within 24 hours.

RESULTS AND DISCUSSIONS

Sampling of water were done in 500milliliter sterile screw-top bottles. Water samples were taken from six families in the counties Buzău, Călăraşi, Ilfov, Ialomiţa, Prahova, Râmnicu Vâlcea and one from a family in Bucharest. Samples were transported in a cooler on ice packs to the Ecology and Microbiology laboratory from Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine

of Bucharest. Split samples from each individual monitoring site were simultaneously tested, using laboratory WTW pH-meter, laboratory WTW turbidimeter and the IDEXX defined substrate methods. Table 1 presents logistics data related to the sampling water sample.

Table 1. Logistics data ground water sample

The place of sampling	Identity of the sample
Gura Teghii Village Gura Teghii Commune Buzău County	GTGTBZ
Drajna Village Dragalina Commune Călăraşi County	DDCL
Tamaşi Village Corbeanca County Ilfov County	TCIF
Orezu Village Ciochina Commune Ialomiţa County	OCIL
Adunaţi Village Adunaţi Commune Prahova County	AAPH
Bogdăneşti Village Bujoreni Commune Râmnicu Vâlcea County	BBRV
Bucharest	B

Organoleptic, physico-chemical indicators for ground water

Table 2 presents the organoleptic, physicochemical indicators of the water samples.

Table 2. Organoleptic, physico-chemical indicators of the ground water samples

(laboratory environmental conditions - T oC = 23 oC, humidity = 54%)

Indicator name	**Test method**	**Maximum allowable values (see L 458/2002 republished in 2011)**	**Quantification limit**	**The determined values**
Color	SR ISO 7887/2012	Acceptable to consumers and no abnormal change	-	White - yellowish
Smell	SR EN 1622/ 2007	Acceptable to consumers and no abnormal change	-	Acceptable to consumers and no abnormal change
Taste	SR EN 1622/ 2007	Acceptable to consumers and no abnormal change	-	Acceptable to consumers and no abnormal change
pH / Temperature	SR EN ISO 10523/ 2012	6,5 – 9,5 unit pH/^{o}C	-	1.GTGTBZ sample – 7,912 unit pH/ 28 ^{o}C 2.DDCL sample - 7,923 unit pH/ 28^{o}C 3.TCIF sample – 7,103 unit pH/ 28^{o}C 4.OCIL sample – 8,124 unit pH/ 28^{o}C 5.AAPH sample - 7,125 unit pH/ 28 ^{o}C 6.BBRV sample - 7,501 unit pH/ 28^{o}C 7.B sample - 6,745 unit pH/ 28 ^{o}C

Turbidity	SR EN ISO 7027/2001	< 5 NTU	-	1.GTGTBZ sample – 0,18 NTU 2.DDCL sample - 0,01 NTU 3.TCIF sample – 0,08 NTU 4.OCIL sample – 0,11 NTU 5.AAPH sample -0,01 NTU 6.BBRV sample - 0,04 NTU 7.B sample - 0,02 NTU

The result obtained with the WTW turbidimeter for the fountain water sample, the recorded value being 0.01 NTU and 0,18 NTU, below the minimum admissible limit according to the standard of <5 NTU.

Table 3 presents the microbiological indicators of the water samples.

Table 3. Microbiological indicators of the groundwater samples

(laboratory environmental conditions - T oC = 23 oC, humidity = 54%)

Indicator name	**Test method**	**Maximum allowable values**	**The determined values**
Total coliform bacteria / 100 ml water sample	Colilert IDEXX	0 coliform bacteria/100 ml sample	1.GTGTBZ sample – 0 coliform bacteria/100 ml sample 2.DDCL sample – 0 coliform bacteria/100 ml sample 3.TCIF sample – 0 coliform bacteria/100 ml sample 4.OCIL sample – 0 coliform bacteria/100 ml sample 5.AAPH sample -0 coliform bacteria/100 ml sample 6.BBRV sample –0 coliform bacteria/100 ml sample 7.B sample - 0 coliform bacteria/100 ml sample
Escherichia coli/ 100 ml water sample	Colilert IDEXX	0 *Escherichia coli*/100 ml sample	1.GTGTBZ sample – 0 *Escherichia coli*/100 ml sample 2.DDCL sample - 0 *Escherichia coli*/100 ml sample 3.TCIF sample – 0 *Escherichia coli*/100 ml sample 4.OCIL sample – 0 *Escherichia coli*/100 ml sample 5.AAPH sample - 0

			Escherichia coli/100 ml sample 6.BBRV sample - 0 *Escherichia coli*/100 ml sample 7.B sample – 0 *Escherichia coli*/100 ml sample
Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*)	Enterolert IDEXX	0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample	1.GTGTBZ sample – 0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample 2.DDCL sample - 0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample 3.TCIF sample – 0Intestinal enterococci (faecal streptococci*Enterococcus faecalis, E. faecium*) /100 ml sample 4.OCIL sample – 0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample 5.AAPH sample -0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample 6.BBRV sample - 0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample 7.B sample - 0Intestinal enterococci (faecal streptococci *Enterococcus faecalis, E. faecium*) /100 ml sample
Escherichia coli/ 100 ml water sample	Enterolert IDEXX	0 *Escherichia coli*/100 ml sample	1.GTGTBZ sample – 0 *Escherichia coli* /100 ml sample 2.DDCL sample - 0 *Escherichia coli* /100 ml sample 3.TCIF sample – 0 *Escherichia coli* /100 ml sample 4.OCIL sample – 0

			Escherichia coli /100 ml sample 5.AAPH sample -0 *Escherichia coli* /100 ml sample 6.BBRV sample - 0 *Escherichia coli* /100 ml sample 7.B sample – 0 0 *Escherichia coli* /100 ml sample

CONCLUSIONS

Sampling of water were done in 500 milliliter sterile screw-top bottles. Water samples were taken from six families in the counties Buzău, Călăraşi, Ilfov, Ialomiţa, Prahova, Râmnicu Vâlcea and one from a family in Bucharest. Samples were transported in a cooler on ice packs to the Ecology and Microbiology laboratory from Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest. Split samples from each individual monitoring site were simultaneously tested, using laboratory WTW pH-meter, laboratory WTW turbidimeter and the IDEXX defined substrate methods.

The physical parameters of the laboratory were: 23 degrees Celsius and 54% humidity. The determinations were made with the laboratory instruments WTW pH meter and WTW turbidimeter.

For microbiological analyzes IDEXX Colilert - 18® and IDEXX Enterolert were used. All the ground water samples incubated 18 hours at 35 degrees Celsius in the Memmert thermostat incubator and after these period results were interpreted.

Following the organoleptic, physicochemical and microbiological analyzes of samples, we identified the following: colour white - yellowish; odour: acceptable to consumers without any abnormal change; taste: acceptable to consumers without any abnormal change; pH range beteewn6.745 and 7.923 pH units / 28 oC; turbidity range between 0.01 NTU and 0.18 NTU; no total coliform bacteria were detected; no Escherichia coli were detected; no intestinal enterococci (fecal streptococci Enterococcus faecalis, E. faecium) were detected; Organoleptic, physicochemical and microbiological results have shown that fountain water falls within the normal limits for these parameters.

Advantages of the IDEXX 18-hour method include minimal labour costs and training, ease of use, ease of interpretation, and more rapid availability of results. IDEXX may be of interest to other communities who need to routinely monitor ground water resources.

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