PREPARATION OF INDICATORS FOR MICROBIOLOGICAL ANALYSIS OF WATER IN SCHOOL LABORATORY

Students: Karlo Strel & Globe group
Mentor: Olivera Tadić, globe teacher
Secondary school Mate Blažina Labin
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Summary

In times of great climate changes, it is extremely important to monitor the condition of natural waters as they affect the development of microorganisms in them, especially pathogenic microorganisms that are dangerous to human health.
Such projects require a large number of samples and microbiological analysis which is not cheap, we decided to find a simpler and cheaper method for testing water.
Since we assumed that this was possible, we have searched the available resources on the Internet and found several methods, among which the method called the "H_2S test" seemed the most suitable.
Research question

Can we make the test and determine the level of microbiological contamination of the water?

Work is divided into: preparation, verification and application.

The results show that the degree of contamination of natural water can be determined. The test can be applied in the field, and a larger number of samples can be taken.
To determine the actual health risk, test results indicating high risk would need to be verified by microbiological analysis.
The assumption that cheaper and simpler tests than the traditional methods of determining water quality can be found has been confirmed.
The answer to the research question is: Yes, we can!

Sažetak

U vrijeme velikih klimatskih promjena izuzetno je važno pratiti stanje prirodnih voda jer one utječu na razvoj mikroorganizama u njima posebno patogenih mikroorganizama opasnih po ljudsko zdravlje.
Naši projekti često obuhvaćaju velik broj uzoraka a mikrobiološka analiza nije jeftina pa smo odlučili pokušati naći jednostavniju i jeftiniju metodu za testiranje vode.
Pretpostavili smo da je to moguće, pa smo pretražili dostupne resurse na internetu i pronašli više metoda od kojih nam se je najprimjerenijom učinila metoda pod nazivom “H_2S test”.
Istraživačko pitanje

Možemo li sami izraditi test i njime utvrditi stupanj mikrobiološke kontaminacije vode?

Rad je podijeljen na: izrada indikator papira, provjera testa I primjena testa

Rezultati rada pokazuju da se testom mogu utvrditi stupnjevi kontaminacije prirodnih voda.
Test se može koristiti na terenu i može se uzorkovati veći broj uzoraka.
Da bi se utvrdili stvarni rizici za zdravlje, rezultate testa koji su ukazali na visok rizik moralo bi se provjeriti mikrobiološkom analizom.
Naša pretpostavka da se može naći jeftinije i jednostavnije testove od konvecionalnih metoda za utvrđivanje kvalitete vode je potvrđena.
Odgovor na istraživačko pitanje je, da možemo!

CONTENT

1. Research Question and Hypothesis

For many years, our GLOBE GROUP has been working on projects using GLOBE HYDROLOGICAL PROTOCOLS to determine water quality (sea, river, stream, spring, well, water supply). Since microbiological analysis is extremely important in determining water quality and human health impacts, we need to utilise the services of LABORATORY VODOVOD LABIN. The experts at VODOVOD LABIN have selflessly helped us with each of our projects.

Nowadays, monitoring the state of the environment, especially water, is extremely important due to major climate changes, as these changes affect the growth and development of microorganisms in the environment and, consequently, pathogenic microorganisms dangerous to human health.

Since our projects often require many samples and microbiological analysis is not cheap, we decided to find a simpler and cheaper method for microbiological analysis of water.
Since we assumed that this was possible, we searched the available resources on the Internet and found several methods, among which the method called the "H_2S test" was the most suitable for our work.

We asked ourselves a research question: Can we make the test and determine the level of microbiological contamination of the water?

2. Introduction and Review of Literature

Microorganisms from the digestive tract of humans and animals are basic indicators of domestic wastewater, but they are also found in industrial wastewater. Among this group of microorganisms, pathogenic microorganisms that can cause diseases are of particular importance. Diseases can be transmitted through drinking water, bathing in polluted water, and especially through the consumption of aquatic products (fish, shellfish).
Bacteria of the normal intestinal flora of humans and animals - coliform bacteria - usually serve as indicators of contamination by these microorganisms. When faecal microorganisms reach a recipient (environment) with different living conditions (temperature, hydrogen ion concentration, ultraviolet radiation), they gradually disappear. The time of extinction is not the same for all microorganisms and depends mainly on the content of dissolved (nutrient) salts in the water (Habuda-Stanić et al. 2007).

In 1975, Allen and Geldreich showed that the presence of coliform bacteria in the water is also associated with hydrogen sulphide (H_2S)-producing organisms.
In 1982, Manja et al. developed a simple paper strip method for screening for bacterial contamination of water bodies.
Bacteria can produce hydrogen sulphide through the anaerobic degradation of cysteine, an amino acid containing the sulfhydryl group.
Members of the *Enterobacteriaceae* group such as *Salmonella, Citrobacter, Clostridia, Klebsiella*, and *Proteus* are capable of producing hydrogen sulphide in conjunction with thiosulfate as a sulphur source and ferric ammonium citrate as an "indicator," resulting in the development of a black precipitate. Hydrogen sulphide is produced by the reduction of thiosulfate and then reacts with the iron salt to form an insoluble black iron sulphide precipitate.

3. Research Methods and Materials *(Including GLOBE Data!)*

We divided our work into three parts:
1. preparation of indicator paper for the H_2S test
2. verification of the test
3. application of the test.

22 students, members of the group GLOBE, participated in the work.
The collaborators in the project were experts from VODOVOD LABIN.

3.1. Production of indicator paper for the H_2S test

A. PREPARATION OF THE INDICATOR REAGENT SOLUTION

a. Prepare a glass or plastic bottle with a volume of 200 mL that has a heat-resistant lid. First, clean the bottle with liquid detergent, rinse with tap water, soak overnight in a 5% bleach solution, rinse with tap water, and dry in an oven at 5°C and refrigerate.
b. Add 100 mL of distilled water to a prepared bottle and then dissolve it in:
bacteriological peptone 40.0 g
dipotassium hydrogen phosphate 3.0 g
ammonium iron (III) citrate 1.5 g sodium thiosulfate 2.0 g
liquid detergent 2.0 mL citrate (optional but increases sensitivity).

Stir the mixture well to dissolve the chemicals.

B. PREPARATION OF THE INDICATOR PAPER

a. Take the filter paper and cut it into strips. Each strip of paper for 10 mL of the test sample must contain 0.5 Ml of indicator solution.
b. Dry and cool the strips in an oven at about 60°C.
c. Reagent impregnated strips may be stored in a dry place
(in an envelope or preferably in a zippered bag) for several months.

C. PREPARING THE TEST TUBE AND TESTING SAMPLES OF WATER

a. Prepare glass or plastic tubes with a volume of 20 mL that have a heat-resistant lid. Label the tubes with a volume of 10 mL.
Place a paper indicator in each tube and close them.
b. Then sterilize the tubes in a hot air oven at approximately 120°C for 60 minutes.
c. After heat treatment, cool the tubes. The tubes should be stored in a dark place until they can be used. Experience has shown that they can be stored in this manner for at least 5 years.
d. Add 10 mL of the test sample to the tube. Place the test samples in a dark place for a total of three days at room temperature. Every 12 hours, the samples are examined for colour changes. Distilled water is used as a control sample. The control sample serves as a measure for comparing the colour change of the test samples

Note: The colour of the control samples will change slightly into pale yellow or light brown due to the colour of the reagent, which is normal.

WHAT DO RESULTS MEAN?

(-) If there is no change in colour, it means that the water is clean and free from bacterial contamination

(+) If the water has turned light brown, there is a possibility that there are bacteria in the water. Wait a few days and check again.

(++) If the colour has turned dark brown, there is some degree of bacterial contamination in the water.

(+++) If the paper strip and water sample are noticeably black, there is a very high risk of bacterial contamination of the water.
(++++) If there is a rapid reaction, i.e. if the water solution and the paper strip turn black overnight, this means that there is a high probability that bacteria are present.

3.2 Review of the H_2S test

To verify the effectiveness of the H_2S test, we decided to test samples of distilled water, water from springs, wells and ponds and compare the results with the microbiological analysis of VODOVOD LABIN.

3.3 Application of the H_2S test

We decided to investigate the quality of spring water on the "Divine Springs" trail using the H2S test and compare the results with the microbiological analysis of VODOVOD LABIN.
To find the springs, we used a topographic map created by members of our Globe group as part of the Divine Springs project presented at the 2020 International Virtual Science Symposium (IVSS). To create the map, we used GLOBE GPS and MUC protocols (Figure 1.).

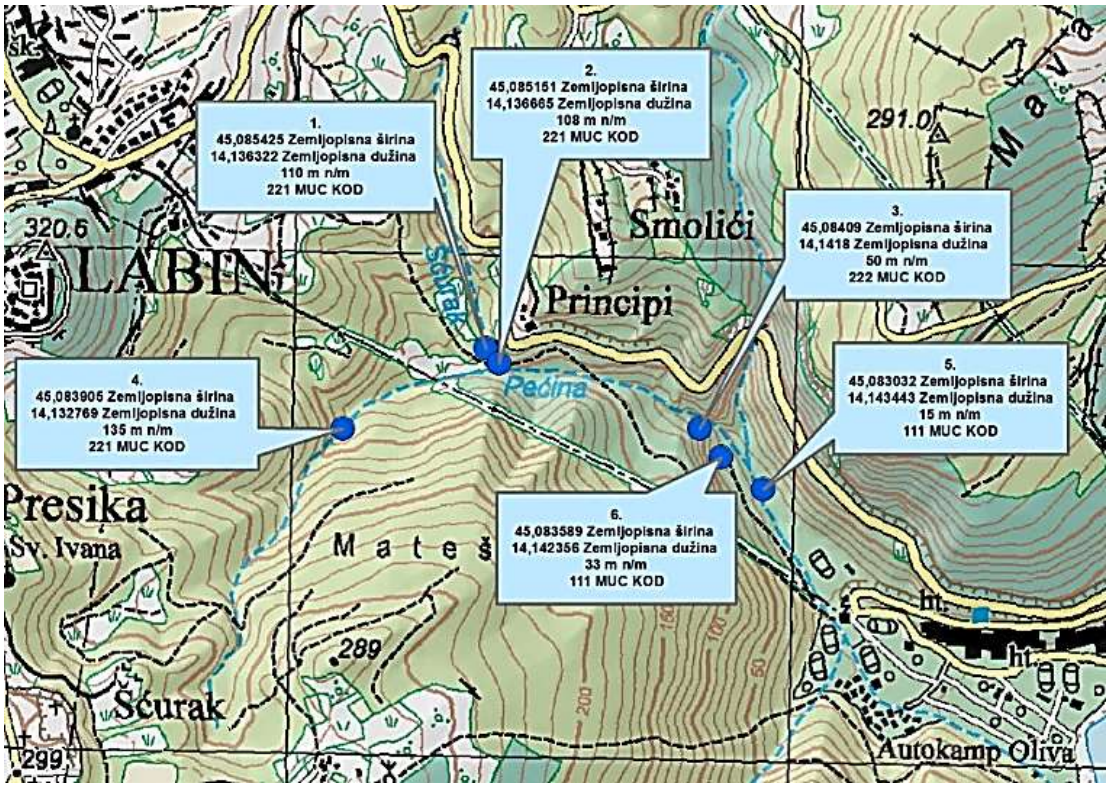

Figure 1. *Topographic map*
Source: *"Divine springs" https://www.globe.gov/documents/10157/66092df6-9206-4923-a1c8-4dc0d3386caf*

4. Results

4.1 Preparation of the indicator paper for the H_2S test

According to the instructions described in the research methods, we prepared the H_2S test (Figure 2.).

Figure 2 *preparation of the H_2S test* ***Source*****:** our design

4.2 Review of the H_2S test

Table 1. shows the time taken to develop the H_2S test and the level of contamination compared to the bacterial count for conventional methods.

ABBREVIATIONS

H_2S - TEST:
The time (in hours) required for H_2S development is divided into:
IC - initial, grey staining,
FC - complete, black colouration.
CONVENTIONAL METHODS:
CFU - colony forming units per 1 mL/ 22˚C,
TC - total coliforms per 100 mL,
EC - Escherichia coli per 100 mL,
EN - enterococci per 100 mL,
PA - Pseudomonas aeruginosa per 100 mL.

Table1. Verification of the H_2S test

SITE	H_2S – TEST			MICROBIOLOGICAL INDICATORS•				
	IC	FC	DEGREE OF CONTAMINATION	CFU	TC	*EC*	*EN*	*PA*
1. DISTILLED WATER	-	-	-	.	.	-	-	-
2. TAP WATER	-	-	-	1	2	0	0	0
3. WELL	48	72	++	145	548	1	172	0
4. PONDS	24	48	+++	>2400	62	5	1	>2420
5. SPRING	24	36	+++	>2400	435	199	488	1

- **VODOVOD LABIN** has analysed the microbiological indicators that cannot be determined in our school laboratory.

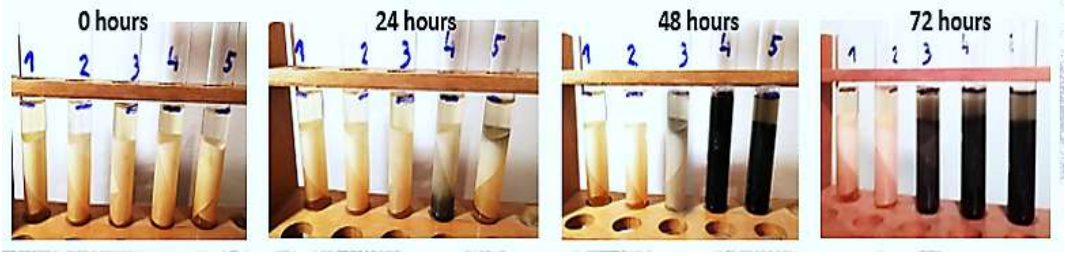

Figure 3 *Checking H_2S test* ***Source:*** *our design*

The degree of contamination is highest in the water from the spring (site 5.), the shortest time required for the appearance of black colour (36 hours) and the highest number of *Escherichia coli* per 100 mL.
Tap water (site 2.) has a negative test which is consistent with microbiological analysis.

4.3. Application of the H_2S test

Table 2. shows the time needed for the H_2S test development and degree of contamination compared to bacteria counts using conventional methods on water from spring (see figure 1.)

Table 2. Application of the H_2S test

SPRING	H_2S – TEST			MICROBIOLOGICAL INDICATORS•				
	IC	FC	DEGREE OF CONTAMINATION	CFU	TC	*EC*	*EN*	*PA*
1.	24	36	+++ 	2020	180	29	3	0
2.	24	48	+++ 	451	166	25	2	0
3.	48	72	++ 	378	34	8	0	0
4.	-	-	- 	64	0	0	0	0
5.	24	36	+++ 	2040	214	29	3	0
6.	24	48	+++ 	328	160	26	2	0

- **VODOVOD LABIN** has analysed the microbiological indicators that cannot be determined in our school laboratory.

The degree of contamination is highest in springs 1 and 5 (the shortest time needed for the appearance of a black colour (36 hours) and the highest number of Escherichia coli per 100 mL. The water from source 4. has a negative H_2S test, which is consistent with the microbiological analysis.

5. Discussion

The results of our work show that the pollution level of natural waters can be determined by the H_2S test. This test can be easily made in the school laboratory, can be used in the field, and many samples can be collected. To determine actual health risks, H_2S test results indicating high risk should be verified by microbiological analysis.

6. Conclusion

The assumption that cheaper and simpler tests can be used instead of traditional methods to determine water quality has been confirmed. The answer to the research question is: Yes, we can!
The test is well suited for testing water for faecal contamination. The major advantages of the H_2S test over other conventional microbiological analyzes are that it is inexpensive and does not require sophisticated equipment to produce or perform the analyzes.
The indicator papers for the H_2S test can be easily prepared in the school laboratory.
H_2S test results are visual and therefore easy for people to understand because a black colour change occurs when bacterial levels in water are high.
In our further work in the GLOBE program, we will use the H_2S test, which we make ourselves in the school laboratory, in studying the quality of natural water bodies in our area, to warn the local community promptly of possible pollution that could be dangerous to health.
Our work was supported by the experts from VODOVODA LABIN, with whom we have been successfully cooperating for many years.

7. Bibliography/Citations

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8. Acknowledgements

We thank our mentor Olivera Tadić who selflessly helps us with our research.
Special thanks go to the experts from VODOVOD LABIN, who have been supporting us professionally for many years.

9. (Optional) Badge Descriptions/Justifications

- Be a **STEM professional**

For many years we have successfully cooperated with experts from VODOVODA LABIN who selflessly help us in our work, especially when researching water quality research.
In their lab, we have learned a lot about water quality testing techniques.

- Be a **Collaborator**

We collaborated with our Globe teachers and mentors on this project.
We also collaborated with other members of the GLOBE group and other students from our school during our fieldwork.
Students collected water samples from springs on the Divine Springs footpath so that they could be analyzed using the H_2S tests and microbiological analysis. This confirmed the assumption that the H_2S test can be used to quickly identify sources that are hazardous to human health.

- **Make an Impact**

Since Labin is a tourist destination rich in surface waters that are often "destinations" of tourists (sea, river Raša, springs DIVINE SPRING, ponds), it is important to inform the local population about the quality of these water resources.
In our further work within the program GLOBE, we will use the H_2S test, which we will prepare ourselves in the school laboratory, in the study of the quality of natural waters in our area, to warn the local population on time of possible pollution that could be dangerous to health.