

THE MICROBIOLOGICAL QUALITY OF WATER, PATHOGENIC MICROORGANISMS IN FOOD PRODUCTS AND FACEL CONTAMINATION IN THE RIVERS OF THE REPUBLIC OF KOSOVO

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ABSTRACT

Microbiological quality of surface water is mainly determined by monitoring the presence of pathogenic microorganisms based on the indicator's identification of fecal contamination.

On the other hand, the definition of the different types of pathogenic microorganisms in rivers and lakes is difficult, time-consuming and more expenditure. For this reason, the study and evaluation of microbiological water quality are conducted by determining the microbial indicators, which provide information about the possibility of fecal contamination of these waters. The test of determining microbial indicators, such as fecal coliforms is fast and low cost, compared with a test for microorganisms and specific pathogens. These microbial indicators of fecal contamination, which are used as an indicator of hygienic water quality must meet certain criteria. In the first place, they should always be present and in large numbers in fecal material of humans or warm-blooded animals, and should be easily detected by simple methods and should not reproduce in natural waters. Also, a special importance has the fact that the presence, cleaning or their removal in water treatment processes should be similar to that of pathogenic microorganisms.

Keywords: water, fecal coliforms, food, contamination.

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INTRODUCTION

The main indicator of the water suitability for various uses is the presence or absence of fecal coliforms and fecal streptococci. These groups of bacteria are considered as the most important indicators of possible contamination of the river water from the discharge of wastewater. Because these bacteria are normally found in the gastrointestinal human and warm-blooded animals. If the water contains such bacteria above the allowed limits, then it can be said that water is contaminated by microbiological standpoint.

Today researchers believe that the higher the number of fecal coliforms and fecal streptococci, the greater is the possibility of the presence of pathogenic micro-organisms. These pathogenic microorganisms live in the gastrointestinal tract of humans and warm-blooded animals such as *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrion*es, and *Hepatitis A* and *D*. *Giardia intestinalis* can contaminate water if feces come into contact with it. At the same time, these contaminated waters may be able to cause serious disease or epidemic among people who use these waters for irrigation of crops, fishing, swimming, etc¹.

Fecal coliform bacteria are aerobic or facultatively anaerobic, gram-negative, oxidase - negative, rod-shaped, nonspore forming. They are traditionally defined as coliforms that ferment lactose (with β galactose) at 44 ± 0.5 °C, in an environment of bile salts within 48 hours to produce acid and gas release. In the absence of molecular oxygen, these microorganisms use fermentation as an alternative way of survival. *Escherichia coli* take part in *Enterobacteriaceae* family of Eubacterial order. They grow well in ordinary terrain at a temperature of 10°C – 45°C.

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The optimum temperature is 37°C². Typical colonies of *Escherichia coli* are usually easily distinguished by their characteristic appearance on different terrains because they ferment lactose.

Intestinal enterococci (*E. faecalis*, *E. faecium*, *E. avium* and *E. gallinarum*) are fecal streptococci that grow in the presence of 6.5% NaCl at a temperature of 45°C. *E. faecium*, *E. faecalis*, and are present in the feces of humans and animals. Agreements between the Council of Europe and European Parliament have made possible the revision of the Surface Water Directive, so that it fits with the current standards, which define microbiological standards for water quality, see Table-1.

Table-1: New Directive 2006/7/EC for Surface Waters³.

Parameter	Excellent Quality (A)	Good Quality (B)	Sufficient Quality (C)
Intestinal enterococci (CFU/100ml)	200(*)	400(*)	330(**)
<i>Escherichia coli</i> (CFU/100ml)	500(*)	1000(*)	900(**)

CFU/100ml = Colony forming units /100ml

(*) based on 95 % of evaluation; (**) based on 90% of evaluation

The Microbiological Quality of the Agricultural Crops that are Consumed Fresh

Good quality of human life is the main objective in the whole world. The healthy life is closely related to the environment in which we live and with food safety, especially with the foods that are consumed fresh.⁴ The fresh crop consumption is steadily increasing, due to the awareness of people about a healthy food diet. It is important that these crops are not contaminated by pathogenic micro-organisms, which have a great impact on public health.⁵

Sources of Agricultural Crops Contamination

Microbiological contamination continues in the same way during the vegetable harvest and continues during trading, when trading is often done on the road, beyond any standards. Microbiological contamination is a very important aspect of contamination of fresh vegetables because it is the main route of transmission of pathogenic microorganisms from food to people who consume these vegetables.

During the cultivation of agricultural products are used different types of organic compost. These include chemical fertilizers (nitrogen, phosphorus and potassium) compound fertilizers, organic fertilizers and animal fecal composed with human fecal⁶. To animal, organic manure may be present pathogens such as *Campylobacter*, *Salmonella*, *E. coli* 0157: H7⁷.

Pesticides are commonly used in the process of cultivating agricultural products. Their use consists in the control of bacteria, moss, insects etc. Usually, pesticides are in powder or liquid form and are divided into: insecticides, fungicides and herbicides, which have a specific action against different populations. Dissolved pesticides may be a suitable environment for the survival and growth of different microorganisms, including pathogens such as *Salmonella*, *Shigella*, *E. coli* 0157: H7 and *L. monocytogenes*.

Birds' feces are recognized as a source of bacterial contaminants, including pathogens of food origin, such as *Salmonella* spp. Animals are another potential source of contamination of vegetables including livestock, birds or different insects.⁸

Urban, industrial and hospital dumping - The riverbed of "Drinii Bardhe" and "Ibri" has been and is the collection and urban discharge of industrial products of the community's activity. These discharges with high contents of various chemical elements as N, P and S are affecting the eutrophication of the area and making a stifling environment.⁹

EXPERIMENTAL

The research was conducted during the period 2014– 2016. In this study were analyzed the determination of fecal coliform and fecal streptococci as an indicator of contamination in samples of fresh vegetables (lettuce, onion and parsley), which were irrigated with water from the rivers "Drinii Bardhe" and "Ibri", and were consumed fresh. Analytical methods of determination: determination of fecal coliforms by the method of MPN (Most probable Numbers) in samples of river water and fresh vegetables¹⁰.

The number of fecal coliforms, as microbial indicators in water samples and fresh vegetables were

determined by statistical evaluation called the most probable number (MPN).MPN is the most probable number of coliform or streptococci in 100 ml water¹¹.

For the river water analysis was obtained from 81 samples, while vegetables from 54 samples from different stations, Fig.-1.

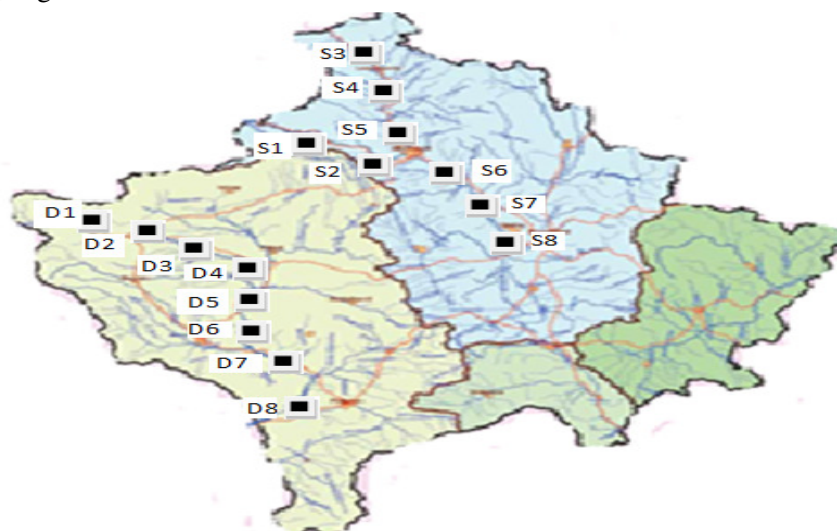


Fig.-1: Sampling Points that showed in the "DriniiBardhe"(D) and "Ibri" (S).

RESULTS AND DISCUSSION

Results for fecal coliform in the water of “DriniiBardhe” and “Ibri” show that fecal coliform values represent a dynamic at seasons and different stations.

In Table-2 and 3 are given the content of *fecal coliform* and descriptive statistics of fecal coliform seasonality in the “Drini Bardhe” river. In the river “DriniiBardhe” the station with the highest values of fecal coliform is D3 station (River Bridge in Klina) compared to D2 and D4, but these two stations represent high values of this bacterial indicator. D3 station (River Bridge in Klina), the Cf values ranging from 43000 bacteria/100 ml (summer 2014) to 12000000 bacteria/100 ml (summer 2016). These values, and the values of two other stations, D2 (Appareled) and D4 (except 3 seasons) are up to 4 times higher than the rates set by Directive 76/160 / EEC and 2006/7 / EC. Station D2 (Appareled), the value of Cf range 28000 bacteria/100 ml (winter 2015) to 460000 bacteria/100 ml (spring-summer 2015), while the station D4 value of Cf ranges from 750 bacteria / 100 ml (spring 2015) to 750000 bacteria / 100 ml (summer 2015).

Table-2: Values of Fecal Coliform (Bacteria/100ml) by MPN Table, Stations of the "DriniiBardhe" River During June 2014-2016

Years	Seasons	D1	D2	D3	D4	D5	D6	D7	D8
2014	Summer	210	75000	43000	1500	200	1500	1400	140000
	Autumn	150	93000	1500000	4600	210	450000	46000	44000
	Winter	140	46000	1400000	14000	1500	150	160	93000
2015	Spring	200	460000	9400000	750	230	2100	2200	7500
	Summer	240	460000	12000000	750000	240	92000	73000	230000
	Autumn	240	93000	750000	23000	210	12000	12000	93000
2016	Winter	230	28000	230000	2300	180	23000	21000	43000
	Spring	230	39000	75000	2300	200	9300	9400	2300
	Summer	240	340000	7200000	44000	210	37000	12000	120000
	Autumn	240	340000	7200000	430000	210	38000	110000	12000

Table- 3: Descriptive Statistics of Fecal Coliform Seasonality in the “Drini Bardhe” River

Fecal Coliforms	Parameter	Winter	Spring	Summer	Autumn
	Average	227271.3	1234685.	1715683	308123.8
	Median	15150.00	20650.00	59000	58500.00

(MPN/100ml)	Maximum	1500000	9300000	12000000	1500000
	Minimum	140.0000	200.0000	210	150.0000
	Dev. Std.	520616.4	3262628.	3876571	543910.9
	Skewness	2.166134	2.257605	2.01877	1.566229
	Kurtosis	5.860500	6.116328	5.435036	3.905145
	Jarque-Bera	8.983671	10.03288	11.11557	3.543861
	Probability	0.011200	0.006628	0.003857	0.170005
	Sum	1818170	9877480	20588190	2464990
	No. of measurements	8	8	12	8

Table- 4: Fecal Coliform Values (Bacteria/100ml) by MPN Table, “Ibri” River Stations During the Period June 2014 - June 2016

Years	Seasons	S1	S2	S3	S4	S5	S6	S7	S8
2014	Summer	1500	4300	430	900	140	430	4300	4800
	Autumn	90	6500	15000	240	110	1500	7500	7900
2015	Winter	70	7500	15000	230	90	1500	1500	1400
	Spring	110	9300	6400	430	40	230	750	800
	Summer	230	12000	23000	2300	150	7500	9300	9400
	Autumn	230	4300	7500	430	120	2300	4300	4500
2016	Winter	210	2400	11000	2100	90	4600	4600	4800
	Spring	40	430	23000	40	30	2400	2300	2400
	Summer	210	930	28000	2100	110	6400	7500	8000

Table- 5: Descriptive Statistics of Fecal Coliform According to Seasons in the River “Ibri”

Fecal Coliform- Iber	Parameter	Winter	Spring	Summer	Autumn
	Average	52221.67	3605.000	54350	10173.7
	Median	35000.00	2200.000	15000	2850.00
	Maximum	150000.0	9300.000	230000	46000.0
	Minimum	150.0000	200.0000	200	21.0000
	Dev.Std.	59124.34	3861.522	78112.17	17910.0
	Skewness	0.739667	0.609719	1.380157	0.638706
	Kurtosis	2.150468	1.677648	3.77638	3.910348
	Jarque-Bera	0.727534	0.808910	3.083287	2.892542
	Probability	0.695053	0.667340	0.214029	0.235447
	Sum	313330.0	21630.00	489150	61042.0
No. of measurements	6	6	9	6	

In Table-4 and 5 are given the content of *fecal coliform* and descriptive statistics of fecal coliform seasonality in the “Ibri” river, show that fecal coliform values represent a dynamic at seasons and at different stations¹². The lowest number of fecal coliform is in Springer 2015 with 40 bacteria/100ml, while higher in the summer of 2016 with 28000 bacteria/100ml, while S3 has a higher level of fecal coliform, and lower level of fecal coliform have in S1.

As well as statistical analysis showing the highest level of coliform fecal we have in the summer.

To study the microbiological contamination of agricultural crops that are irrigated with water, from the river “DriniiBardhe” and “Ibri” were selected 3 types of leafy vegetables, which are consumed fresh:

- Green salad- *Lactucasativa*.
- Onion- *Allium cepa*.
- Parsley- *Petroselinum hortense*.

Seasonal dynamics of fecal coliform in fresh vegetables (green salad, fresh onion and parsley), which are irrigated with water from the rivers of “DriniiBardhe” and “Ibri”.

The obtained results from the study period (June 2014 - 2016), for fecal coliform in fresh vegetables,

which are irrigated with water from the “DriniiBardhe” river appears in the following table.

Table-6: Values of Fecal Coliform (Bacteria/100ml) by MPN Table, the Fresh Vegetables and Water of the River “DriniiBardhe” in Green Houses During June 2014-2016.

Years	Seasons	Salad	Onion	Parsley	Water for Irrigation
2014	Summer	43000	28000	39000	7500000
	Autumn	1500000	930000	1600000	150000
2015	Winter	210000	210000	23400	150000
	Spring	4300	28000	39000	9300000
	Summer	460000	430000	640000	12000000
2016	Autumn	23000	21000	20000	750000
	Winter	21000	15000	23000	240000
	Spring	28000	23000	21000	75000
	Summer	430000	460000	350000	43000

In the Table-6 it is observed that the level of fecal coliform in any fresh vegetables at every station of the river “DriniiBardhe” is different, but the trend of progress in different seasonal periods is almost the same. This means that in the same season, high values are observed in three types of vegetables for every station and in another season these may increase or decrease. This is explained by the fact that in each station 3 types of vegetables irrigated with the same river water with certain bacteriological parameters. Likewise, the type of soil, chemical fertilizers and pesticides used are the same in every station. Contaminated river water used for irrigation may be one of the main contaminants of fresh vegetables. At sampling station D8, lower values of fecal coliforms are: salads - 4300 bacteria/100 ml (spring 2014); 15000 onions (winter 2015); parsley 21000 bacteria/100 ml (autumn 2014). While higher values of Cf in three types of vegetables are reached in autumn 2014 (salads 1500000 bacteria/100 ml, onions 930000 bacteria/100 ml and parsley 1600000 bacteria/100 ml). These Cf values in this station are 2-3 times higher than the maximum allowable level of 1000 bacteria /1 g¹³.

In Table-7 we notice that Cf high levels in samples of 3 types of vegetables that are consumed fresh can be explained by the fact that they are irrigated with contaminated water of the river “Ibri”, which at this station highest value has reached in summer 2014 (11000000 bacteria/100 ml), while the lowest value reached in the summer of 2015 (43000 bacteria /100 ml). Contaminated water in the river “Ibri” may be one of the main factors of bacterial pollution, contaminants along with other factors such as fertilization, soil, pesticides, etc.

Table-7: Values of Fecal Coliform (Bacteria/100ml) by MPN Table in Fresh Vegetables and Water of the River “Ibri”.

Years	Seasons	Salad	Onion	Parsley	Water for irrigation
2014	Summer	300	7500	9300	11000000
	Autumn	6400	3900	4300	44000
2015	Winter	2300	2800	21090	150000
	Spring	7500	6400	6400	7500
	Summer	24000	43000	21000	230000
2016	Autumn	2100	2000	2000	93000
	Winter	1500	1400	1400	46000
	Spring	1100	900	900	2300
	Summer	9300	9300	7500	15000

CONCLUSION

Based on the results of microbiological analysis of water samples of the rivers " Drinii Bardhe" and "Ibri", and of fresh vegetables, which are irrigated with water from these rivers, brought out the following conclusions:

- The presence of fecal coliforms in the "Drinii Bardhe" and "Ibri" river is an indicator of bacteriological water quality.

- Values of fecal coliform in the aforementioned rivers ranged from 140 - 12000000 bacteria/100ml.
- Fecal coliform values in D8 station ranged from 1.500 - 1500000 bacteria/100ml of fresh vegetables and 43000- 11000000 bacteria/100ml to the water of the "Ibri" river, used for their irrigation.
- At the D8 station, average values of fecal coliforms in 100% vegetable analyzed samples were above the permitted levels, while average values in the D6 station.

Recommendation

To improve the quality of water of two rivers "Drinii Bardhe" and "Ibri" considering their environmental values, economic and social factors, it is recommended that:

- An immediate prohibition of wastewater discharges urban and industrial discharges into the waters of these two rivers.
- Stopping by the local authorities of animal grazing on the "Drinii Bardhe" and "Ibri" river.
- Build the implants to treat wastewater before being discharged into rivers or lakes.

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