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MICROBIAL POLLUTION IN DRINO RIVER (GJIROKASTRA'S DISTRICT) AND ITS IMPACT ON FRESH VEGETABLES

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Drino River.

SYNOPSIS

One of environmental challenges facing Albania recently is the microbial quality of surface waters and fresh vegetables that can be irrigated with them. Drino River is one of the most important water resources in Gjirokastra's district with environmental, social and economic values. The aim of our work during 2012 – 2013 periode was the monitoring of bacterial pollution of Drino's water and its impact on fresh vegetables. Microbiological examinations of water and lettuce samples, as well as the physical – chemical examinations of river's water collected from 6 stations were performed in four seasons: Summer - Autumn 2012, Winter - Spring 2013. The results obtained from the analysis of microbial indicators FC/FS vary for FC from 460 to 9,3 $x10^6$ bacteria/100ml and for FS from 90 to$1.5x10^4$ bacteria/100ml for the Drino's water, while for the lettuce vary for FC from $4,3 \times 10^4$ to $1,5 \times 10^6$ bacteria/100ml and for FS from 2300 to 9.3×10^4 bacteria/100ml.

This situation comes from the fact that urban discharges flow directly into the river without any previous treatment. As a consequence this pollution has a great impact on public health. We recommend the urgent treatment of urban wastewater before their discharging into the river.

INTRODUCTION

Water pollution is a major global problem which requires ongoing evaluation and revision of water resources policy at all levels (Puto, 2011). Aquatic environments are usually polluted along the rivers from direct and indirect discharges. The quality and the pollution of rivers have been very problematic recently in the southern region of Albania. One of them is Drino River (Fig. 1).

Drino River runs through Albanian territory in a principle direction from southeast to northwest. Its length is 84.6 km and its source is in the northwestern part of Joannina regional unit, Greece. The climatology of the catchment is the Mediterranean one with, nevertheless, significant diferences within the catchment. According to climatology classification the upper part of the river flows in the Mediterranean hilly southeastern zone, while the middle part of the river flows in the central plain Mediterranean zone. The main characteristics of this climate are the balmy humid winters and the hot dry summers. Two factors that significantly affect climatological changes in these areas are a) the closeness to the sea b) the elevation over the sea level.

Figure 1:
View of the Drino River in Albania.

Drino is one of the most important water resources in southern region of Albania. It's a very important river in Gjirokastra district with enviromental, social and economic values. Its water is used for irrigation, fishery, tourism etc.

Drino is characterized by biological diversity of ecosystems and landscapes, offering variety of plant populations, vegetations, invertebrate animals, as well as indicator organisms of the enviromental situation and biodiversity (Shkurti, 2009). Although it is considered among low impacted rivers in Albania, deforestation, erosion, use of fertilizers in agriculture, urban discharges, untreated wastewater, alteration of natural habitats and gravel excavation are increasing during two last decades.

Monitoring of biological and physico – chemical characteristics was used to determine the microbial pollution of Drino river, as well as its impact on fresh vegetables that can be irrigated with its water. The object of this monitoring has been a measurement of faecal coliforms and faecal streptoccoci in Drino River water during 2012 - 2013, as well as the faecal coliforms and faecal streptoccoci in lettuce that is irrigated by the water of this river.

Freshwaters polluted by fecal discharges from peoples and animals may transport a variety of pathogenic microorganisms. Pathogenic microorganisms found in non-treated wastewater have the ability to reproduce easily due to the large amount of available nutrients, thereby affecting the environment and presenting a great risk to health (Pusch et al., 2005). Water quality is mainly determined by the monitoring of the presence of pathogenic microorganisms, based on the identification of fecal contamination indicators (FC and FS) (Puto, 2012). Through the use of microbial indicators for the quality of waters, mainly for fecal contamination, we receive information for determining the amount of the presence of pollutants in general and the presence of fecal contaminants of serious impact on the health of the population that uses these waters for irrigation, fish growing etc.

The higher level of bacterial indicators (FC and FS) in river water speaks for a high level of its fecal contamination. WHO and various states have developed different standards, which specify mandatory and recommended norms for the quality of water (World Health Organisation).

In February 2006, the European Parliament and the Council of the European Union approved Directive 2006/7/EC on the quality of washing waters in regard with their microbiological norms to be met by European Union member countries until the year 2015 (Directive 2006/7/EC; Council Directive 76/160/EEC) (Tab. 1).

Table 1: Water quality standards for inland waters according to Directive 2006/7/EC.

PARAMETERS	Excellent quality	Good quality	Sufficient quality
Escherichia coli/100ml	200	400	330
Enterococci/100ml	500	1000	900

Pollution is a very important aspect of this contamination study since this is the main way that pathogenic microorganisms are transmitted through water and fresh vegetables to humans. Many diseases in humans are caused by the pollution of fresh vegetables, diseases arising from the bacteria, viruses and parasites already found on them (Rai & Tripathi, 2007; Tyrell et al., 2006).

MATERIAL AND METHODS

Microbiological examination of water and lettuce samples collected from 6 stations (4 water samples in Drino River and 2 lettuce samples in Mashkullore's greenhouses and Tepelena's fields) were performed in four seasons: Summer -

Autumn 2012, Winter - Spring 2013 in the Laboratory of Biotechnology Department, FNS, University of Albania.

In addition, with the goal of a better assessment of the water quality of Drino River, four stations (Pacarela, Kordhoca, River Bridge and Virua) are analysed using some physical and chemical parameters related to water quality. These parameters are measured in situ. The device that is used to measure pH, dissolved oxygen and conductivity in water is a portable multiparameter MULTI 340.

There were analyzed in total 48 samples: 16 water samples for *Faecal coliforms* (FC) and 16 other water samples for *Faecal steptococci* (FS), and 8 lettuce samples for *Faecal coliforms* (FC) and 8 other lettuce samples for *Faecal steptococci* (FS).

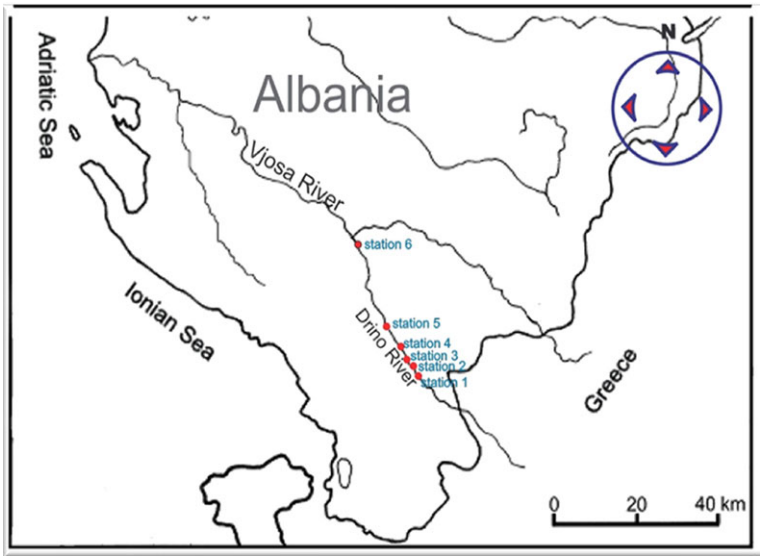


Figure 2:
Map of the Drino River in Albania.

The selection of sampling stations was based on the monitoring scheme of liquid discharges. The sampling stations are (Figure 2):

1. **Pacarela** - Drino river (after the industrial waste discharges of leather shoe factory)
2. **Kordhoca** - Drino river
3. **River Bridge** - (after the discharge of industrial and urban pollution in Gjirokastra city)
4. **Virua** - (after the urban discharges and untreated wastewater of different restaurants in this area)
5. **Mashkullore's greenhouses** - (lettuce irrigated by the water of Drino River)
6. **Tepelena's fields** - (lettuce irrigated by the water of Drino River)

Water samples were taken in 250 ml sterilized glass containers and along with the lettuce samples were transported in cooling holders in the temperature 4^{0}C according to the European standard rates. The analyses were done within 24 hours. The indicator bacteria in water and lettuce samples were detected via the Multiple-Tube Fermentation Technique or the Most Probable Numbers (MPN) Technique. Dilutions in 9 ml test tubes were made depending on the degree of pollution. Tamponated phosphate (Butterfield's phosphate buffer) was used as a diluent. Three test tubes for each dilution were inoculated with 1 ml of test water.

Faecal coliforms (FC) were determined by inoculating 10ml, 1ml, 0.1ml, 0.01ml, etc. (up to 6 dilutions) of the water samples first in LSB (Lauryl pepto broth) preliminary test, at 35^{0}C for 24 - 48 hours. The results were recorded after 24 ± 2 hours for gas production. The test tubes which had not released gas were re-incubated for another 24 hours and then re-examined for gas production. Meanwhile for all positive tubes (which had produced gas), the confirmation test was performed by carefully mixing all test tubes that had produced gas into the LSB ground *(Lauryl pepto broth)* and then transfering it to 1-3 anza suspensions in tubes with ECB ground (*EC broth)*. They were incubated at 44.5 ± 0.2° C for 24 ± 2 hours. Positive reaction is indicated by the gas production in the Durham pipes.

Faecal streptococci (FS) were determined by inoculating 10ml, 1ml, 0.1ml, 0.01ml, etc. (up to 6 dilutions) of the water samples first in ADB *(Azide Dextrose Broth),* preliminary test, at 35^{0}C for 24-48 hours. The test tubes which had not a purple coloured precipitate were incubated for another 24 hours and then re-examined for precipitate production. Meanwhile for all positive tubes (which had produced a purple coloured precipitate), the confimation test was performed by carefully mixing all test tubes that had produced a purple coloured precipitate into the ADB (*Azide Dextrose Broth*) and then transfering it to 1-3 anza suspensions in tubes in EVAB (*Ethyl Violet Azide Broth*), conformation test, at 35^{0}C for 24 hours. The presence of a purple colored precipitate at the bottom of the test tube indicates the presence of faecal streptococci.

The number of Faecal coliforms and Faecal streptococci (MPN) in water and lettuce samples were calculated according to the relevant table.

RESULTS AND DISCUSSIONS

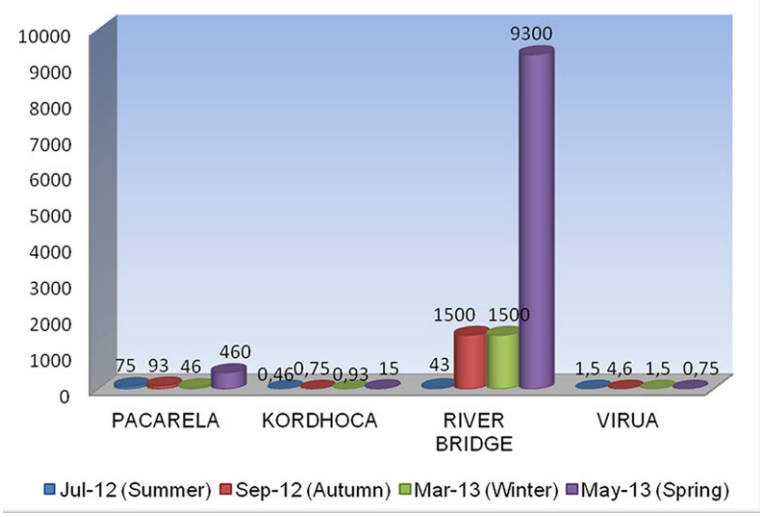
There were analyzed in total 48 samples from 6 stations in four seasons: Summer - Autumn 2012, Winter - Spring 2013, 16 water samples for *Faecal coliforms* (FC) and 16 other water samples for *Faecal steptococci* (FS), and 8 lettuce samples for *Faecal coliforms* (FC) and 8 other lettuce samples for *Faecal steptococci* (FS).

From the analysis of water samples of Drino River, we could be observed the differentiation of *Faecal coliforms* and *Faecal streptococci* according to the seasons and at various points of the river.

Figure 3:
View of the River Bridge station.

Table 2: Feacal Coliforms in water samples in 4 seasons.

		MPN/100ml/1000		
STATIONS	Jul – 12	Sep - 12	Mar - 13	May - 13
	(Summer)	(Autumn)	(Winter)	(Spring)
PACARELA	75	93	46	460
KORDHOCA	0.46	0.75	0.93	15
RIVER BRIDGE	43	1500	1500	9300
VIRUA	1.5	4.6	1.5	0,75



Graph 1:
The dynamics of Faecal Coliforms at 4 stations on the Drino River in 4 seasons.

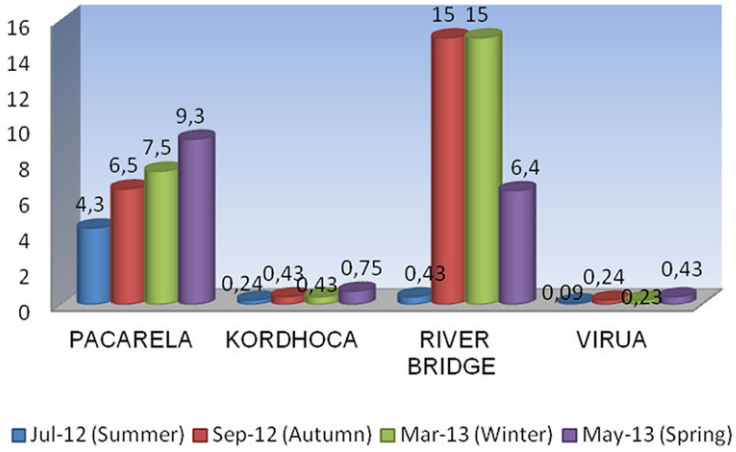
Table 2 and Graph 1 show the microbial values of FC in 4 stations along the Drino River. The cleanest water was in the upper flow of the river (Kordhoca station)

with 460 bacteria/100ml. The stations in its downstream flow were dirty, and sometimes they passed the permitted rates of the EU standards for surface waters. The most polluted is River Bridge station with $9.3x10^6$ bacteria/100ml (Figure 3). This level of pollution is 3-4 times higher than that defined by the EU directives of the permitted rates for surface waters.

Table 3 and Graph 2 show the microbial values of FS in 4 stations along the Drino River. The station with the highest pollution was River Bridge station with MPN $1.5x10^4$ bacteria/100ml, while the one with the lowest pollution was Virua station with 90 bacteria/100ml. Perhaps this situation in Virua station is explained by natural water springs flowing in it.

Table 3: Faecal Streptococci in water samples

MPN/100ml/1000				
STATIONS	Jul-12 (Summer)	Sep-12 (Autumn)	Mar-13 (Winter)	May- 13 (Spring)
PACARELA	4.3	6.5	7.5	9.3
KORDHOCA	0.24	0.43	0.43	0.75
RIVER BRIDGE	0.43	15	15	6.4
VIRUA	0.09	0.24	0.23	0.43

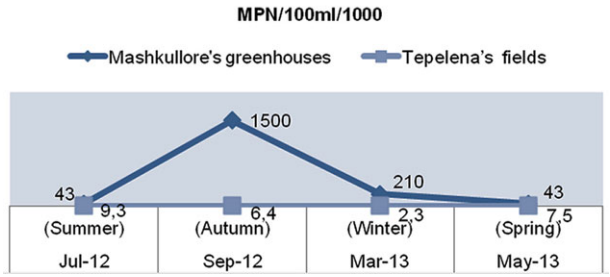


Graph 2: The dynamics of Faecal Streptoccoci at 4 stations on the Drino River in 4 seasons.

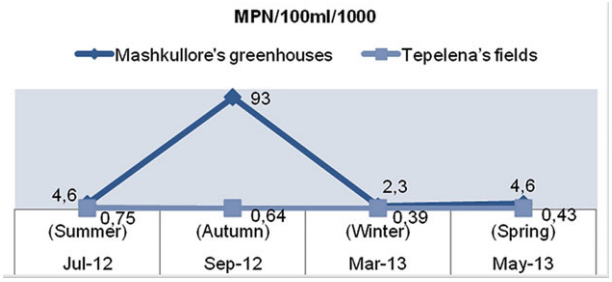
The values of MPN for CF and SF in River Bridge station (after the discharge of industrial and urban wastewater in Gjirokastra city) are several times higher than the permitted levels and this situation is a high potential risk on public health. Non-modified human wastes, industrial and urban wastes are discharged into the river without any previous treatment causing the highest pollution in this station.

At Mashkullore's greenhouses and Tepelena's fields where the lettuce is irrigated with the Drino River water, samples were taken and analysed, and the

values of MPN for CF and SF were high during all the seasons (Graphs 3 & 4). According to the literature and rates for food safety, and good water quality for vegetable irrigation, the number of coliforms and streptococci should not exceed 200 coliforms/100 ml of water (Puto, 2012).



Graph 3:
The dynamics of Faecal Coliforms in lettuce samples at 2 stations in 4 seasons.



Graph 4:
The dynamics of Faecal Streptoccoci in lettuce samples at 2 stations in 4 seasons.

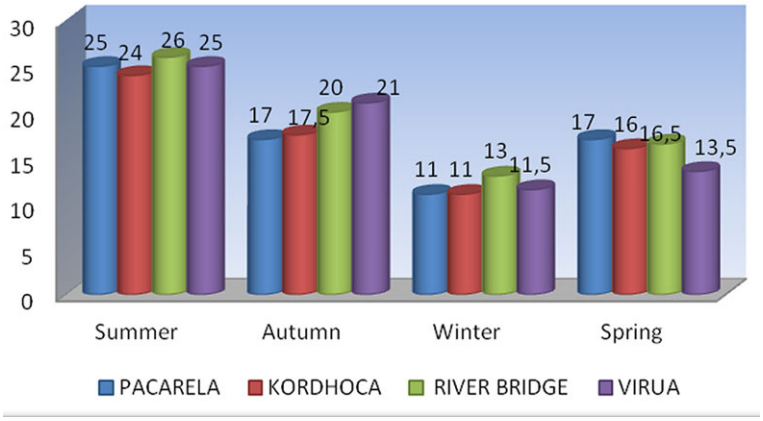
The highest values of MPN for FC and FS were in September (autumn season) at Mashkullore's greenhouses compared with the other seasons. So the hightest values for FC and FS were respectively 1.5 x 10^6 bacteria/100ml and 9.3 x 10^4 bacteria/100ml. These high values can be explained by the high temperatures of this period (over 40 ^{0}C).

The presence of Faecal Coliforms and Faecal Streptoccoci is an indication of faecal pollution that comes from river water used for irrigation. The fresh vegetables are also contaminated by fertilization with fresh organic liquid fertilizer, from livestock grazing around them and from aerosols.

Considering that this water is used for the irrigation of vegetables in these areas, it is possible that their contamination at such high levels has a consequence of great impact on public health. Consequently, these fresh vegetables may be a threat for the consumer and may be considered a serious risk to public health (Rai & Tripathi, 2007; Tyrell et al., 2006).

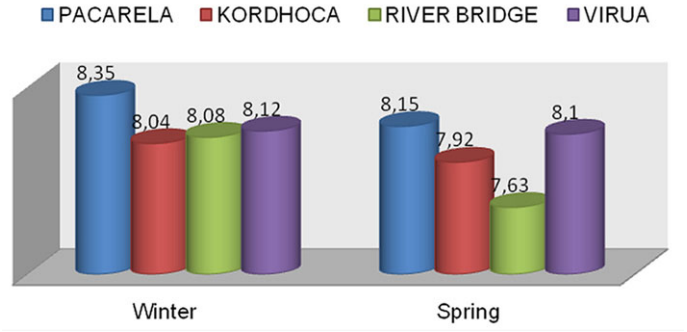
There are presented variations of some physical-chemical parameters of water in 4 stations.

In graph 5 is presented the variation of water temperature in 4 points for 4 seasons of our study period. Minimal and maximal temperatures vary from 11^{0}C to 26^{0}C.

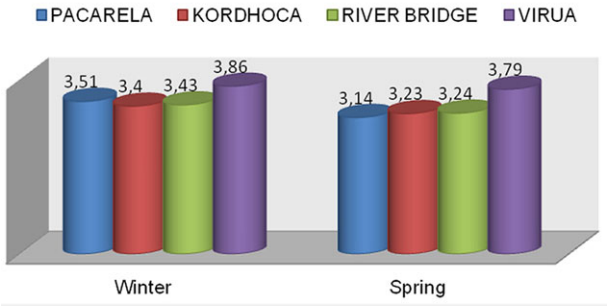


Graph 5:
Water temperature (t^{0}C) in 4 seasons.

Regarding to pH values (graph 6) it varies from 7.63 - 8.35. These are normal values according to European norms. The lowest value of ph is observed at station 3, while the highest value at station 1.



Graph 6:
pH in 2 seasons.

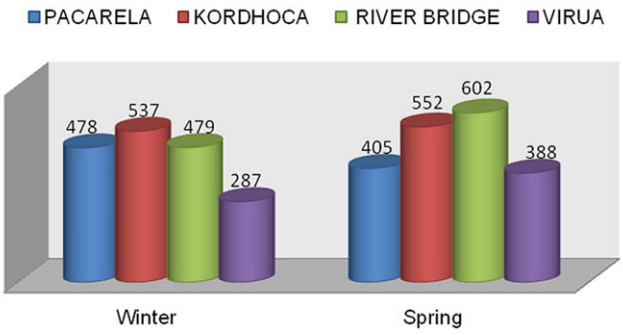


Graph 7:
The variation of dissolved oxygen D.O. (mg/l).

Regarding to the dissolved oxygen as a very important quality parameter that determines significantly the health condition of the water, we observe that the minimal and maximal values of it varied from 3.14mg/l to 3.86 mg/l (graph 7). According to the literature dissolved oxygen concentrations in unpolluted water normally range between 8mg/l and 10 mg/l (Watson *et al.*, 1985). So we can say

that at all sampling stations its values are below the allowed limit rate, which undoubtedly affects the living organisms that live in the water of the river.

In the graph 8 is presented the variation of electric conductivity at 4 points for 2 seasons (winter – spring). Electrical conductivity of water is a useful and easy indicator of its salinity or total salt content (Morrison *et al.,* 2001). Minimal and maximal values of E.C vary from 287µS/cm to 602µS/cm. These are normal values according to European norms, because mid range conductivity (200µS/cm to 1000µS/cm) is the normal background for most major rivers.



Graph 8: The variation of electric conductivity E.C. (µS/cm).

CONCLUSIONS

The analysis made in four seasons (Summer - Autumn 2012, Winter - Spring 2013) show that Drino River water is heavily polluted from a microbial perspective, according to Europian standards. River Bridge station (after the discharge of industrial and urban pollution in Gjirokastra city) results with the highest pollution. The highest values of MPN for FC and FS are respectively 9,3 x10^6 bacteria/100ml in spring 2013 and 1.5 x 10^4 bacteria/100ml in autumn 2012. These values of MPN for FC and FS in River Bridge station are several times higher than the permitted levels. This situation is a high potential risk on public health and it comes from the fact that industrial and urban discharges flow directly into the river without any previous treatment.

The presence of *Faecal Coliforms* and *Faecal Streptoccoci* in fresh vegetables is an indication of faecal pollution that comes from river water used for irrigation. In lettuce the values of MPN vary for FC from 4,3x 10^4 to 1,5x 10^6 bacteria/100ml and for FS from 2300 to 9.3 x 10^4 bacteria/100ml. These values of pollution are very high, according to the literature that the number of coliforms and streptococci should not exceed 200 coliforms/100 ml of water used for vegetable irrigation.

Actually, in Albania there is a lack of knowledge and precautions about the water used for irrigation. The users of this water should be aware of the

consequences and the risks for the public health that come with irrigation using polluted water.

We strongly recommend stopping the discharge of urban discharges and untreated wastewater to the Drino River.

Also, we recommend urgent precautions for the wastewater treatment before their discharge into the river.

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RESUME

Ndotja mikrobike e lumit Drino (në qarkun e Gjirokastrës) dhe ndikimi i tij tek perimet e freskëta

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Përmbledhje

Një nga sfidat mjedisore vitet e fundit në Shqiperi është cilësia e ujërave sipërfaqësore dhe e perimeve të freskëta që ujiten me to. Lumi Drino është një nga burimet më të rëndësishme ujore në qarkun e Gjirokastrës me vlera mjedisore, sociale dhe ekonomike. Uji i këtij lumi përdoret për ujitje, peshkim, turizëm etj. Monitorimi i karakteristikave biologjike të ujit të lumit Drino përdoret për të përcaktuar cilësinë e ujit dhe ndotjen mikrobike të tij, si dhe ndikimin e tij në mjedis dhe në shëndetin publik.

Objekti i punës tonë gjatë periudhës 2012 – 2013 ka qene monitorimi i ndotjes mikrobike të ujit të lumit Drino nga shkarkimet urbane të patrajtuara, si dhe monitorimi i ndotjes mikrobike të perimeve të freskëta që ujiten me ujin e këtij lumi.

Analizat mikrobiologjike të mostrave të ujit dhe të sallatës, si dhe analizat fiziko – kimike të mostrave të ujit të lumit Drino të marra ne 6 stacione (4 stacione në lumin Drino dhe 2 stacione për mostrat e sallatës) janë bërë në katër stinë: Verë – Vjeshtë 2012 dhe Dimër – Pranverë 2013. Koliformet fekale (FC) dhe Streptokoket fekale (FS), si indikatorë të ndotjes mikrobike janë zbuluar me anë të teknikës së tubave të shumëfishtë ose teknikës MPN.

Rezultatet e marra nga analizat e indikatorëve mikrobike FC/FS variojnë për FC 460/9,3x10^6 baktere/100ml dhe për FS 90/1.5x10^4 baktere/100ml për ujin e lumit Drino, ndërsa për sallatën këta indikatorë mikrobikë variojnë për FC 4,3x10^4/1,5x10^6 baktere/100ml dhe per FS 2300/9.3 x 10^4 baktere/100ml.

Kjo situatë vjen si rezultat i derdhjes së shkarkimeve urbane në lumin Drino pa asnjë trajtim paraprak. Si rezultat, kjo ndotje mikrobike ka një impakt të madh në shëndetin publik. Ne rekomandojme trajtimin urgjent te mbetjeve urbane para derdhjes së tyre në lumë.