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IMPACT OF THE WATER QUALITY OF THE ERZENI RIVER ON THE MICROBIAL SAFETY OF FRESH VEGETABLES

ABSTRACT

The Erzeni River flows to a length 109 km, traversing the regions of Tirana and Durrës, with an area of 760 km^2 and finally flows into the Adriatic Sea. In this way, the physical, chemical and biological qualities of the river water are present in the agricultural activity of these regions. Often the agricultural products, as a result of the microbial contamination and pathogens in them, have an impact on public health as a result of certain diseases originating from contaminated foods. The microbial quality of the water used for irrigation in agriculture, and especially that used for fresh vegetables, is important to monitor. The poor quality of the river water used for irrigation is one of the reasons for the presence of microbial pathogens in fresh vegetables such as salad vegetables, tomatoes and onions. The microorganisms present in these products come mainly from the agricultural land and irrigation water. In this paper, the presence of microbial pathogens (faecal coliforms), and the influence of the physical and chemical factors, at seven points along the Erzeni River during 2011 have been analysed. Also, in three of these points the influence of the river water used for irrigation in salad vegetables was analysed. From this, the results showed that the river water was mostly polluted by faecal coliforms (CF) above the numbers allowed by the international norms. This is because of the untreated river water in two major regions: Tirana and Durrës. Also, there is an impact from the microbial contamination of the contaminated soil caused by the misuse of manure and nitrates, keeping cattle along the river, and from pets, etc. Consequently, the salad vegetables irrigated by the river water are very polluted by microbial pathogens, specifically CF. Microbial contamination at levels 3-4 times the permitted levels of urban pollution requires treatment and continuous monitoring of the river water as one of the immediate tasks having an impact on public health

Keywords: fresh vegetables, microbial safety, coliforms

INTRODUCTION

Today a good quality of life is the main target worldwide. A healthy way of life is closely related to the environment in which we live, and the care and safety of food quality, especially those foods with little or no processing such as

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fruits and vegetables. It is important that food products are not contaminated by pathogenic microorganisms, which have a major impact on public health. Today, even in Albania, the protection of human health is very important as it is related to lifestyles, the environment and eating habits. After 1990, political and socio-economic developments changed the lifestyles, environment and agricultural and livestock production of Albania greatly. Our country, after nearly twenty years, is facing new and significant health and environmental challenges closely related to the quality of human life. The cultivation of agricultural products in the private economies of Albania, often individual and involved in not only manufacturing but also marketing, occurs in the most inappropriate of environments when compared to other countries, which determine their contamination levels using a variety of microorganisms and pathogens. This contamination, especially in fresh agricultural products that are consumed untreated, brings consequences for public health because of the diseases it can spread. One of the pollutants causing the microbial contamination of vegetables is the water used for irrigation. Therefore, the microbial quality of the water is important, and a critical problem that must be assessed (APHA-AWWA-WEF, 1995). This importance is related to the fact that the water used for irrigation, contaminated with faecal pathogens from untreated urban waste that flows into it, naturally contaminates the products which are irrigated by it. The goal of this study was to determine the quality of the Erzeni River water, which is used by the residents, at some of its points for the irrigation of fresh vegetables, bringing consequences to public health by evaluating microbial contamination (DIRECTIVE 2006/7/EC, Harrington, 2009).

What is the importance of safe food?

Every year the Albanian consumer becomes more aware and has more demands for a "safe food", which means that food products, including the agricultural ones, should be manufactured and marketed in a safe way and must be certified within the EU standards. These microbiological parameters are considered as quality indicators for safety with a public health impact. 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 and Tripathi, 2007; Tyrell, S., Knox, J. and Weatherhead, 2006).

Sources and routes of the contamination of river water and vegetables

The coliform bacteria belong to a nonpathogenic group of microorganisms found in the human intestines, as well as in warm-blooded animals, which help in the digestion of food. They usually pass from the discharge of untreated urban waste into surface waters. The presence of faecal coliforms in the water indicates that it is contaminated by faecal material, and at the same time the water can be contaminated by pathogens such as bacteria, viruses or parasites. Gastroenteritis, hepatitis A and typhoid fever are some of the diseases caused by water contaminated with pathogens. Coliform bacteria are good indicators of the possible contamination of the water source (APHA-AWWA-WEF, 1995;

DIRECTIVE 2006/7/EC, 2006). The routes of spreading pathogenic microorganisms to surface water are mainly from untreated urban discharge and from the sewage flowing into them. The cattle that graze along the rivers and on land close to the rivers are another cause of pollution. When the land is irrigated by this water, microbial contaminants are passed to the vegetables. Another kind of planted parcel pollution is the aerosols around them, as well as the soil and fresh fertilizer used during the spring by farmers that grow vegetables. Contamination continues in the same way during the harvest and after it. In addition to this, the contamination of vegetables continues during trading times when often their marketing is made across the roads without any consideration of the conditions. Even those who trade across the roads near the rivers, in order to keep their products cool, sprinkle them with the polluted river water.

The benefits and the value of vegetables

The OBSH and the Agriculture and Food Organization (FAO) have set parameters for the benefits of fresh vegetables and fruits in nutritive diets, and they recommend eating a quantity of at least 400 g per day. The adequate intake of fresh fruits and vegetables is important for human health, mainly for the prevention of heart disease, cancer and diabetes, which are three diseases with the greatest mortality rate for humans in the world today. The consumption of vegetables and fruits, by the year 2020, is predicted to increase from 24% of the diet to 27%. Therefore this study was conducted in order to protect consumers and their health. The criteria for a quality food, and to avoid diseases originating from their contamination, is cause for the evaluation of contamination sources, one of which is the water used for irrigation (Harrington, 2009). The Erzeni River water, which is used by the villagers near the river for the irrigation of agricultural products, has been subjected to analysis. The Erzeni River is localized in the central part of Albania with an area of 663 km^2, and it is characterized by a Mediterranean climate with extensive rainfall during the winter season. The Erzeni River, with a length of 109 km, expands along the two largest cities of Albania, Tirana and Durrës, with a population density of about 50% of the whole country.

The main source of the upstream of Erzeni River is Shengjergj, Tirana, and it flows into the Adriatic Sea at the Lalzi Bay. Consequently, it has a considerable impact to the environment and public health of this area. Actually, the situation regarding the sewage which is discharged throughout this area is very critical. The collection and treatment of sewage has never been done, and so through the years has resulted in a growing pollution level in the Erzeni River water. What really complicates the situation is that some channels and streams discharge their water into the river, in this way increasing the degree of pollution, as is happening in the Zalli stream, Gerdeci stream, Tana channel and Rubjek reservoir.

In order to determine the faecal pollution of the Erzeni River and to evaluate its quality, the coliform microorganisms, as indicators of water

pollution, were enumerated. Entire surfaces of this area over the length of the Erzeni River are agricultural lands with constant agricultural activity. Besides the pollution from the sewage that flows untreated directly into the river, the urban and industrial wastes of Tirana and a good part of Durrës on the banks of this river are also discharged.

Figure 1: Map of the Erzeni River in Albania

The object of this monitoring has been a measurement of the coliform bacteria in the Erzeni River water during 2011, as well as the heterotrophs and coliforms in the salad vegetables that are irrigated by the water of this river at three main points: Ndroq, Sallmone and Rrushkull. Various aspects of this monitoring have been analysed involving the sources of water contamination and the vegetables that are irrigated with river water, or contaminated in other ways not only during irrigation but also after their harvest and marketing. All of these have been performed in order to assess the safety of the food with an impact on public health. The vegetables are also contaminated by fertilization with fresh organic liquid fertilizer, from livestock grazing around them and from aerosols. Pollution is a very important aspect of this contamination study since this is the main way that pathogenic microorganisms are transmitted through food to humans.

MATERIAL AND METHODS

The microbial parameters analysed in the Laboratory of Biotechnology Department, FNS, University of Albania, were examined in a certain number of water samples and salad samples during 2011. The water samples were taken at 11 points along the Erzeni River and in three of these points, Ndroq, Rrushkull and Sallmone, there were analyses conducted of the physical and chemical parameters, as well as a microbial analysis for heterotrophs, coliforms and streptococci in the salad vegetables during 5 months of 2011, from February to June. The samples taken and the tests used to determine these parameters were done according to the European standard rates. The water samples were taken with 250 ml sterilized bottles, and 2 L of water were taken for the physical and chemical parameters. Our water samples, along with the salad vegetable samples, were brought to the laboratory overnight while maintained in refrigerated conditions at 4^{o} C. The analysis was done within 24 hours. The indicator bacteria 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) or tamponated water peptone was used as a diluent. Three or five test tubes for each dilution were inoculated with 1 ml of test water. The incubation was done at 350° C for 48 ± 2 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 LTB ground and then transferring it to 1-3 Anza suspensions in tubes with EC ground. They were incubated at 44.5 ± 0.2° C for 24 ± 2 hours. Then they were examined for gas production. The number of faecal coliforms (MPN) was calculated according to the relevant table. There were 15 samples of salad taken over 5 months, and one sample for each point in Ndroq, Sallmone and Rrushkull was analysed. The vegetable samples were taken at three points in the fields where the irrigation was being done using Erzeni River water in the Tirana and Durrës areas. The methods used for the vegetable tests were the Multiply Tube Test and plating for faecal coliforms, and the media PCA and TSA were used for the heterotrophic microorganisms. The incubations were done at 35-37º C for 24 hours.

RESULTS AND DISCUSSION

In total, 44 samples of water were analysed for faecal coliforms from 11 points of the river during 2011, four times for each season (spring, summer, autumn and winter). The differentiation of coliforms according to the seasons and at various points of the river could be observed from the analysis of our water samples. Table 1 shows the water quality standards when using the faecal coliform indicators CFU, which refers to the CFU for coliforms in 100 ml of surface water, according to ISO 7899-1.

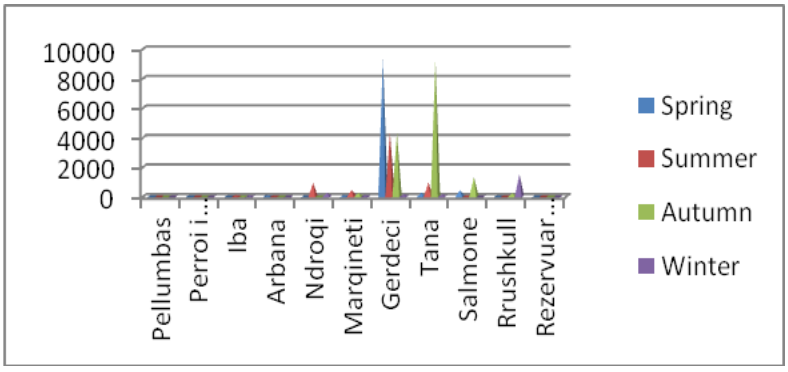
Table 1: Water quality standards for rivers, ISO 7899-1

Total coliforms	Very good quality	Good Quality	Bad Quality	Very Bad Quality
CFU/100 ml	1,250	2,500	5,000	10, 000

The points with the cleanest water were those in the upper flow of the Erzeni River (Table 2). The points in its downstream flow were very dirty, and sometimes they passed the permitted rates of the EU standards for surface waters.

Table 2: Faecal coliforms at 11 points in the Erzeni River during 2011

MPN/100 ml /1000				
	Spring	Summer	Autumn	Winter
Pellumbas	0.4	2.3	0.23	2.3
Perroi i Zallit	4.3	23	9.3	24
Iba	2.3	93	43	93
Arbana	93	43	9.3	24
Ndroqi	9.3	930	23	240
Marqineti	93	430	230	93
Gerdeci	9300	4300	4300	150
Tana	240	930	9300	150
Salmone	430	75	1300	230
Rrushkull	43	23	230	150
(Rubjek)	9.3	0.93	0.93	4.3



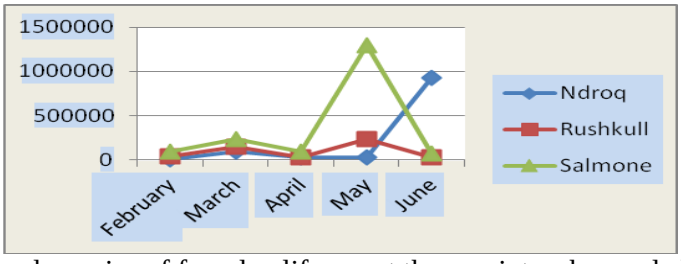
Graph 1: The dynamics of faecal coliforms at 11 points on the Erzeni River

Figure 2: Photograph of the Ndroq point

At three points where the salad vegetables were irrigated with the river water, samples were taken and analysed, and the pollution coliform levels were high. According to the literature and rates for food safety, and good water quality for vegetable irrigation, a proper value should approach that of drinking water and the number of coliforms should not exceed 200 coliforms/100 ml of water (Table 3 and Graph 2). The seasons WERE also considered, excluding the Ndroq point during the winter when the water results were of a good quality, and in all points during all seasons the water was of a poor quality.

Table 3: Faecal coliforms at three points where salad vegetables irrigated by the Erzeni River were analysed

	February	March	April	May	June
Ndroq	2,400	93,000	24,000	23,000	930,000
Rrushkull	43,000	150,000	23,000	230,000	23,000
Sallmone	93,000	230,000	93,000	1,300,000	75,000



Graph 2: The dynamics of faecal coliforms at three points where salad vegetables irrigated by the Erzeni River were analysed

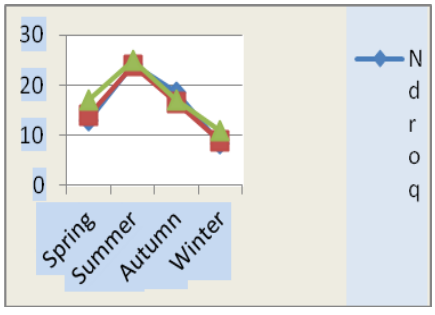
In addition, with the goal of a better assessment of the water quality of the Erzeni River, three points (Ndroq, Rrushkull and Sallmone) were analysed using some physical and chemical parameters related to water quality. These parameters, as seen from the Tables, in some seasons of the year are at very high values when compared with the rates as a result of the pollution of the river water. The temperature in Rrushkull reached the river water temperature, up to 25^{o}C, in the summer, and the suspended matter reached 7,229 mg/l. The phosphorus values during the autumn and spring reached the values of 10 gr/ml from 1 g/ml, which is the rate in the surface waters. These numbers indicate contamination and a very bad quality of water.

Table 4: The variation of temperature during 2011

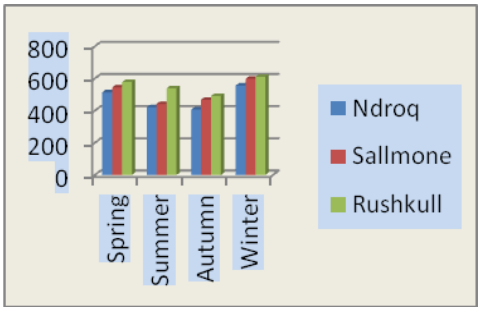
	Temperature ^{0}C			
	Spring	Summer	Autumn	Winter
Ndroq	13	24.1	18.55	8.5
Sallmone	14	24	16.5	9
Rrushkull	17	25	17	11

Table 5: The variation in conductivity during 2011

	Conductivity (mg/l)			
	Spring	Summer	Autumn	Winter
Ndroq	514	421	407	557
Sallmone	544	442	468	598
Rrushkull	578	539	491	611



Graph 3: Temperature



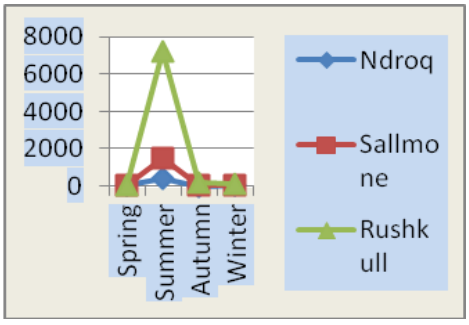
Graph 4: Conductivity

Table 6: The variation of suspended matter during 2011

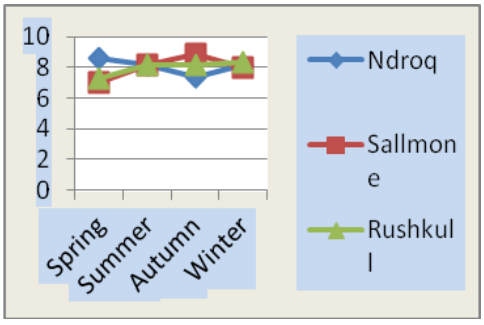
	Suspended matter (mg/l)			
	Spring	Summer	Autumn	Winter
Ndroq	14.8	386	8	18.4
Sallmone	11.6	1521	53.6	41.2
Rrushkull	63.6	7229	157.6	108.8

Table 7: The variation of pH during 2011

	pH			
	Spring	Summer	Autumn	Winter
Ndroq	8.57	8.18	7.35	8.15
Sallmone	6.99	8.17	8.85	8
Rrushkull	7.25	8.19	8.15	8.34



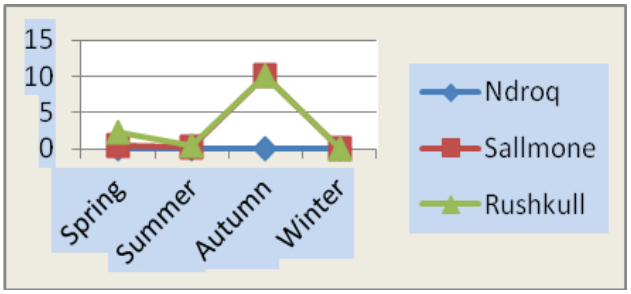
Graph 5: Suspended matter



Graph 6: The variation in pH

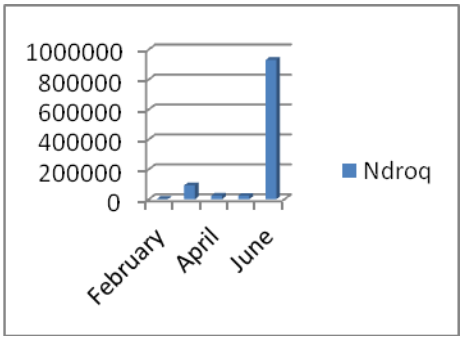
Table 8: Phosphorous (g/ml)

	pH			
	Spring	Summer	Autumn	Winter
Ndroq	0.112	0.14	0.009	0.005
Sallmone	0.419	0.22	10.1	0.01
Rrushkull	2.355	0.42	10.2	0.008

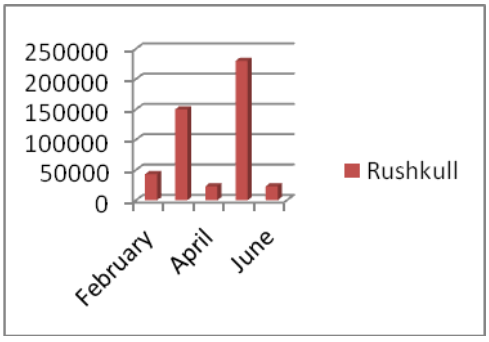


Graph 7: Phosphorous

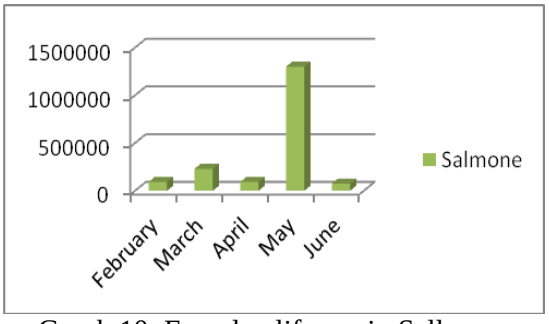
Graphs 8, 9 and 10, show the faecal coliforms of the three points whose water irrigates the salad vegetables for the months that we analysed the heterotrophs and coliforms (from February to June). Only in February were low coliform numbers observed, while in the 4 following months the number was above the permitted levels for irrigation, considering it to be unsuitable for use in vegetable irrigation, especially those vegetables that are consumed fresh.



Graph 8: Faecal coliforms in Ndroq



Graph 9: Faecal coliforms in Rrushkull

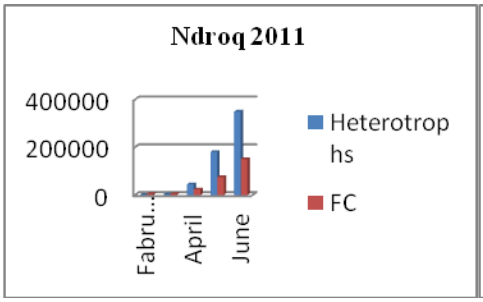


Graph 10: Faecal coliforms in Sallmone

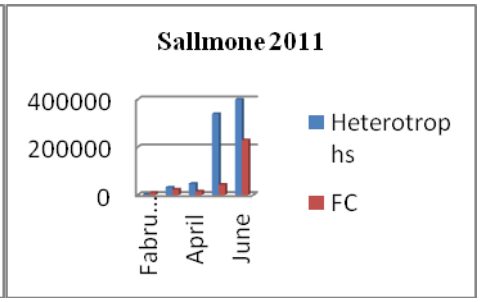
Heterotrophic bacteria and faecal coliforms in salad

In total 15 samples of salad vegetables were taken and tested from the gardens where plant irrigation was done using the Erzeni River water. The control vegetables were irrigated by tap water. All of the samples were tested for

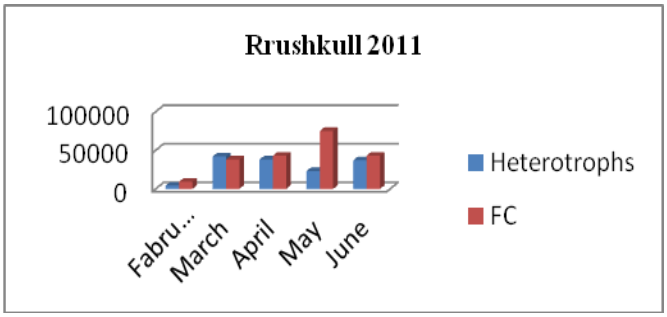
the presence of heterotrophs and faecal coliforms, and the results are shown in Graphs 11, 12 and 13.



Graph 11: Heterotrophs in Ndroq



Graph 12: Heterotrophs in Sallmone



Graph 13: Heterotrophs in Rrushkull

The results showed that all of the tested salad vegetable samples were contaminated with bacteria. The level of the heterotrophic bacteria varied from 400 bacteria/g to 350,000 bacteria/g of salad vegetables in Ndroq. Faecal coliforms varied from 4,300-150,000/100 ml. The level of heterotrophic bacteria varied from 2,500 bacteria/g to 400,000 bacteria/g of salad vegetables in Sallmone. The faecal coliforms varied from 9,300-230,000/100 ml and the level of heterotrophic bacteria varied from 4,300 bacteria/g to 42,000 bacteria/g of salad vegetables in Rrushkull. Faecal coliforms varied from 9,300-400,000/100 ml. The high amount of bacteria found in these samples comes from polluted water as well as from the soil. The presence of faecal coliforms is an indication of faecal pollution in the river water used for irrigation.

The presence of faecal coliforms remains a strong indicator for the presence of pathogens that are very dangerous to human health.

CONCLUSIONS

The analyses made show that the Erzeni River is heavily polluted from a microbial perspective. Faecal coliform levels in this water extend from $2.3x10^3$ in Rrushkull, up to $1.3x10^6$ MPN/100 ml in Sallmone, indicating that the level of

pollution is 3-4 times higher than that defined by the EU directives of the permitted rates for surface waters.

In salad vegetables the level of heterotrophic bacteria varied from 400 bacteria/g to 350,000 bacteria/g of salad vegetables in Ndroq. Faecal coliforms varied from 4,300-150,000 bacteria/g. The level of heterotrophic bacteria varied from 2,500 bacteria/g to 400,000 bacteria/g of salad vegetables in Sallmone. Faecal coliforms varied from 9,300-230,000/100 ml. The level of heterotrophic bacteria varied from 4,300 bacteria/g to 42,000 bacteria/g of salad vegetables in Rrushkull. Faecal coliforms varied from 9,300-400,000/100 ml.

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.

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.

It is urgently recommended that we should intervene in the processing of sewage before it is discharged into the river, as well as intervene in the other multiple urban and agricultural discharges.

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Klementina PUTO

UTICAJ KVALITETA VODE RIJEKE ERZEN NA MIKROBNU BEZBJEDNOST SVJEŽEG POVRĆA

SAŽETAK

Rijeka Erzen teče dužinom od 109km, prolazeći kroz predijele Tirane i Drača, sa površinom sliva od 760 km2 i uliva se u Jadransko more. Na ovaj način, fizičkohemijski i biološki kvalitet njene vode učestvuje u poljoprivrednoj aktivnosti ovih regiona. Često poljoprivredni proizvodi, usljed njihovog mikrobnog zagađenja i patogena utiču na javno zdravlje i za posljedicu imaju određene bolesti čiji je uzrok zagađena hrana. Veoma je bitno nadzirati mikrobni kvalitet vode korišćene za navodnjavanje poljoprivrednih kultura, a pogotovo svježeg povrća. Loš kvalitet rječne vode korišćene za navodnjavanje je jedan od razloga za prisustvo mikrobnih patogena u svježem povrću poput salate, paradajza, luka itd. Mikroorganizmi koji se nalaze u ovim proizvodima uglavnom potiču iz poljoprivrednog zemljišta i vode za navodnjavanje. U ovom radu je analizirano prisustvo mikrobnih patogena (fekalnih koliforma) i uticaj fizičkohemijskih faktora na sedam lokacija duž rijeke Erzen u 2011. godini. Osim toga, na tri od datih lokacija je analiziran uticaj rječne vode korišćene za navodnjavanje salate. Ova ispitivanja su dokazala da je rječna voda najvećim dijelom zaražena fekalnim koliformima. Uzrok tome je neprerađena rječna voda u dvije glavne oblasti: Tirani i Draču. Na mikrobno zagađenje zemljišta takođe utiče nepravilna upotreba đubriva i nitrata, kao i stočarstvo u neposrednoj blizini rijeka. Stoga, salata navodnjavana rječnom vodom biva dosta zagađena mokrobskim patogenima. Mikrobno zagađenje koje je 3-4 veće od dozvoljenog nivoa gradske zagađenosti, iziskuje tretman i stalni nadzor rječne vode kao jedan od hitnih zadataka koji utiču na javno zdravlje.

Ključne riječi: svježe povrće, mikrobna bezbjednost, koliformi.