

MICROBIOLOGICAL QUALITY OF SURFACE WATER AND SAFE VEGETABLE PRODUCTION

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Abstract

Good microbiological quality of irrigation water has a great importance for health-safety agricultural production. In order to avoid microbiological contamination of crops, the microbiological quality of the water supplying the crops should be monitored. The aim of this paper is investigation of the microbiological quality of the water from 5 channels and one pool from the municipality of Surcin which can potentially be used for irrigation, as well as determination the microbiological quality of the vegetables which are irrigated from the pool. Microbiological parameters of water and vegetables quality are determined by the standard methods prescribed by the Rulebook on parameters of surface waters (MPN method) and Rulebook of food hygiene of production of Republic of Serbia. The values of the tested parameters in one of the five investigated channels, Surcin channel water, were higher in relation to the limit values of the Rulebook. The increased levels of total and fecal coliforms, *Escherichia coli*, *Salmonella*, and *Shigella* were found. The Surcin channel water requires appropriate treatment before its use for crop irrigation. The values of other tested waters did not go beyond the limit values prescribed by the Rulebook, but for the safer agricultural production their treatment is recommended. Microbiological analyzes of vegetables were also done. The total and fecal coliform bacteria, as well as *E. coli*, *Salmonella* sp. and *Shigella* sp., were not detected in the tested vegetable samples.

Key words: *microbiological quality of irrigation water, coliform bacteria, E. coli, Salmonella sp.*

Introduction

Consumption of fresh fruits and vegetables is essential for healthy nutrition and prevention of various diseases. The World Health Organization (WHO) underline necessary for consumption at least 400 g of raw fruits and vegetables daily. However, these products could be microbiologically contaminated and pose a risk for human health. Based on that, it is necessary to take care about microbiological safety of fresh vegetables and fruits. It implies the absence of human pathogens, because consuming raw vegetables that are microbiologically contaminated can cause many health problems and sometimes can even lead to death. Human pathogens could be associated with gastrointestinal diseases and cause many health diseases such as dysentery, hepatitis, hemolytic uraemic syndrome, typhus (Halablab et al., 2011).

Microbiological contamination of fresh vegetables can occur at any point of production chain and the sources of contamination could be numerous, but one of the most common is contaminated irrigation water or faecal contamination from humans or animals (Halablab et al., 2011). The surface waters are used for irrigation but they are very often polluted (Uyttendaele et

al., 2015) and that contamination comes from runoff, animal faeces, sewage (Gemmell and Schmidt, 2013).

Irrigation water is the good environment for many human pathogens such as viruses (adenovirus, norovirus, enterovirus, rotavirus), human pathogen bacteria (*Campylobacter sp.*, *E. coli*, *E. coli* O157: H7, *Bacillus cereus*, *Salmonella sp.*, *Shigella sp.*) (Ahmed et al., 2014). These microorganisms are able to colonize vegetable plant tissue through irrigation water and contaminate edible parts of plants because plant root niches are a good environment for their survival. Thus, irrigation water from two rivers in Norway had higher level of *E. coli* compared to national regulatory standard (Paruch et al., 2015). The research of Mcheik et al., (2018) showed huge microbiological contamination in the 33 well water samples taken from 5 sites. All water samples were contaminated with total coliforms and most of them with fecal coliforms, *E. coli* and *S. aureus*. These waters were used for irrigation which caused negative effect on microbiological safety of vegetables. The high microbial loads of coliforms and *S. aureus* were noticed at lettuce, mint, dandelion, spinach, coriander and radish. Such a large number of bacteria on mentioned leafy species of vegetables is a consequence of the larger surface area exposed to contaminated irrigation water. Also, the reason for this is entrance and penetration human pathogen bacteria in plant inner tissues, root and leaves (Kljujev et al., 2018a; Kljujev et al., 2018b). Also, there is evidence that surface of leafy vegetables could be a host for potential human pathogens (Dees et al., 2015). The enteric-bacterial-contaminated irrigation water has impact on the microbiological safety of vegetables. The large number of coliforms and *E. coli* in channel irrigation water leads to contamination vegetables. The high number of coliforms and *E. coli* was at spring onion, cabbage, carrot and tomato (Douti et al., 2021).

It is very important to take care about microbiological quality of irrigation water (Jeong et al., 2016; De Pascale et al., 2011), especially those which is used for irrigation of vegetables that are eaten in raw. It is necessary to regularly control these waters in order to enable safe vegetable production.

Materials and methods

Sampling of water and vegetables for microbiological analysis was done on the territory of the Surcin, municipality near Belgrade in Serbia. The microbiological analysis included 6 water samples and 3 vegetable samples. Out of 6 water samples, 5 samples were originated from channel water and another one was from pool used for irrigation. The locations for water samples were: Channel "Surcin", Channel "Donje polje", Channel between P.S "Donje polje" and P.S Petrac", Channel "Petrac", Channel "Galovca", Pool "I.S.C.". The lettuce sample was taken from open field but hot peppers and cucumbers samples were taken from greenhouse. Analyzed vegetables are irrigated with water from Poll "I.S.C."

The channel "Galovica" covers an area of about 70 000 ha and its capacity is 24 m³ of water per second. It flows into the river "Sava" near the settlement of New Belgrade and it brings water to the "Galovica" pumping station. The channel "Petrac" covers an area of 8 000 ha, it has capacity 8 m³ water/sec. and its length is 24 496 m. Both channels ("Galovica" and "Petrac") run in parallel at a distance of 65 m from each other and they are interconnected by a connecting side channel for water inlet and outlet. The water in these channels represents an excess of water and has a important role in irrigation especially in summer. There are two big companies (PKB Corporation A.D. Belgrade and Farm "July 7"-Jakovo) which use waters of Channel "Donje polje" and Channel between P.S "Donje polje" and P.S "Petrac" for irrigation their fields with

corn, sunflower and other mainly industrial plants during the summer. Also, the water sample was taken from the pool of the agricultural farm "Iceberg Salat Center". This company uses water from well of 25 meters depth for irrigation.

The microbiological quality of lettuce (*Lactuca sativa*), hot pepper (*Capsicum annum*) and cucumber (*Cucumis sativus*) was examined. Vegetable samples were taken from the agricultural areas of the company "Iceberg Salat Center". Lettuce was grown in the open field and hot peppers and cucumbers were grown in greenhouses.

Microbiological analysis included determination of: aerobic heterotrophs (psychrophilic and mesophilic), total and fecal coliforms, *Escherichia coli*, *Salmonella* sp., *Shigella* sp. Determination of indicator microorganisms (their presence and number) was done inoculated appropriate nutrient media by direct and diluted samples. The number of heterotrophs was determined on Nutrient Agar (Torlak, Serbia). The water samples were diluted in Peptone Water (Torlak, Serbia) and after medium inoculation, incubation was done at 26°C, 5-7 days (mesophiles), 22°C, 5-7 days (psychrophiles). After incubation, colony counts were done.

The detection of total, fecal coliforms and *E.coli* was done using MPN method. A series of test tubes with MacConkey Broth (Torlak, Serbia) and Durham tubes were inoculated with 1 ml of samples diluted in peptone water, and it was done in triplicate. Incubation was done at 37°C for 48 hours. The tubes with changed medium color and bubbles in Durhams were taken as positive. Based on the number of positive tubes, the most probable number of coliform bacteria in 100 ml of water is determined using the MPN table. The confirmation test using EMB agar (Biomerieux, France) was done. The detection of *Salmonella* sp. and *Shigella* sp. was done using Selenite Broth (Biomerieux, France) for enrichment and SS Agar (Biomerieux, France) for confirmation. Incubation was done at 37°C for 24^h. The appearance of typical black colonies is confirmed as *Salmonella* sp. and uncolored and colonies were estimated as *Shigella* sp. The vegetable samples were tested to presence of aerobic heterotrophs, total and fecal coliform bacteria, *E. coli*, *Salmonella* sp. and *Shigella* sp. The 20g of vegetable pieces was homogenized with Peptone Water on a shaker 250 rpm for 30 min. The 1 ml of dilution was used for inoculation of Nutrient Agar and selective media MacConkey Broth, Selenite Broth, SS Agar, EMB Agar. Incubation was done at 22°C, 37°C and 44°C for 24 – 48^h. The number of bacteria is represented as cfu/ml and MPN/100 ml for water samples and cfu/g DM and MPN/gDM (DM- Dry Matter) for vegetable samples. The obtained results were interpreted according to Rulebook on parameters of ecological and chemical composition of surface waters and parameters of chemical and quantitative status of groundwater ("Official Gazette of RS", no. 74/2011) and Rulebook on general and special conditions of food hygiene at any stages of production, processing and trade ("Official Gazette of RS", No. 72/10).

Results and discussion

Aerobic heterotrophic bacteria are indicators of water status and quality. Their presence in large numbers indicates that the water is loaded with biodegradable organic pollutants. Our results showed that the number of total aerobic heterotrophs depends on the sampling location and the highest number was detected in Channel "Surcin".

Table 1 . The number of aerobic heterotrophs in water samples

Location	Unit	Temperature (t°)	
		22°C	37°C
Channel "Surcin"	cfu/ml	551	368
Channel "Donje polje"	cfu/ml	14	14
Channel between P.S "Donje polje" and P.S "Petrac"	cfu/ml	338	99,5
Channel "Petrac"	cfu/ml	24	36
Channel "Galovca"	cfu/ml	12	9,5
Pool "I.S.C."	cfu/ml	24,5	18.5

The number of psychrophilic bacteria was 551 cfu/ml, and mesophilic was 368 cfu/ml which indicate that water is loaded with organic matter. The smallest number of heterotrophic bacteria was noticed in Channel "Galovica" (12 cfu/ml and 9.5 cfu/ml.) (Table 1). The results of the tested vegetable samples showed the high number of aerobic heterotrophs at lettuce (103 cfu/g DM and 54 cfu/g DM) (Table 2).

Table 2. The number of aerobic heterotrophs on vegetable samples

Vegetable sample	Unit	Temperature (t°)	
		22°C	37°C
Lettuce	cfu/g DM	103	54
Hot pepper	cfu/g DM	14,5	0
Cucumber	cfu/g DM	9	5

Determination of total coliforms (TC), fecal coliforms (FC) and *E.coli* is important for microbiological quality of irrigation waters and vegetables. The coliform bacteria indicate to fecal contamination. The highest number of coliform bacteria (total and fecal) was detected in water of Channel "Surcin", more that 1 100 000 (MPN/100 ml) and according to the Rulebook on parameters of ecological and chemical composition of surface waters and parameters of chemical and quantitative status of groundwater ("Official Gazette of RS", no. 74/2011) this water belongs to IV-V class.

Based on our results, the water of Channel "Surcin" should not be recommended for irrigation crops without appropriate treatment. Channel "Surcin" is located about 200 m from the municipality center near markets, feed factory, livestock farm and many other facilities. It is assumed that all waste, generated from the mentioned facilities, arrive into the channal, as well as the local population often leave their waste into the Channel "Surcin". Also, there is an inflow of sewage water into the Channel "Surcin" under the bridge near downtown which indicates that the water is much polluted.

The water from Channel "Donje polje", Channel between P.S "Donje polje" and P.S "Petrac", Channel "Petrac" and Pool "I.S.C." belong to II and III class according to the above-mentioned Rulebook. These waters could be used for irrigation according to the Regulation on Water Classification ("Official Gazette of RS", No. 5/68). The smallest number of coliforms was

noticed in Channel "Galovca" water 430 MPN/100 ml which means that this water belongs to I-II class and it is safe for irrigation crops (Table 3).

Also, the number of fecal coliforms as well as *E. coli* was the highest in Channel "Surcin" water. The *E. coli* was also detected in waters of Channel "Donje polje" and Channel between P.S "Donje polje" and P.S "Petrac" but its number was much smaller. Other investigated waters were free for *E. coli*. Our results are in agreement with other authors which detected total coliforms in channel water ranged between 3.74 and 3.92 (logCFU/100ml) while the fecal coliforms results ranged from 3.39 and 3.82 (logCFU/100ml), respectively. The *E. coli* ranged from 3.17 to 3.54 (logCFU/100ml), while *Salmonella* sp. was not detected (Douti et al., 2021).

According to above-mentioned Rulebook ("Official Gazette of RS", No. 74/2011) for presence of fecal coliforms in groundwaters, Channel "Surcin" water belongs III-IV class. The large number of *E. coli* in this water indicates its huge contamination with fecal matter and it will be a big risk if this water should be used for irrigation, especially vegetables which are eaten in raw. The presence of *Salmonella* sp. and *Shigella* sp. was confirmed in waters of Channel "Surcin" and Channel "Donje polje" (Table 3). The study of Mcheik et al. (2018) showed that fecal contamination was in the range from 92.4% to 17.5% out of 33 total well water samples and *S. aureus* was in the range from 78.7% to 14.5%.

The number of total coliform bacteria in vegetable samples (lettuce, hot pepper, cucumber) was insignificant, less than 30 MPN/gDM each sample. The pathogens, *E. coli*, *Salmonella* sp. and *Shigella* sp. were also not detected and which indicates no fecal contamination and vegetables are safe for consumption. Unlike our results, Mcheik et al. (2018) noticed high level of faecal coliforms and *E. coli* at vegetables which were irrigated with contaminated water and the highest level of bacteria was at lettuce (6.0 and 5.0 log₁₀CFU.g⁻¹ fresh weight). Also, other authors found out high level of *E. coli* in vegetable samples irrigated with polluted water. The average number of *E. coli* for tomato samples were 3.27 logCFU/10g, for carrot samples 3.33 logCFU/10g and for spring onion and cabbage were 3.50 and 3.34 respectively (Douti et al., 2021).

Table 3. The number of coliforms (MPN/100 ml) and presence of pathogens in water samples

Water sample	Channel "Surcin"	Channel "Donje polje"	Channel between P.S "Donje polje" and P.S "Petrac"	Channel "Petrac"	Channel "Galovca"	Pool "I.S.C."
TC	>1.100.000	7.300	2.400	2.400	430	930
FC	70	< 30	< 30	< 30	< 30	< 30
<i>E. coli</i>	1100	200	36	0	0	0
<i>Salmonella</i>	+	+	-	-	-	-
<i>Shigella</i>	+	+	-	-	-	-

According to the German standard for irrigation waters (DIN 19650), the water of the Channel "Surcin" should not be used for irrigation crops which are consumed in raw. Also, according to mentioned German standard, waters from Channel "Donje polje" and Channel between P.S "Donje polje" and P.S. "Petrac" would not be used for irrigation, but our Regulation on water

classification ("Official Gazette of RS", No. 5/68) allows using these water for irrigation (II-III class).

The presence of *Salmonella* sp. and *Shigella* sp. in waters of Channel "Surcin" and Channel "Donje polje" indicate for fecal contamination and their presence in the groundwater is not specified by our law for waters which could be potentially used for irrigation. Considering worldwide epidemics caused by consumption of *Salmonella*-contaminated fresh products, it could not recommend these waters for irrigation. According to the Rulebook on general and special conditions of food hygiene at any stages of production, processing and trade ("Official Gazette of RS", No. 72/10) it is not allowed presence of *Salmonella* sp. in food. *Salmonella* sp. and *Shigella* sp. were not detected in tested vegetable samples which indicate good microbiological quality of investigated vegetables.

Conclusion

Based on the microbiological analyzes of water, it could be concluded that investigated water have medium to good microbiological quality. The Surcin channel water has very poor microbiological quality and this water is a good environment for many human pathogens. It is not usable for irrigation, especially for irrigation vegetables which are eaten in raw. This water should undergo certain purification processes in order to get microbiologically safety water for irrigation.

The other tested waters could potentially be used for irrigation but appropriate water treatment should be recommended in order to improve microbiological quality of water and achieving health safety agricultural production.

The water from the well of the company "Iceberg Salat Center", which is otherwise used for irrigation, has satisfactory microbiological quality as well as vegetables which are healthy safe for consumption.

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