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Microbial Quality of Fresh Vegetables and Irrigation Waters in Central Serbia*

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Abstract

The usage of fresh fruits and vegetables has risen over the past decades. Due to environmental problems, agricultural products can be contaminated during cultivation and other activities. The aim of this paper was to determine the microbial quality of soil, irrigation water and fresh vegetables in the central part of the Republic of Serbia. Sampling was performed in the summer of 2015 at the Agricultural high-school Svilajnac (AHS), Suburb of Svilajnac (SS), Village Gložane (GL), and Village Grabovac (GR). The microbial diversity of the soil and presence of potential human pathogens was determined using agar plate technique and MPN method, respectively. MPN method was also used for determination of total and fecal coliform bacteria in irrigation water samples. Nutrient agar was uses for determination of aerobic psychrophilic and mesophilic bacteria in irrigation water samples and vegetables and Cetrimide agar for *Pseudomonas aeruginosa* determination. Chemical characterization of irrigation water was performed using standard methodology. High microbial diversity as well as presence of pathogenic bacteria were noticed in the examined soil samples. The water cation and anion content was under the limits of permissible levels for irrigation waters. Although patogenic bacteria were detected in irrigation water samples, the obtained values were under the limits proposed by several international guidelines. In order to eliminate the possible contamination sources, principles of good agriculture practice must be implemented in production and manipulation processes.
Key words: water quality, vegetables, irrigation water, soil, coliform bacteria, contamination

Резюме

В предходните десетилетия употребата на пресни плодове и зеленчуци нараства. Поради проблеми в заобикалящата ни среда, земеделските продукти могат да бъдат заразени в периода на тяхното отглеждане, свързано с определени дейности. Целта на тази статия е да се определи микробното съдържание в почвата, поливната вода и пресните плодове и зеленчуци в централната част на Република Сърбия. Събирането на проби е осъществено през лятото на 2015 в Земеделската гимназия Свилайнац (AHS), предградието на Свилайнац (SS), село Гложане (GL) и село Габровац (GR). Микробното разнообразие на почвата и присъствието на възможни човешки патогени е определяно съответно чрез техники на плаки с агар гел и метода MPN. Методът MPN е използван също за определяне на общите и фекалните колиформи в пробите от поливната вода. За определянето на аеробни психрофилни и мезофилни бактерии от поливната вода и зеленчуците е използвана хранителна агарона среда, а Цетримидов агар за определянето на *Pseudomonas aeruginosa*. Химическото характеризиране на поливната вода е направено по стандратна методология. Голямо микробиално разнообразие, а така също наличието на патогенни бактерии е отбелязано в изследваните почвени проби. Съдържанието на катиони и аниони е под допустимите нива за поливните води. Въпреки наличието на патогенни бактерии във водните проби, получените стойности са под допустимите гра-

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ници, предлагани в няколко международни наръчници. За да се избегне възможно контаминиране е необходимо в процесите на производството и обработката да се прилагат принципите на добрата земеделска практика.

Introduction

Consumption of fresh fruits and vegetables has been increasing in the past decades (Leon *et al.*, 2009; Betts, 2014) and provides benefits for the health of consumers (Dauchet *et al.*, 2005). However, consumption of these products is one of the factors connected to human gastrointestinal problems (Pachepsky *et al.*, 2011). Fresh products can be microbiologically contaminated during production, packing, preparation, and other activities. It is evident that irrigation water, which includes different surface waters, groundwaters and/or aquifers, is one of the major sources of contamination (Uyttendaele *et al.*, 2015). Thus, microbial quality of irrigation water, as well as, its contamination potential, can be affected by the design of wells, substrate characteristics, depth of groundwaters, etc. (Gerba, 2009).

In recent years, the accidence of foodborne outbreaks connected with fresh vegetables contamination has increased worldwide (Mukherjee *et al.*, 2006). Several reports indicated the contamination of fresh vegetables with pathogenic bacteria, such as *Salmonella* (Greene *et al.*, 2008), *Escherichia coli* (Friesema *et al.*, 2007), *Listeria* (Abadias *et al.*, 2008), etc. As described previously, the major source of these pathogenic microorganisms are contaminated irrigation water, but their incidence may be linked with the usage of contaminated seeds (Berger *et al.*, 2010), or organic fertilizers as a soil amendment (Franz *et al.,* 2008). Compared to traditional production, agricultural organic production has increased the risk to public health due to organic manure application instead of mineral fertilizers, and avoidance of pesticides treatments (Oliveira *et al.,* 2010). One of the aims of this cultivation method is reduction of the potential microbiological contamination of fresh agricultural products; however, in some cases, lack of scientific arguments to support this statement was noticed (McMahon and Wilson, 2001).

Vegetable production is one of the most important branch of agriculture of the Republic of Serbia (Novković *et al.,* 2012). Due to the favourable agro-ecological conditions for vegetable production in the Republic of Serbia (especially in the central part), it is necessary to support the production of high-quality fresh products (Puškarić *et al.*, 2009). The aim of this work was to estimate the microbial quality of soil, irrigation water and fresh vegetables in the central part of the Republic of Serbia – Pomoravlje District.

Material and Methods

Sampling of soil, irrigation water and vegetables (tomato – *Solanum lycopersicon*; pepper - *Capsicum annuum*; and carrot – *Daucus carota*) was performed in the summer of 2015 at several locations in the Pomoravlje District (Table 1).

The total number of bacteria and *Azotobacter* sp. used for determination of the microbiological characteristics of soil was estimated on tryptic soya agar (Torlak, Serbia), and Fyodorov's agar, respectivelly. Most-probable-number (MPN) procedures were used to estimate numbers of total coliform (TC) and fecal coliform (FC) bacteria. *E. coli* was identified with Eosin Methylene Blue Agar (EMB Agar, Biomerieux, France) at the temperature of 37ºC for 24 h, while *Streptococcus faecalis* was estimated on Bile Esculin Agar (Torlak, Serbia) at the

Table 1. Examined locations in Pomoravlje District

Locations	Description of location	Type of sample	Type of production	Type of gardening
1	Agricultural high-school Svilajnac (AHS)	Groundwater/soil/tomato, pepper	conventional	greenhouse
2	Suburb of Svilajnac (SS)	Groundwater/soil/tomato, pepper	conventional	open field
3	Village Gložane (GL)	Groundwater/soil/tomato, carrot	organic	open field
4	Village Grabovac (GR)	Groundwater/soil/tomato, pepper, carrot	organic	greenhouse

temperature of 44ºC for 24 h

The ammonia nitrogen content in irrigation water was determined using EPA 350.1 method, nitrates, nitrites and sulphates by EPA 300.1 method (Hautman and Munch, 1997), COD by dichromate oxidation method (APHA, 1995), total phosphorus by EN ISO 11885 method, and Fe, K, Mn, and Na content by ICP-OES method.

In irrigation water samples and vegetables samples, the number of aerobic psychrophilic and mesophilic bacteria was determined using cultivation methods on Nutrient agar (Torlak, Serbia) at the temperatures of 22ºC for 72 h, and 37ºC for 48 h, respectively. Determination of TC, FC, *E. coli*, and *S. faecalis* in irrigation water and vegetable samples was performed using the same techniques for its abundance in soil. The presence of *P. aeruginosa* in irrigation water and vegetables was detected using Cetrimide agar (HiMedia, India) at the temperature of 42ºC for 24 h.

The population density of the total number of bacteria and *Azotobacter* sp., as well as psychrophilic and mesophilic aerobic bacteria in soil/vegetables and irrigation water samples was expressed as colony forming units (CFU) per gram, or mililiter, respectivelly. The microbiological quality of vegetables was estimated using the criteria proposed by PHLS (2000) and EC 2073/2005.

Results and Discussion

The number of bacterial species in soil may be enormous (Dykhuizen, 1998); they play an important role in the soil ecosystem (van Elsas *et al*., 2006). Although microbial diversity of soil has a positive effect on the abundance of pathogenic bacteria, this effect depends on soil characteristics (van Elsas *et al.,* 2012) and agricultural management (Messiha *et al*., 2009).

The results of this study showed a different number of microorganisms in the soil, depending on the location of sampling, type of production and gardening, and vegetable species (Table 2).

In soil samples from village Grabovac, where organic production of vegetables was applied, the highest total number of bacteria was noticed compared to soil samples from other locations. Also, the highest abundance of *Azotobacter* sp. was noticed in Grabovac village in soil under tomato ($6.83x10^4$ CFU/g). At all examinated locations, the number of *Azotobacter* sp. was higher in the soil under tomato compared to other vegetables. Other authors suggest the specific role of *Azotobacter* sp. in tomato production, which can be linked with the synthesis of auxins, cytokinins and similar stimulative compounds (Azcorn and Barea, 1975). The results presented in Table 2 show presence of fecal contamination in most samples. The highest value of total and fecal coliform bacteria, as well as *E. coli*, was noticed in soil under tomato at SS location. A high number of these bacteria was also recorded at AHS location. Recent studies of agricultural soils have recorded fecal contamination (Faissal *et al*., 2016; Palacios *et al*., 2017). Some studies suggested that pathogenic bacteria can be transported from soil, through roots, to the shoots of vegetables (Solomon *et al*., 2002), or from contaminated roots to shoots (Bernstain *et al*., 2007). On the other hand, Sharma *et al*. (2009) confirmed that pathogens found in the roots were not detected in the shoot tissue of spinach.

Besides microbiological parameters, the quality of water can be evaluated using chemical indicators (Reche *et al*., 2016). Groundwaters are a valuable source that sustains life on Earth (D'Ales-

Table 2. Microbiological characteristics of soil

Sample	Vegetable	Total number of bacteria ($x10^7$CFU/g)	*Azotobacter* sp.($x10^4$CFU/g)	TC	FC	*E. coli*	*S. faecalis*
				MPN/g			
AHS	tomato	2.29	2.29	>1279	>1279	>1279	<3
	pepper	2.59	0.00	>1447	>1447	57	<4
SS	tomato	1.48	3.00	>1507	>1507	>1507	<4
	pepper	1.66	2.52	<4	<4	<4	<4
GL	tomato	3.06	5.44	57	57	57	<4
	carrot	1.46	3.84	1279	1279	34	<3
GR	tomato	4.81	6.83	59	59	34	<4
	pepper	4.47	4.89	36	36	26	<4
	carrot	8.63	3.19	>1158	>1158	>1158	<3

Table 3. Chemical characteristics of irrigation water

Sample	pH	NH_3	NO_2^-	NO_3^-	SO_4^{2-}	COD	total P	Fe	K	Mn	Na
	mg/l										
AHS	7.56	<0.05	<0.020	34.5	73.8	<10	0.091	<0.010	1.92	0.003	19.9
SS	7.57	0.05	<0.020	48.2	83.1	<10	<0.055	0.231	2.59	0.003	20.3
GL	7.46	<0.05	<0.020	45.1	231.8	<10	<0.055	0.011	5,78	0.015	25.5
GR	7.35	<0.05	<0.020	46.8	58.8	<10	0.055	<0.010	2.18	<0.001	22.9

sandro *et al*., 2017) and its composition is affected by hydrological, geochemical and biological processes (Custodio, 2010). In our study, good chemical quality of all groundwater samples, according to Serbian legislation (Službeni glasnik RS 50/2012) was recorded (Table 3).

In all samples, neutral to slighly alkaline reaction of irrigation water was recorded. The water cation and anion content was under the limits of permissible levels for irrigation waters according to proposed legislation. Similar results were obtained for the microbiological characteristics of water samples (Table 4).

Except for the SS sample, low numbers of psychrophilic and mesophilic bacteria in iriirgation water samples were recorded. Absence of *P. aeruginosa* in all samples and low presence of *E. coli* in one sample (GR) was recorded. The number of total and fecal coliform bacteria was also under the permissible level according to Serbian legislation. The values obtained in this research were also under the limits proposed by several international guidelines for quality of irrigation waters (British Columbia Ministry of environment, 1998; Canadian Council of ministers of the environment, 1999; DIN 19650, 1999).

Most bacteria initially detected on fresh vegetables originated from soil ecosystems (Barth *et al*., 2009). The quality of fresh vegetables depends on irrigation water quality and application of good agriculture practices; unfortunatelly, the contact with soil particles, irrigation water and fertilizers, impede the control of vegetable contamination by pathogenic microorganisms (de Oliveira *et al*., 2011).

Our results showed different levels of fresh vegetables contamination, depending on the various factors (Table 5).

The presented results show a higher level of contamination at AHS location - in pepper samples compared to tomato, while at SS location vice versa. In organic production fields, higher numbers of psychrophilic and mesophilic bacteria in carrot samples compared to other vegetables were recorded. A large number of these bacteria was detected in leafy vegetables, carrots and sprouds (Abadias *et al*., 2008), which is in accordance with our results. The number of aerobic bacteria in tomato was from 0.7 to 468x10^3 CFU/g, while in carrot from 209 to 386x10^3 CFU/g. These values for tomato were lower compared with other reports, where the average total aerobic plate count was 4x10^7 CFU/g (Razzaq *et al.,* 2014). Also, Jeddi *et al.* (2014) indicate that in most of the samples (70%) the number of aerobic bacteria in ready-to-eat salad containing tomato, carrot, lettuce, cucumber and coleslaw was between 10^6 and 10^7 CFU/g. On the other hand, Razzaq *et al*. (2014) reported a lower number of aerobic bacteria in carrot (4x10^4 CFU/g) compared to our research.

Table 4. Microbiological characteristics of irrigation water

Sample	aerobic		*P. aeruginosa*	coliform		*E. coli*	*S. fecalis*
	psychrophilic	mesophilic		total	fecal		
	(x 10^3 CFU/ml)			MPN/100 ml			
AHS	0.05	0.04	n.i.	9.2	0.0	0.0	9.2
SS	11.30	31.70	n.i.	>16	>16	0.0	0.0
GL	0.58	0.08	n.i.	0.0	0.0	0.0	0.0
GR	0.60	0.70	n.i.	2.2	2.2	2.2	0.0

n.i. – not identified

Table 5. Microbiological quality of fresh vegetables

Sample	Vegetable	AP	AM	TC	FC	*E. coli*	*S. faecalis*	PS
		(x 10^3 CFU/g)		MPN/100g				
AHS	tomato	0.7	1.9	1,100	1,100	6.0	<300	-
	pepper	315	153	4,300	4,300	<300	4,600	+
SS	tomato	468	310	>110,000	>110,000	>110,000	<300	-
	pepper	43	43	1,100	360	<300	<300	-
GL	tomato	4.7	2.0	<300	<300	<300	<300	-
	carrot	386	356	110,000	110,000	<300	<300	-
GR	tomato	23	18	>110,000	110,000	<300	<300	-
	pepper	136	15	>7,500	610	610	<300	-
	carrot	290	209	>9,300	>9,300	<300	<300	-

Legend: AP - aerobic psychrofilic; AM - aerobic mesophilic; TC - total coliform; FC - fecal coliform; PS – *P. aeruginosa*

Contamination by coliform bacteria was detected in all examined samples. The highest level of contamination was noticed in tomato samples at AHS and GR location, and in carrot at GL location. The presence of *E. coli* was the highest in tomato samples at SS location, and *S. fecalis* in pepper at AHS location. The comparison of the results obtained in our research to the results of Razzaq *et al.* (2014) show that the total number of coliforms obtained on tomato samples was higher, but in the case of carrot it was lower. Our results are similar to the results obtained by Shenge *et al*. (2015), who noticed that in most tomato samples the number of total coliforms was about 5 Log MPN/100g. In the same research, in most of tomato samples, *E. coli* was not identified. Similar observation was achieved by Arthur *et al.* (2007). *E. coli* and *S. fecalis* were also present in all examined vegetable samples. Bohaychuk *et al.* (2009) confirmed the absence of *E. coli* in organically and conventionally grown tomato samples, and its presence was noticed only in conventionally grown carrot samples, which differs from our results. In most samples, contamination of pepper was lower compared with tomato and carrot. This observation is in contrast with the results of Almuktar and Sholz (2015), who indicated insignificant microbiological contamination of chillies.

In most samples, absence of *P. aeruginosa* was detected, which is in acordance with several previous studies where contamination of vegetables by *P. aeruginosa* was not detected. According to critiria proposed by PHLS (2000) based on the total aerobic bacteria number, the microbiological quality of all tomato and pepper samples from SS location was acceptable. The results showed that according to EC 2073/2005, most of the vegetable samples can be used for human consumption.

This results indicate the good microbiological quality of the examined samples. The principles of good agriculture, manufacturing and hygiene practice must be applied by producers in order to eliminate the possible sources of contamination and to improve the quality of vegetables production.

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