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Sanitary assessment of soil in the municipality of Čačak based on the presence of *Escherichia coli* and *Salmonella* sp.

Leka Mandić[1*], Dragutin Đukić[1], Marijana Pešaković[2], Vesna Đurović[1]

[1] *University of Kragujevac, Faculty of Agronomy in Čačak, Cara Dušana 34, Čačak, Serbia*
[2] *Fruit Research Institute in Čačak, Kralja Petra 18, Čačak, Serbia*

**Corresponding author: lekamg@kg.ac.rs*

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A B S T R A C T

The aim of this study was to assess the sanitary status of soils in the Municipality of Čačak, based on the presence of *Escherichia coli* and *Salmonella* sp. The examination involved testing soils previously exposed to different forms of degradation, including soils near industrial zones and landfills, soils close to congested roads,soils exposed to floods, and soils exposed to the uncontrolled use of agrochemicals and agricultural machinery.

The data obtained indicate that the examined soils are mostly unpolluted or slightly to moderately polluted and that therefore they will not have a negative effect on plant production and the health of humans and animals.

Keywords: soil, Escherichia coli, Salmonella sp., sanitary assessment.

И З В О Д

У раду је разматран санитарни статус земљишта на територији града Чачка, на основу присуства и бројности *Escherichia coli* і *Salmonella* sp. Испитивањем су обухваћена земљишта која су у претходном периоду билиа изложена неком виду деградације: земљишта у близини индустријских зона и депоније; земљишта поред фреквентних саобраћајница; земљишта која су била изложена поплавама и земљишта са неконтролисаном употребом агрохемикалија и пољопривредних машина.

Добијени подаци указују да су испитивана земљишта углавном незагађена или слабо до умерено оптерећена овом врстом загађења и да због тога неће имати негативног ефекта на биљну продукцију и здравствени статус животиња и људи.

Кључне речи: земљиште, Escherichia coli, Salmonella sp., санитарна оцена.

1. Introduction

Microorganisms are the most important chain in the metabolic activity of soil. They are involved in a number of biological processes, and their numbers and activity have a significant effect on the production capability and the ecological, sanitary and hygienic status of soil (Winding et al., 2005; Yamahara et al., 2012; Mandić, 2019). The total numbers of saprophytes and certain physiological groups of microorganisms, and their enzymatic activity are important indicators of potential and effective fertility of soil (Nannipieri et al., 2003; Đukić and Mandić, 2006). Recently, in addition to the aforementioned characteristics of soil, the assessment of its sanitary and epidemiological status and the identification of soil pollution microbial indicators have been emphasised. Sanitary and epidemiological assessments of soil rely on the use of sanitary and bacteriological indicators –the presence of the causal agents of intestinal infections, pathogenic bacteria, enteroviruses, and sanitation indicator microorganisms; sanitary parasitological indicators – the presence of the causal agents of parasitic diseases (geo-helminthiasis, amoebiasis); sanitary toxicological indicators –the contents of chemical pollutants; sanitary chemical indicators (sanitary number, organic matter content), and the presence of fly larvae and pupae (Đukić et al., 2019; Kalwasińska et al., 2012).

One of the most important indicators of the sanitary status of soil is the presence of the faecal bacteria *Escherichia coli* and *Salmonella* sp. These bacteria are not typical inhabitants of soil and therefore they are not covered by national regulations on soil quality assessment. In most cases, they are not found in pure soils or their number is very low (coli titre is above 1). As determined by Đukić et al. (2011), values of coli titre are 1.0–0.01 in slightly contaminated soils,0.01–0.001 in moderately contaminated soils, and up to 0.001 in extremely contaminated soils.

Their numbers and survival in the soil depend on several factors (Yamahara et al., 2012), which have to be considered due to the possibility of their penetration into cultivated plants (Đukić at al., 2009) or groundwater, and the resulting harmful effects on the health status of higher organisms and humans in the food chain(Grisey et al., 2010).

The aim of this research is the sanitary assessment of soil in the Municipality of Čačak based on the

presence and numbers of Escherichia coli and Salmonella sp.

2. Materials and methods

Soil testing was performed at the end of 2018 using samples of soils previously exposed to some form of degradation in the Municipality of Čačak. When choosing sampling sites, consideration was given to the fact that suburban and urban areas are mostly polluted from industries, landfills and traffic congestion, and that the most frequent sources of pollution of agricultural soils are the uncontrolled use of agrochemicals (mineral fertilisers, pesticides etc) and agricultural machinery or the influence of weather-related natural disasters such as floods. With this in mind and aiming to facilitate the interpretation and analysis of results, the chosen sites were classified into four groups, as follows: soils near industrial zones and landfill (3 sites), soils near congested roads (3 sites), soils exposed to floods (2 sites), and soils exposed to the uncontrolled use of agrochemicals and agricultural machinery (2 sites) –Tab. 1.

The soils were sampled using an agrochemical probe from the surface layer up to 30 cm depth. When sampling, GPS coordinates of the sites were determined.

Three separate soil samples were taken from each site aseptically and transferred to three sterile bags. Air-dried samples were ground and passed through a 2 mm sieve. Microbiological tests (determination of counts of *E. coli* and *Salmonella* sp.) were performed in the Microbiological Laboratory of the Faculty of Agronomy in Čačak. The numbers of *E. coli* and *Salmonella* sp. were determined as colony forming units (CFUs) on agar plates by the Serial Dilution Plate method. The media used for the enumeration of *E. coli* and *Salmonella* sp. were Endo agar and SS agar (*Salmonella Shigella Agar*), respectively. Plates were incubated at 37^{O}C for 24–48 hours.

The determination of the presence of the aforementioned bacteria was based on colony characteristics and microscopic evaluation. The obtained values were calculated at 1.0 g absolutely dry soil. The soil pollution status was assessed following the standards reported by Đukić et al. (2011).

Table 1
Sites chosen for the sanitary assessment of soils in the Municipality of Čačak

Item	Site	Number of analysed samples
I Soils near industrial zones and landfill		
1.	Old industrial zone	9
2.	New industrial zone	9
3.	Landfill	9
II Soils close to congested roads		
4.	Soils close to roadswith dense traffic, outside the city zones (locality I)	9
5.	Soils close to roadswith dense traffic, outside the city zones (locality II)	9
6.	Soils close to roadswith dense traffic, within the city zones	1
III Soils exposed to floods		
7.	Agricultural soil exposed to floods (locality I)	4
8.	Agricultural soil exposed to floods (locality II)	4
IV Soils exposed to the uncontrolled use of agrochemicals and agricultural machinery		
9.	Orchards	3
10.	Arable land	3

3. Test results and discussion

The counts/presence of the tested groups of microorganisms in the soils of the tested sites is presented in Table 2.

Table 2
Sanitary assessment of the examined sites in the Municipality of Čačak, based on average counts of *E. coli* and *Salmonella* sp.

Sample number	GPS (N)	GPS (E)	*E. coli* (10^2/g)	*Salmonella* sp. (25 g)
Solis near the old industrial zone				
1	43 53.485 43 53.477	20 22.094 20 22.105	8.1**	-
2	43 53.525 43 53.516	20 22.129 20 22.150	5.3**	-
3	43 53.573 43 53.559	20 22.167 20 22.196	7.1**	-
4	43 53.375 43 53.362	20 22.142 20 22.143	3.3**	-
5	43 53.359 43 53.372	20 22.083 20 22.078	10.0**	-
6	43 53.312 43 53.315	20 22.008 20 21.988	10.0**	-
7	43 53.186 43 53.184	20 22.251 20 22.244	1.2*	-

8	43 53.165 43 53.163	20 22.206 20 22.212	-	-
9	43 53.139 43 53.131	20 22.174 20 22.178	-	-
Soils near the new industrial zone				
10	43 53.790 43 53.795	20 25.660 20 25.632	5**	-
11	43 53.831 43 53.830	20 25.708 20 25.726	1.8**	-
12	43 53.888 43 53.874	20 25.769 20 25.797	0.6*	-
13	43 53.857 43 53.853	20 25.824 20 25.842	0.6*	-
14	43 53.829 43 53.826	20 25.914 20 25.939	0.7*	+
15	43 53.781 43 53.783	20 25.929 20 25.911	2.1**	+
16	43 53.802 43 53.808	20 25.788 20 25.767	0.1*	+
17	43 53.742 43 53.732	20 25.872 20 25.875	22.1***	-
18	43 53.759 43 53.763	20 25.780 20 25.748	-	-
Soils near the landfill				
19	43 52.616 43 52.629	20 23.111 20 23.080	3.1**	-
20	43 52.642 43 52.639	20 22.988 20 23.013	10.0**	-
21	43 52.841 43 52.838	20 22.828 20 22.814	-	-
22	43 52.585 43 52.591	20 23.026 20 23.000	-	-
23	43 52.571 43 52.568	20 23.146 20 23.122	0.3*	-
24	43 52.853 43 52.862	20 22.776 20 22.774	1.3*	-
25	43 52.578 43 52.590	20 22.919 20 22.894	50.3***	-
26	43 52.520 43 52.527	20 23.135 20 23.118	6.2**	-
27	43 52.863 43 52.864	20 22.725 20 22.713	2.4**	-
Soils close to roads with dense traffic, outside the city zones (locality I)				
28	43 56.004 43 56.024	20 24.449 20 24.461	0.4*	-
29	43 55.969 43 55.982	20 24.581 20 24.582	0.9*	-
30	43 55.973 43 55.989	20 24.653 20 24.656	0.9*	-
31	43 55.292 43 55.287	20 24.457 20 24.444	0.2*	-
32	43 55.313 43 55.310	20 24.361 20 24.346	4.6**	+
33	43 55.328 43 55.348	20 24.282 20 24.282	1.8**	-
34	43 55.038 43 55.033	20 24.446 20 24.441	0.7*	-
35	43 55.049 43 55.055	20 24.405 20 24.411	0.1*	-
36	43 55.059 43 55.069	20 24.360 20 24.366	0.9*	-
Soils close to roads with dense traffic, outside the city zones (locality II)				
37	43° 51.755 43° 51.741	20 32.660 20 32.685	8.2**	-
38	43° 51.688 43 51.674	20 32.847 20 32.868	8.3**	-

39	43 51.401 43 51.385	20 33.193 20 33.209	10.0**	-
40	43 51.346 43 51.341	20 33.182 20 33.213	6.4**	-
41	43 51.305 43 51.293	20 33.152 20 33.142	17.1***	+
42	43 51.549 43 51.553	20 32.768 20 32.742	2.2**	+
43	43 51.626 43 51.620	20 32.563 20 32.584	9.3**	-
44	43 51.614 43 51.630	20 32.807 20 32.820	3.1**	-
45	43 51.683 43 51.678	20 32.603 20 32.595	8.0**	-
Soils close to roads with dense traffic, within the city zones				
46	43 52.762 43 52.753	20 21.237 20 21.232	3.3**	-
Agricultural soils exposed to floods (locality I)				
47	43 54.072 43 54.063	20 24.299 20 24.289	1.0*	-
48	43 53.981 43 53.971	20 24.455 20 24.464	0.7*	-
49	43 53.583 43 53.602	20 24.481 20 24.461	0.3*	-
50	43 53.948 43 53.960	20 24.873 20 24.843	0.7*	-
Agricultural soils exposed to floods (locality II)				
51	43 51.747 43 51.727	20 27.835 20 27.817	5.1**	-
52	43 51.493 43 51.500	20 28.326 20 28.298	20.2***	-
53	43 51.696 43 51.673	20 28.334 20 28.315	8.3**	-
54	43 51.278 43 51.299	20 28.500 20 28.486	5.2**	-
Soil exposed to the uncontrolled use of agrochemicals and agricultural machinery (orchards)				
55	43 56.147 43 56.136	20 20.346 20 20.373	1.6*	-
56	43 55.840 43 55.822	20 21.410 20 21.404	-	-
57	43 54.981 43 54.997	20 21.855 20 21.861	3.1**	-
Soil exposed to the uncontrolled use of agrochemicals and agricultural machinery) (arable land)				
58	43 55.850 43 55.871	20 19.561 20 19.552	4.5**	-
59	43 56.226 43 56.227	20 22.054 20 22.066	5.0**	-
60	43 54.860 43 54.858	20 21.189 20 21.168	10.0**	-

* slightly polluted; ** moderately polluted; *** extremely polluted/contaminated
+ presence; - absence

The obtained data on the sanitary status of soil (presence/numbers of E. coli and Salmonella sp.) indicate that the examined soils are mostly uncontaminated or slightly to moderately contaminated. Exceptions are soils near the new industrial zone, soils near the landfill, soils close to roads with dense traffic (locality II) and floodplains at locality II (samples nos. 17, 25, 41 and 52, respectively), classified, by the presence of E. coli, asextremely contaminated soils. The occurrence of somewhat higher numbers of E. coli at these sites may be the result of an increased amount of fresh organic waste, faeces of sick animals and birds, and other uncontrolled activities reported by other authors (Yamahara et al., 2012). With regard to Salmonella sp., significant differences were not observed.

Since these groups of microorganisms are not capable of long-term persistence in soil (Recorbet et al., 1993; Gagliardi and Karns, 2002; Đukić et al., 2009, 2011), and since their counts are not significantly high, their current status is not expected to have a crucial effect on the productive capacity of soil and the quality of plant production. However, to prevent the inflow of these microorganisms into the soil and their expansion,

care must be taken to ensure proper preparation and use of organic fertilisers, acceptable irrigation water quality, proper municipal and industrial waste management, and the use of other sanitary measures which directly or indirectly affect the level of soil contamination.

4. Conclusion

The present study reveals that the soil of the Municipality of Čačak exposed to some form of degradation (industrial zones, landfill, soil exposed to floods, soils near congested roads, soil exposed to the uncontrolled use of agrochemicals and agricultural machinery) is mostly uncontaminated or slightly to moderately contaminated with *E. coli* and *Salmonella* sp.

The sporadic occurrence of a larger number of *E. coli* in certain soil samples may be attributed to its uncontrolled expansion due to the vicinity of congested roads, industrial zones and landfills.

The studied groups of microorganisms will not have a negative effect on plant production and human and animal health since they are not capable of long-term persistence (survival) and since their numbers are not significantly high.

In order to prevent and mitigate soil contamination and the expansion of these microorganisms in the soil, care must be taken to ensure the use of microbiologically safe manure and the implementation of sustainable industrial and municipal waste management.

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